Toxicology Assessment
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ABBREVIATIONS

ng	Nanogram	**nM**	Nanomolar
µg	Microgram	**mM**	Millimolar
mg	Milligram	**sec**	Second
kg	Kilogram	**min**	Minute
mL	Millilitre	**h**	Hour
L	Litre	**m**	Metre
GI	Gastrointestinal	**SC**	Subcutaneous
IM	Intramuscular	**LH**	Luteinising hormone
IP	Intraperitoneal	**mg/kg bw/d**	mg/kg bodyweight/day
IV	Intravenous	**ppb**	Parts per billion
PO	Oral	**ppm**	Parts per million

ADI	Acceptable Daily Intake
AP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
ALT	Alanine aminotransferase (SGPT)
BUN	Blood urea nitrogen
ChE	Cholinesterase
CPK	Creatinine phosphokinase
DDM	4,4'-Diaminodiphenylmethane
DMSO	Dimethyl sulfoxide
EUP	End Use Product
GLP	Good Laboratory Practice
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
LOEL	Lowest Observed Effect Level
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MRL	Maximum Residue Limit
NOEL	No Observable Effect Level
NTE	Neuropathy target esterase
OP	Organophosphorus pesticide
2-PAM	Pyridine-2-aldoxime methiodide
P-2-S	2-pyridine-aldoxime methyl methanesulfonate
TGAC	Technical Grade Active Constituent

ACPH	Advisory Committee on Pesticides and Health
NHMRC	National Health and Medical Research Council
NDPSC	National Drugs and Poisons Scheduling Committee

SUMMARY

Introduction

Chlorpyrifos is a broad-spectrum organophosphorus insecticide that has been used in Australia for over 30 years. The current Acceptable Daily Intake (ADI) for chlorpyrifos is 0.003 mg/kg/d, based on the no-observed effect level (NOEL) for plasma cholinesterase inhibition of 0.03 mg/kg/d in a human volunteer study, and using a 10-fold safety factor. The current poisons schedule classification for chlorpyrifos is Schedule 6, with a cutoff to Schedule 5 when used in preparations at concentrations of 5% or less of chlorpyrifos, when in aqueous preparations containing 20% or less of micrencapsulated chlorpyrifos, or in controlled release granular preparations containing 10% or less of chlorpyrifos. Potting or soil mixes containing 100g per cubic metre or less of chlorpyrifos are exempt from poisons scheduling.

Chlorpyrifos is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under Australia's Existing Chemicals Review Program (ECRP). A number of additional data submissions on the toxicology of chlorpyrifos were received from industry and the public following the ECRP data call-in process, and these data, together with all previously submitted data, have been assessed in detail. The detailed report is summarised below.

The toxicology of chlorpyrifos has also been periodically reviewed by the Joint WHO/FAO Meeting on Pesticide Residues (JMPR), most recently in 1999, and the JMPR ADI is 0.01 mg/kg bw/day, based on erythrocyte cholinesterase inhibition in a human volunteer study with an NOAEL (no-observed adverse effect level) of 0.1 mg/kg bw/day, and using a 10-fold safety factor, and on the NOAEL of 1 mg/kg bw/day for the inhibition of brain cholinesterase activity in mice, rats and dogs, and using a 100-fold safety factor. The Meeting also allocated an acute reference dose of 0.1 mg/bw on the basis of the NOAEL of 1 mg/kg bw for inhibition of erythrocyte cholinesterase activity in a study in which volunteers received a single oral dose of chlorpyrifos and with a safety factor of 10.

Metabolism and toxicokinetics

^{36}Cl-chlorpyrifos (50 mg/kg bw), given to male Wistar rats by intubation, was eliminated rapidly, predominantly in the urine (90% of the dose) and faeces (10% of the dose). The urinary metabolites were identified as 3,5,6-trichloro-2-pyridyl phosphate (3,5,6-TCP phosphate) (75-80%), 3,5,6-trichloro-2-pyridinol (3,5,6-TCP) (15-20%) and traces of chlorpyrifos; this indicates that desethylation is a significant metabolic pathway in rats. Residue levels were highest in liver and kidney at 4 h post dose but the half life was less than 20 h in these tissues. The longest residue-half-life of 62 h was recorded in fat (Smith et al, 1967).

A single intubated dose of ^{14}C-ring-labelled-chlorpyrifos (19 mg/kg) was administered to male Sprague-Dawley rats. By 72 h, 83-87% of the total dose had been eliminated, mainly as TCP in the urine (68-70%), faeces (14-15%) and respired air (0.15-0.39%). Residues at 72 h were about 1.7% of the total dose and while highest in fat were less than 1 ppm in any tissue (Branson & Litchfield, 1971a).

Female Fischer 344 rats were dosed with chlorpyrifos by oral gavage or by inhalational exposure in nose-only or whole-body chambers. Another group was exposed to oral gavage doses of ^{14}C-labelled chlorpyrifos to estimate recovery efficiency. Oral dosing lead to 30-80% recovery in urine with large inter-individual availability. Inhalational exposure, whether nose-only or whole body, led to average urinary excretion, on an exposure adjusted basis, of between 0.28 to 0.58 µg of 3,5,6-TCP per ppb of chlorpyrifos in air. The whole-body-exposed rats absorbed more chlorpyrifos than would be expected to occur by the inhalation route alone. Grooming activity by rats probably contributed to the larger oral intake of chlorpyrifos in animals exposed in whole-body chambers. The total combined recovery of radiolabel from urine, faeces, and cage-wash was 99.8% of the administered dose (Nolan et al, 1986).

Single doses of ^{14}C-ring labelled chlorpyrifos (19 mg/kg) or 3,5,6-TCP (7 mg/kg) were administered by oral gavage to Sprague-Dawley rats. The radiolabel was analysed in blood samples, expired air and excreta. The major urinary metabolite of 3,5,6-TCP and chlorpyrifos was 3,5,6-TCP. The biological half-life of each chemical was estimated to be between 8-17 h. By 72 h more than 98% of each compound had been eliminated, mainly in the urine (68-70% for chlorpyrifos, 73-76% for 3,5,6-TCP) and faeces (14-15% for chlorpyrifos, 6-7% for 3,5,6-TCP). Elimination as CO_2 was a minor (<1%) pathway. Residue levels were uniformly low in the brain, while the highest residue levels occurred in the bone and intestine following 3,5,6-TCP administration, and in fat and intestine following chlorpyrifos administration. Administration of a single oral dose of ^{14}C-labelled 3,5,6-TCP or chlorpyrifos did not indicate accumulation of these compounds in rat tissues (Branson & Litchfield, 1970, 1971b).

Groups of male and female Fischer 344 rats were dosed with ^{14}C-labelled chlorpyrifos as a single oral dose of 0.5 or 25 mg/kg, or as 15 consecutive daily doses of 0.5 mg/kg/d followed on day 16 by 0.5 mg ^{14}C-chlorpyrifos/kg bw. By 3 days post-exposure, essentially all of the radioactivity had been recovered (>97%), mainly in the urine (84-92% of administered dose), with 6-12% in faeces. Multiple doses induced a slight increase (6-7%) in urinary excretion as compared to a single dose at the 0.5 mg/kg bw/day dose level. At sacrifice, the maximum total residues were 0.2% of the administered dose, seen at the 25 mg/kg bw, and residues occurred mainly in perirenal fat and liver of males and in the ovaries and fat of females. The half-life for excretion was 12.4 h for males and 23.2 h for females at 25 mg/kg, probably reflecting a sex-difference in absorption rates. Analysis of urine for metabolites failed to reveal any unchanged chlorpyrifos. The major metabolites were 3,5,6-TCP (12%), its glucuronide conjugate (80%) and tentatively, a sulphate conjugate (Nolan et al, 1987).

Laying hens (36/group) were fed chlorpyrifos at 0 (two groups), 0.3, 1, 3 and 10 (three groups) ppm in their diet for 30-45 days before sacrifice. After 30 days, all control birds and 24 birds from each dose level were sacrificed. The remaining 12 hens at each dose level were fed treated diet for a total of 45 days, and eggs were collected from these birds during the treatment period. At 10 ppm, the additional two groups of birds were allowed to recover from treatment for 7 and 21 days respectively after the 30-day treatment period, and were then killed. Chlorpyrifos residues were not detected in any tissue except fat; 3,5,6-TCP residues were recorded in liver (0.15 ppm), kidney (0.33 ppm) and in eggs (<0.05 ppm) at the 10 ppm feed-level, but no residues were detectable after 7 days withdrawal period (Dishburger et al, 1972).

Two goats were fed ^{14}C-ring labelled (positions 2 and 6) chlorpyrifos twice daily via capsule for 10 days at a level equivalent to 15 to 19 ppm in the feed. The majority (80.3%) of the total ^{14}C activity was recovered in the urine, with smaller amounts in faeces (3.6%), gut (0.9%), tissues (0.8%), and milk (0.1%). The major urinary analyte was the ß-glucuronide conjugate of 3,5,6-TCP with smaller amounts of free 3,5,6-TCP. The major (>75%) residue in fat was chlorpyrifos (0.12 ppm), while 3,5,6-TCP was the major residue in liver and kidney (Glas, 1981a). A very similar pattern of elimination was seen in a study of lactating goats fed ^{14}C-ring labelled chlorpyrifos twice daily by capsule; very little ^{14}C activity (mainly chlorpyrifos) was recovered in milk (0.05 to 0.14%) (Glas, 1981b).

Groups of 3 pigs (2 male, 1 female) were fed basal rations containing 0, 1, 3 or 10 ppm chlorpyrifos for 30 days; tissue samples were collected after withdrawal periods of 0, 7 and 21 days. Initially, residues were predominantly in the fat tissues, with progressively lower levels seen in muscle, liver and kidney; these values had all declined to non-detectable by 7 days withdrawal (McKellar et al, 1972). Weanling pigs of mixed sex were given daily oral doses (equivalent to 75 ppm in the diet) of ^{14}C-labelled 3,5,6-TCP for 7 days; urine and tissue samples were collected after withdrawal periods of up to 7 days. The principal urinary excretion product was unchanged 3,5,6-TCP. The liver and kidney contained the highest initial residues, and while these residues decreased rapidly after withdrawal, the liver exhibited the slowest rate of clearance (Bauriedel & Miller, 1981).

Calves were fed 3,5,6-TCP in the feed at concentrations up 100 ppm for 28 days. Tissue samples were taken at day 28 or after a 3, 7, or 21-day withdrawal period. Residue levels were highest in liver and kidney with low levels seen in fat and muscle. These residue levels showed a rapid decline after the return to untreated feed (Glas, 1977a). One calf fed 2-methoxy-3,5,6-trichloropyridine for 28 days showed significant (>2 ppm) residues of 3,5,6-TCP but not 2-methoxy-3,5,6-trichloropyridine in liver, kidney, and fat (Glas,1977b).

Calves were fed triclopyr in the feed at concentrations up 1000 ppm for 28 days. Tissue samples were taken at day 28 or after a 3, 7, or 21-day withdrawal period. At the 1000 ppm dose level, triclopyr residues were high (4.3 ppm) in kidney only and required 21 days to return to background. Residues of 3,5,6-TCP were much higher, averaging 0.4 ppm in muscle, 1.0 ppm in fat, 5.9 ppm in liver and 11.7 ppm in kidney at the 1000 ppm feeding level. These residues showed a rapid decline in muscle and fat (3 days) and a slower decline in liver and kidney (21 days) (Glas, 1977c).

Female calves were fed one capsule of chlorpyrifos per day for 30 days. The doses were equivalent to daily dry-matter dietary concentrations of up to 100 ppm. Tissue samples were taken at day 30 or after a 1-5 week withdrawal period. There was a dose-related increase in chlorpyrifos residues in all tissues but especially in fatty tissues, whereas 3,5,6-TCP was found predominantly in the liver and kidney. Chlorpyrifos residues in fat were 0.93 ppm at 7 days, and 0.02 ppm at 35 days after withdrawal of the 100 ppm dose (Dishburger et al, 1972, 1977).

Human subjects poisoned with chlorpyrifos formulations exhibited significant inhibition of serum and RBC cholinesterase activities, with RBC cholinesterase activity the fastest to recover. Chlorpyrifos was detected in serum samples only and at low concentrations compared to diethylphosphorus metabolites that were excreted mainly in the urine. The urinary diethylphosphorus metabolites were excreted with first-order kinetics, with an average elimination half-life of 6.10 $\pm$ 2.25 h in the fastest and of 80.35 $\pm$ 25.8 h in the slowest elimination phase (Drevenkar et al, 1993).

Human male volunteers received an oral dose of chlorpyrifos (0.5 mg/kg) and one month later a dermal dose (5 mg/kg). There were no signs or symptoms of chlorpyrifos toxicity in any volunteers; however dosing led to significant inhibition of pre-dose plasma cholinesterase levels (85% inhibition for the oral, and up to 30% inhibition for the dermal dose). Erythrocyte cholinesterase inhibition was not significantly inhibited following either oral or dermal doses of chlorpyrifos. The half life of 3,5,6-TCP appearance in the blood was 0.5 h after oral dosing and 22.5 h after dermal dosing, but both routes had an elimination half life of 26.9 h. The mean predicted total absorption following oral administration was 72 $\pm$ 11%, and following dermal administration was 1.35 $\pm$ 1.0% (Nolan et al, 1982, 1984).

Acute toxicity

Numerous median lethal dose studies have been carried out using chlorpyrifos technical. The acute toxicity profile of chlorpyrifos technical is tabulated below. In general, the signs of acute chlorpyrifos intoxication in animals were consistent with cholinesterase inhibition, and included inactivity, salivation, dyspnoea, flaccid paralysis, vomiting, piloerection, exothalmia and diarrhea.

Species	Strain	Sex	Study Type	Vehicle	Outcome (95% CI or range)
Rat	albino	F	oral	not specified	Lowest LD50: 96 mg/kg (72-140)
Mouse	Smith Webster	M	oral	1% aqueous gum tragacanth	Lowest LD50: 102 mg/kg (94-110)
Rabbit	NZ white	M+F	dermal	undiluted	Lowest LD50: 1580 mg/kg (828-2606)
Rat	SD	M+F	dermal	undiluted	Lowest LD50: >2000 mg/kg
Rat	SD	F	inhalation	65% xylene	Lowest LC50: 78 mg/m^3 (57-108)
Rat	SD	M+F	inhalation	undiluted	Lowest LC50: >230 mg/m^3 (4 h)
Rabbit	NZ white	F	Eye Irritation	undiluted	Slight to moderate eye irritant
Rabbit	NZ white	M	skin irritation	undiluted	Slight skin irritant
Guinea pig	Dunkin-Hartley	F	skin sensitisation	maize oil	Non-sensitising

Acute toxicity of end-use products

The acute toxicity of chlorpyrifos formulations was related to the concentration of active ingredient in the end-use products. The signs associated with intoxication were similar to those seen with the TGAC.

Short-term, repeat-dose toxicity

In a two-week dose-ranging study conducted using young adult CD-1 mice, Pyrinex Technical (96.3% purity) was administered at dietary concentrations of 0 (control), 75, 150, 300, 600, and 1200 ppm (0, 14.3-18.5, 30.1-32.4, 56.4-66.6, 88.7-103.8 and 127.9-181.1 mg/kg/d, respectively). Mortality and treatment-related clinical signs, including tremor, ocular opacity, ocular staining, hunching and lacrimation were mainly confined to animals in the 1200 ppm group. Marked reductions in body weights and food consumption were observed in animals treated at 600 ppm and above. In males, cholinesterase activity

was reduced at all test doses. At 75 ppm, plasma cholinesterase activity was reduced by >95%, erythrocyte cholinesterase activity by almost 40%, and brain cholinesterase activity by about 50%. Inhibition of erythrocyte cholinesterase activity was not dependent upon dose, with approximately 35% inhibition seen at all dose levels. Plasma and brain cholinesterase activity was reduced in a dose-related manner, with plasma cholinesterase inhibition of 99% and brain cholinesterase inhibition of about 89% at the high dose. Similar patterns of cholinesterase inhibition were seen in females. Based on the results of this study, doses of 5, 50, 200, 400, and 800 ppm were selected for a 13-week dietary study in mice (Crown et al, 1984).

Male and female CD-1 mice were given chlorpyrifos (95.7% purity) in the diet at 0 and 15 ppm (40/sex/group), with 20 animals/sex/group sacrificed after one week, and 20 animals/sex/dose treated for a total period of 4 weeks. Doses equated to 2.7 and 3.4 mg/kg for males and females, respectively. There were no significant in-life clinical signs or unscheduled deaths. Food consumption was unaffected by treatment, but the body weight gain of male mice was decreased by 25% at the end of the 4-week study period. Organ weight analysis did not reveal any findings that were related to treatment and there were no significant differences between groups. Pathology findings at necropsy were within normal limits for all groups. At one week, plasma cholinesterase activity was depressed by 88 to 91% and RBC cholinesterase was depressed by 40 to 53%. At 4 weeks, plasma cholinesterase activity was depressed by 91% and RBC cholinesterase was depressed by 53%. Brain cholinesterase activity was unaffected by treatment at either assay period. There was no sex difference in the changes observed in plasma and erythrocyte cholinesterase activity (Davies et al, 1985).

In a 2-week dietary range-finding study in rats used to establish dose levels for a 13-week dietary study, technical chlorpyrifos (95.5% purity) was administered to six groups of animals (5/sex/dose). Dietary concentrations were 0 (controls), 10, 30, 84, 240, and 694 ppm for 14 days (0, 1.4-1.9, 4.1-5.2, 11.7-13.7, 34.3-39.4, and 65.8-94.8 mg/kg/d, respectively). At the high dose, clinical signs of intoxication were reported, consisting of irritability, hunching, tremor, ataxia, urogenital staining, pigmented orbital secretion, failure to groom, and proneness. No treatment-related clinical signs were observed at other dose levels. Treatment-related mortality was confined to high-dose animals. Body weights were reduced in males and females at 694 ppm and at 240 ppm in females and food consumption was also reduced at 694 ppm. Dose-related, biologically-significant decreases in blood cholinesterase activity were seen at all test doses. On the basis of these findings, the dose levels selected for the 13-week study were 0.5, 10, and 100 ppm (Crown et al, 1984).

Plasma, RBC and brain cholinesterase activities were markedly reduced in rats following dietary administration of chlorpyrifos for 14 days at doses of 5 and 10 mg/kg/d. Inhibition of cholinesterase activity occurred in the absence of clinical signs of intoxication. Feed consumption, body weights, and gross pathological findings were similar in control and treated groups. Statistically-significant increases in absolute and relative adrenal weights (about 20% compared with controls) were observed in females only at 10 mg/kg/d (Liberacki et al, 1990).

In a summary only, it was reported that two rhesus monkeys were given doses of 2 mg/kg chlorpyrifos by stomach tube for three consecutive days. Blood samples were taken at intervals of 24 h and cholinesterase activity was measured. No change in behaviour or clinical signs of cholinergic stimulation were observed in either monkey during the three-day observation period. A sharp decrease in plasma cholinesterase activity was observed 24 h after the initial dose (to 17-46% of control), with slightly

greater reductions after the second and third doses (to 6-23% of control). Erythrocyte cholinesterase activity decreased slightly following the first dose (to 85-95% of control), with greater reductions after the second and third doses (to 66-89% of control) (Coulston et al, 1971).

Chlorpyrifos technical (95.8% purity) was administered orally via gelatine capsule to groups of purebred beagle dogs at doses of 0 (control; lactose only), 0.01, 0.03, 0.5, and 5 mg/kg/d for 4 weeks, using 2 animals/sex/group (only 1 animal/sex/group at 0 and 0.5 mg/kg/d). No deaths occurred during the study, and no clinical signs associated with treatment were observed. All groups gained weight steadily during the treatment period, and food consumption was similar in all groups. Rapid inhibition of plasma cholinesterase activity was observed at 0.5 and 5.0 mg/kg/d at all sample intervals, and inhibition was both dose- and time-dependent. At 0.03 mg/kg/d, plasma cholinesterase activity was reduced, but due to the intra-group variation in results, and the small group sizes in this study, it was considered that the inhibition of plasma cholinesterase at the 0.03 mg/kg/d dose level was not biologically significant. Biologically significant inhibition of erythrocyte and brain cholinesterase activity was observed at 5 mg/kg/d. Macroscopic post mortem examination did not reveal any findings that were considered to be of toxicological significance. Under the conditions of this study, no treatment-related effects were observed at 0.03 mg/kg/d, while plasma cholinesterase activity was inhibited at 0.5 mg/kg/d, and erythrocyte and brain cholinesterase activities were inhibited at 5 mg/kg/d (Harling et al, 1989).

When a chlorpyrifos formulation (61.5% chlorpyrifos, 35% xylene) was applied to the skin of the back and abdomen of 2 rabbits/dose at 5, 10, 25 and 50 mg/kg for 20, 4, 3, and 1 applications respectively, no skin irritation was recorded. Both plasma and RBC cholinesterase measures were significantly inhibited by dermal application under each of the dose regimens; plasma values recovered more quickly than RBC values which were still significantly inhibited at day 40 after the 3, 4 and 20-day exposure patterns (Pennington & Edwards, 1971).

In a dermal study in Fischer 344 rats, animals were dosed with chlorpyrifos (100% purity) at 0, 0.1, 0.5, or 5 mg/kg/d in a corn oil vehicle, for a total of 15 days over a 21-day period. No treatment-related effects were observed at any dose, and plasma, RBC and brain cholinesterase activities were not inhibited. Gross and microscopic pathological examinations did not reveal any treatment-related findings, and body weights and organ weights were unaffected by treatment. In the 4-day range finding component of this study, decreases in plasma ChE activity (45%) and RBC ChE activity (16%) were observed at 10 mg/kg/d, in the absence of clinical signs of intoxication (Calhoun & Johnson, 1988; Calhoun & Johnson, 1989).

In a two-week dermal application of a chlorpyrifos aerosol formulation, five dogs received ten applications of 0.087% Dursban in dichlorophene to their backs (with the fur brushed against the grain) over a period of 2 weeks, with each application lasting 30 seconds. There were no mortalities or abnormalities in the analysis of clinical observations, body weight gain, opthalmoscopic examination, haematology or clinical chemistry parameters. RBC cholinesterase activity was not decreased by treatment, but plasma cholinesterase activity fell from the commencement of treatment, before returning to normal at 72 days post-treatment. There were no clinical signs related to the fall in plasma cholinesterase activity (Sharp & Warner, 1968).

Fischer 344 rats received technical chlorpyrifos (95% purity) by nose-only inhalation exposure for 6

h/day for 5 days at a target concentration of 20 ppb. No mortality or treatment-related clinical signs were reported during the study. Under the conditions of this study, the nose-only exposure of female rats to chlorpyrifos concentrations of 0.34 mg/m^3 (23 ppb) for 6h/day, over 5 days, resulted in a significant decrease in plasma cholinesterase activity. No effect on erythrocyte or brain cholinesterase activity was seen in females, and plasma, erythrocyte and brain cholinesterase activity was unaffected in males (Newton, 1988).

Chlorpyrifos was administered via nose-only exposure to female Fischer 344 rats at concentrations of 0 or 12 ppb (equivalent to 0 or 172 µg/m^3) as a time weighted average for 6 hours/day, 5 days/week, for two weeks. No clinical signs of intoxication or treatment-related changes in body weights were reported during the study, and all rats survived until the scheduled termination. Clinical chemistry examination did not reveal any treatment-related changes in haematology or urinalysis parameters, including plasma, brain, and erythrocyte cholinesterase activity (Landry et al, 1986).

In a dose range finding study, chlorpyrifos technical was administered by whole-body exposure (6 h/day, 5 days/week, for two weeks) to groups of Wistar rats at target concentrations of 0 (air control), 10, 100, and 400 mg/m^3. Exposure of all high-dose animals was terminated after 5 exposures due to the moribund condition of the animals, with rapid weight loss and deterioration in the general condition of the animals observed, and all surviving animals were sacrificed on day 9. No mortality was observed at other exposure levels. No NOEL was demonstrated in this study, based on the significant inhibition of plasma, erythrocyte, and brain cholinesterase activities at doses of 10 mg/m^3 and above, in males and females. Clinical signs of toxicity (tremors, salivation, lachrymation), decreases in food consumption, and decreases in body weight were observed at doses of 94 mg/m^3 and above, but these effects were not observed at 10 mg/m^3. Increased adrenal weights (94 and 388 mg/m^3), and forestomach effects including thickening and congestion (388 mg/m^3) were observed, but no treatment-related pathology was observed at 10 mg/m^3 (Kenny et al, 1988).

Fischer 344 rats were exposed to time-weighted average concentrations (nominal) of 0, 1, or 5 ppb (0, 0.014, or 0.072 mg/m^3) of chlorpyrifos vapour for 6 h/day, 5 days/week for two weeks. The mean daily time weighted average (TWA) analysed concentrations were 0.7 and 5 ppb, respectively, at the nominal concentrations of 1 and 5 ppb, respectively. Two control groups (A and B) were used in this study, and as no statistically-significant differences in cholinesterase activities were determined between the two control groups, the test group cholinesterase activities were compared with the combined control group values. Statistically-significant decreases in plasma, RBC and brain cholinesterase activity were observed at 5 ppb. The reductions in brain and RBC cholinesterase activity at 5 ppb did not reach 20% when compared with any of the control groups, and these effects were not considered to be of toxicological significance. The inhibition of plasma cholinesterase activity was >20% in females at 5 ppb. A statistically-significant decrease in plasma cholinesterase activity at 0.7 ppb in females compared with the combined control group was considered to be incidental to treatment. There were no treatment-related mortality, clinical signs of toxicity or changes in body weights during the study. No treatment-related effects were noted on body weights or organ weights, and no lesions were observed at gross pathological examination. No significant treatment-related effects were observed at 0.7 ppb (approximately 0.01 mg/m^3) (Landry et al, 1985; Streeter et al, 1987).

Four humans and three rabbits were exposed for 5 minutes to chlorpyrifos formulation M-2995 (61.5% chlorpyrifos, 34.5% xylene), using an ULV cold aerosol fog generator delivering 3.8 L/h. The humans

were exposed at a distance of 8 m wearing plastic coveralls but with heads and hands exposed for 2 subjects, and heads, hands and arms exposed for 2 subjects. The rabbits were exposed at a distance of 8 m at 1.3 m height (2 rabbits) or 0.8 m height (1 rabbit). Exposure was terminated at 5 minutes due to the eye and lung irritation induced by the formulation. The air sampling recorded breathing-space concentrations of chlorpyrifos of about 108 mg/L (range 83-133) for humans and rabbits. There was no depression of plasma or RBC cholinesterase in 24 h post-exposure samples from the human subjects. At 24 h post-dose, rabbits recorded a decrease in plasma cholinesterase (up to 33%) and RBC cholinesterase (up to 12%), but by 72 h post-dose these values had recovered to near control values (Pennington & Edwards, 1971).

Subchronic toxicity (TGAC)

Mice (12/sex/dose) were given chlorpyrifos in the diet at 0, 5, 50, 200, 400, and 800 ppm (equivalent to dose ranges of 0, 0.7-1.3, 7.1-13.5, 32.4-53.4, 40.9-135.7, and 112.5-300.7 mg/kg bw/day, respectively) for 13 weeks. Dose-related mortality and ocular opacity were seen at 400 and 800 ppm. Body weight was reduced by 10 to 15% in males and females at 800 ppm throughout the study. Plasma cholinesterase activity was markedly decreased at all doses in males and females; equivocal decreases in erythrocyte cholinesterase activity were observed in both sexes; brain cholinesterase activity was inhibited in a dose dependent manner at doses of 200 ppm and above in males, and at doses of 50 ppm and above in females. Absolute and relative organ weights were relatively unaffected by treatment, although relative liver weights were increased in females at 200, 400, and 800 ppm. Clinical signs included urogenital staining at doses of 200 ppm and above in males and 800 ppm females. Ocular opacities occurred in a single high-dose male and in one female at 400 ppm and four females at 800 ppm. Dose-related histopathological findings were seen in the adrenals (including lipogenic pigmentation at doses of 200 ppm and above in females and at 400 ppm and above in males), and the eyes (acute or subchronic keratitis observed in two males and four females at 800 ppm, and in a single female at 400 ppm). No treatment-related neoplasia was observed. Based on plasma cholinesterase inhibition at all doses, an NOEL was not established for this study. The LOEL, based on inhibition of plasma cholinesterase was 5 ppm (calculated to be equal to 0.7 mg/kg/d). Erythrocyte cholinesterase determinations were variable, and the effect of treatment on this parameter was equivocal. The NOEL for brain cholinesterase inhibition was 5 ppm (equal to 0.7 mg/kg/d), based on this inhibition of activity at 50 ppm (equal to 7.1 mg/kg/d) (Crown et al, 1987).

Rats (10/sex/group) were fed chlorpyrifos at concentrations of 0, 0.1, 1, 5, or 15 mg/kg/d for 13 weeks. At the highest dose (15 mg/kg/d), treatment-related effects consisted of a decrease in bodyweight and bodyweight gain (males), increased fatty vacuolation of the adrenal zone fasciculata (males), and changes in haematology and clinical chemistry parameters (decreased RBC in both sexes; increased platelet counts and reduced serum total protein, albumin, globulin, alanine transaminase and alkaline phosphatase in the males; decreased serum glucose and increased urinary specific gravity in the females). Plasma and erythrocyte cholinesterases were depressed at 1 mg/kg/d and above. Depression of brain cholinesterase activity occurred at 5 and 15 mg/kg/d. The NOEL was 0.1 mg/kg/d in both males and females based on plasma and erythrocyte cholinesterase depressions and the NOEL for brain cholinesterase was 1 mg/kg/d (Szabo et al, 1988).

Rats (10/sex/group) were started on diets containing 0, 0.001, 0.003, 0.03 or 0.1% chlorpyrifos (0, 10,

30, 300, and 1000 ppm, respectively). Dosing of the group receiving 0.1% chlorpyrifos was discontinued after 4 weeks due to the severity of toxicity signs. Plasma cholinesterase activity in both sexes was reduced in a dose-related manner and was significantly lower than controls at all dose levels, erythrocyte cholinesterase activity was similarly significantly reduced at all dose levels, while brain cholinesterase displayed a clear dose-related depression in both sexes. There were no significant adverse haematological or clinical chemistry findings, but very few parameters were examined. No histopathological lesions were found that could be linked to treatment with chlorpyrifos. The NOEL for brain cholinesterase inhibition was 0.001% (LOEL 0.003%). This was estimated to be equivalent to a NOEL of 1 mg/kg/d with an LOEL estimated to be 3 mg/kg/d. There was no NOEL for RBC or plasma cholinesterase inhibition, with effects seen at 0.001% (1 mg/kg/d) and above (Beatty, 1964, and Beatty & McCollister, 1971).

Rats (10/sex/group) were initially exposed to chlorpyrifos in the diet at doses of 0 and 0.3 mg/kg/d for 13 weeks (groups 1 and 2), and 1.0, 3.0 and 10 mg/kg/d (groups 3, 4, and 5) for 4 weeks. There were no deaths during the study. After 4 weeks of intake, there was a clear dose-response for RBC and plasma cholinesterase inhibition with no NOEL evident and at the higher dose levels, animals displayed clinical signs of toxicity. After 4 weeks, animals in groups 3-5 were removed from exposure and allowed to recover for a 3-week period, during which time plasma and RBC cholinesterase activities returned to control values. These groups were then fed chlorpyrifos at doses of 0, 0.03 and 0.1 mg/kg/d for 13 weeks. After 13 weeks intake, there was no unequivocal effect at 0.03 mg/kg/d on any of RBC, plasma or brain cholinesterase in either sex, but at 0.1 mg/kg/d, treatment significantly reduced RBC and brain cholinesterase in males, and RBC and plasma cholinesterase in females. The NOEL for plasma, RBC and brain cholinesterase activity was 0.03 mg/kg/d, based on significant inhibition of one or more of these activities after 13 weeks intake at 0.1 mg/kg/d in one or both sexes (Anon, 1968).

Rats (20/sex/dose) were given chlorpyrifos in the diet at 0, 0.03, 0.15 or 0.75 mg/kg/d for 6 months, and an interim sacrifice (5 animals/sex/dose) was conducted after three months. Unscheduled deaths (16 animals) due to chronic murine pneumonia occurred in all dose groups. There were no treatment-related changes to body weight gain, food consumption, haematology or clinical chemistry values. Cholinesterase inhibition occurred at the high dose (0.75 mg/kg/d) in both red blood cells (50%) and plasma (65%), but there was no inhibition of cholinesterase activity in the brain at any dose. The NOEL in this study was 0.15 mg/kg/d, based on inhibition of plasma and erythrocyte cholinesterase activity at 0.75 mg/kg/d (Coulston et al, 1971).

Rats (20/sex/dose) were given chlorpyrifos at dietary concentrations of 0 (control), 0.5, 10, and 200 ppm for 13 weeks. Under the conditions of this study, no mortality or significant clinical signs were observed at any dose. Clinical chemistry, urinalysis and ophthalmoscopy examinations did not reveal any findings that were considered to be related to treatment. At 200 ppm (approximately 13 mg/kg/d), reduced body weights and erythrocyte count, haematocrit and haemoglobin, and increased food consumption were observed. Biologically and statistically-significant reductions (>20% cf. controls) in plasma cholinesterase activity were observed in males at 12 weeks at doses of 0.5 ppm (approximately 0.03 mg/kg/d) and above, while in females, cholinesterase activity was reduced at doses of 10 ppm (approximately 0.6 mg/kg/d) and above. The LOEL for this study was 0.5 mg/kg/d, calculated to be equal to 0.03 mg/kg/d, based on the inhibition of plasma cholinesterase at all doses in males (Crown et al, 1985).

Rats (10/sex/group) were exposed nose-only to 0, 5.2, 10.3 or 20.6 ppb (0, 75, 147 or 296 µg/m^3) chlorpyrifos for 6 h/day, 5 days/week for 13 weeks. No treatment-related mortalities and only minimal clinical signs were observed during the study. Body weights, urinalysis, haematology and clinical chemistry were unaffected by treatment. No effects on plasma, erythrocyte or brain cholinesterase activity were seen at any dose. Gross and histopathological examination did not reveal any effects associated with the administration of chlorpyrifos in high-dose animals. Under the conditions of this study, no adverse, treatment-related effects were seen in rats exposed to chlorpyrifos by nose-only inhalation, at doses up to 20.6 ppb (296 µg/m^3), for six hours/day, five days/week, for 13 weeks (Corley et al, 1986).

Rats (15/sex/dose) were exposed nose-only for 6 h/d, 5 days/week, for 13 weeks at target concentrations of 0 (control), 5, 10, and 20 ppb (0, 0.07, 0.14 and 0.28 mg/m^3 respectively). There was no treatment-related mortality or ophthalmoscopic changes. Haematological and clinical chemistry examinations did not reveal any effects that were considered to be treatment-related. Plasma cholinesterase activity was inhibited by 23% in high-dose males ($p<0.01$), but other changes in cholinesterase activity were considered not to be treatment-related. Pathology and histopathology examinations did not reveal any effects of treatment. The NOEL for this study was 10 ppb, based on a decrease in plasma cholinesterase activity in terminal sacrifice males at 20 ppb (0.28 mg/m^3) (Newton, 1988).

Young adult beagle dogs (2/sex/dose) were given daily doses of chlorpyrifos orally by capsule at doses of 0, 0.03, 0.10, 0.30 and 1.0 mg/kg/d for 90 days. Plasma cholinesterase activity was inhibited at all dose levels, and so the LOEL for plasma cholinesterase inhibition was 0.03 mg/kg/d. The NOEL for RBC cholinesterase inhibition was 0.03 mg/kg/d, based on inhibition at doses of 0.10 mg/kg/d and above, and the NOEL for brain cholinesterase inhibition was 1.0 mg/kg/d (Blackmore, 1968).

Technical chlorpyrifos was administered orally by capsule to purebred beagle dogs (4/sex/dose) once daily for 13 weeks, at doses of 0 (control), 0.01, 0.22, and 5 mg/kg/d. There were no unscheduled deaths or unequivocal clinical signs during the study. There was a reduction in group mean body weights in males and females in the high-dose group. Examinations of haematological, clinical chemistry, urinalysis and ophthalmoscopic parameters did not reveal any findings that were considered to be treatment-related. Pathological and histopathological examinations did not reveal any macroscopic or microscopic findings that could be attributed to treatment. Under the conditions of this study, there was no NOEL for inhibition of plasma cholinesterase activity, with biologically and statistically-significant reduction of activity seen in females at doses of 0.01 mg/kg/d and above. In males, plasma cholinesterase activity was reduced at doses of 0.22 mg/kg/d and above. Erythrocyte cholinesterase activity was inhibited in males and females at 0.22 mg/kg/d and above, with an NOEL of 0.01 mg/kg/d for this effect, and brain cholinesterase activity was inhibited in both sexes at 5 mg/kg/d, with an NOEL for this effect of 0.2 mg/kg/d (Harling et al, 1989).

Rhesus monkeys (1 or 2/sex/group) were given 0, 0.08, 0.40 and 2.0 mg/kg/d technical chlorpyrifos in 1% aqueous gum tragacanth by stomach tube for a period of 6 months. There were no treatment-related changes in body weight gain, food consumption, clinical observations, haematology, clinical chemistry or histopathological examinations. Plasma cholinesterase activity was depressed at all doses, erythrocyte cholinesterase activity was depressed at 0.4 and 2.0 mg/kg. A LOEL of 0.08 mg/kg/d was

seen for plasma cholinesterase inhibition at 16 weeks but this concentration was a NOEL at 24 weeks. A NOEL of 0.08 mg/kg/d was seen for RBC cholinesterase inhibition at 16 and 24 weeks and a NOEL of 0.4 mg/kg/d for brain cholinesterase inhibition. However this study was not deemed adequate for regulatory purposes due to the low numbers (sometimes one) of animals tested at each point (Coulston et al, 1971).

White leghorn chickens were administered chlorpyrifos in drinking water at 1 ppb, 1 ppm and 100 ppm for up to 84 days. Only plasma cholinesterase was reported due to technical difficulties with the blood sampling. Significant inhibition (54% decrease) of plasma cholinesterase was recorded on day 84 at 100 ppm (Stevenson, 1965).

Subchronic Toxicity (TCP)

Rats (10/sex/group) were fed diets containing 0, 0.01, 0.03, 0.1, 0.3 or 1.0 % of 3,5,6-trichloro-2-pyridinol (TCP) for 90 days. There were no treatment-related effects at 0.01, 0.03 or 0.10% TCP on body weight gain, mortality, food consumption, haematology values, BUN, ALP, and average body and organ weights. Based on reduced bodyweight gain in both sexes at 1.0%, the NOEL for TCP was 0.10% in the diet for 90 days (1000 ppm, or approximately 50 mg/kg/d) (Beatty, 1964).

Rats (15/sex/group) were fed 0, 10, 30 or 100 mg/kg/d sodium-TCP (3,5,6-trichloro-2-pyridinol) in their diets for 91 days. The dietary concentration was varied to allow for increasing body weight, and ranged from ca. 85, 250 and 825 ppm at day 0, through to ca. 160, 460 and 1540 ppm at day 84 for the 10, 30 and 100 mg/kg/d groups respectively. Significantly increased bodyweight gains were observed in 30 mg/kg males from day 48 onwards. There were no treatment-related changes in haematology, urinalysis or blood chemistry values. There were increases in absolute heart weight in 100 mg/kg/d males, and increased absolute and relative liver and kidney weights in both sexes at 100 mg/kg/d. There were no gross or histopathological findings due to treatment. Based on the increases in relative liver and kidney weights, the NOEL for sodium-TCP was 30 mg/kg/d in the diet for 90 days (Barna-Lloyd & Szabo, 1985).

Beagle dogs (2/sex/group) were administered TCP (3,5,6-trichloro-2-pyridinol) in the diet at levels of 0, 26 and 80 mg/kg/d for a period of 93 days. At termination, haematological findings were normal; however no NOEL could be established as there was elevated serum alkaline phosphatase activity and histopathological evidence of liver damage in both sexes at both doses, as well as increased testes weights in males at both doses (Copeland, 1964).

Dogs (3/sex/group) were administered TCP (3,5,6-trichloro-2-pyridinol) in the diet at levels of 1, 3, 10 or 30 mg/kg/d for a period of 91 days. There was no apparent toxicity associated with the administration of 1, 3 or 10 mg/kg TCP (3,5,6-trichloro-2-pyridinol). At the highest dose of 30 mg/kg there was evidence of liver toxicity with elevated levels of AST, ALP and ALT, but there were no unequivocal signs of liver damage in the histopathological examination (Emerson & Gerbig, 1970).

Chronic toxicity

The dietary administration of technical chlorpyrifos to CD-1 mice for up to 78 weeks at doses of 5, 50,

and 250 ppm did not have any adverse effect on survival, and no increase in neoplastic findings were attributed to treatment. Treatment-related clinical signs (ocular opacities, excessive lachrymation, hair loss on head) were observed at 250 ppm (approximately 31.7 mg/kg/d), and reductions in body weights and food consumption were also seen at the high-dose level. Plasma cholinesterase activity was inhibited at doses of 5 ppm (0.7 mg/kg/d) and above in this study, and no NOEL was demonstrated for this effect. Brain and erythrocyte cholinesterase activities were inhibited at 50 ppm (6.1 mg/kg/d) and above, and the NOEL for these endpoints was 5 ppm (0.7 mg/kg/d). Non-neoplastic effects were observed in the livers of high-dose males (slight subchronic pericholangitis, histiocytic proliferation, and centrilobular hepatocytic fatty vacuolation), and in the eyes of high-dose males and females. No treatment-related lesions were seen at doses of 50 ppm (6.1 mg/kg/d) and below. Based on the inhibition of plasma cholinesterase activity at all dose levels in this study, no NOEL could be established. The LOEL, based on inhibition of plasma cholinesterase activity, was 5 ppm (0.7 mg/kg/d) (Gur et al, 1991).

In a study in which CD-1 mice were maintained on diets containing 0, 0.5, 5 and 15 ppm of chlorpyrifos (99.6%) for 105 weeks (approximately 0.05, 0.5 and 1.5 mg/kg/d), body weights and absolute and relative organ weights were generally not significantly affected by treatment. Mortality was also generally unaffected by treatment. There were no clinical signs that indicated an effect of treatment, and pathology reported no findings that indicated an effect related to treatment. A significant increase was observed in the incidence of spindle cell hyperplasia of the adrenal gland (both sexes) in animals ingesting 0.5 ppm chlorpyrifos and in males only from the group ingesting 5 ppm chlorpyrifos. This finding was not considered to be treatment-related as there was a high background incidence in aging mice, and there was no dose-response relationship. Sciatic nerve preparations recorded vacuolation in controls (2/56 males and 3/54 females); at 0.5 ppm (5/54 males and 8/55 females); at 5 ppm (2/56 males and 2/52 females); and at 15 ppm (6/52 males and 8/53 females). The incidences of alveologenic adenomas and hyperplastic nodules in the liver were significantly elevated in the intermediate (5 ppm) male group, but not in any other treatment group. These findings were considered to be incidental. This study was considered to be barely adequate for assessment of carcinogenicity and inadequate for chronic toxicity assessment, as it had some serious shortcomings in design, methodology and data collection. No clinical pathology was performed, including no measurement of cholinesterase activity. No clinical signs other than physical examination were reported. Individual data were not presented for several parameters including histopathology. Intestinal parasites (nematodes) were present in all groups. No clear evidence of treatment-related carcinogenicity was seen in this study (Warner et al, 1980).

Rats were fed chlorpyrifos at 0, 0.01, 0.03, 0.1, 1.0 or 3.0 mg/kg/d (dietary levels not provided) in their diet for 2 years. This study was considered to be inadequate for assessment of carcinogenicity, and also inadequate for assessment of chronic effects other than cholinesterase inhibition in a chronic study as there were serious shortcomings in data collection, the gross pathology and histopathology reports were inadequate and no clinical signs were reported. The NOEL for plasma and RBC cholinesterase inhibition was 0.1 mg/kg/d based on significant inhibition in both sexes at 1.0 mg/kg/d. The NOEL for brain cholinesterase inhibition was 1.0 mg/kg/d based on significant inhibition in both sexes at 3.0 mg/kg/d. The study was inadequate to enable a NOEL for carcinogenic effects to be established (McCollister et al, 1971).

A supplementary report provided some of the data identified as lacking/inadequate in the original study

report outlined above (McCollister et al, 1971). However the "in life" individual animal observations were very limited and did not include basic observations such as abnormal behaviour or gait. Individual body weight records, gross pathology findings and histopathology findings were included in this supplement; histopathology was limited and variable with only a small number of tissues examined consistently in each rat. The histopathology results were not presented as summary tables of incidence and severity. The conclusions of the evaluation of the original study remain unchanged by this supplement (McCollister et al, 1985).

Fischer F344 rats were exposed to chlorpyrifos in the diet at 0, 0 (vehicle control), 0.2, 5 and 100 ppm for two years. Mortality was not affected by treatment, nor was the incidence of clinical signs or palpable masses. Body weights in 100 ppm males and females were reduced compared with controls. A variety of non-neoplastic and neoplastic lesions were recorded, and occasionally the incidence of these lesions displayed a positive trend with treatment. However the incidence of lesions was generally within the range of historical laboratory controls or within the range of published NTP values. There were no neoplastic lesions which recorded an unequivocal or statistically-significant dose relationship. In this study, the NOEL for plasma cholinesterase activity was considered to be 0.012 mg/kg/d (0.2 ppm) based on significant (>20%) inhibition relative to the control value at 0.3 mg/kg/d (5 ppm) and above. No significant treatment-related neoplastic or non-neoplastic lesions were observed at any dose. No NOEL was set for RBC cholinesterase inhibition in this study due to data inadequacies. The NOEL for inhibition of brain cholinesterase was 0.3 mg/kg/d based on significant inhibition at 6 mg/kg/d (Crown et al, 1988).

In a study in which chlorpyrifos was administered to rats at 0.05, 0.1, 1.0 and 10 mg/kg/d for up to 2 years, high-dose males showed: a consistent decrease in body weight gain relative to controls in the absence of reduced food consumption; depression of plasma (56-87%), erythrocyte (20-40%) and brain (56-58%) cholinesterase activities; and an increase in the weight of adrenal glands, characterised microscopically by an exacerbated fatty vacuolation of the zona fasciculata. Effects similar to those seen in males were also observed in females at 10 mg/kg/d, but were generally less pronounced in females. For example, there was a transient decrease in bodyweight gain relative to controls with no reduction in food consumption; depression of plasma (82-95%), erythrocyte (generally $\leq$20%) and brain (57-61%) cholinesterase activities; and an increase in adrenal weight at the terminal sacrifice, but with no associated histopathological lesions. At 1 mg/kg/d, the only noted effect attributable to treatment was an inhibition of plasma (39-71% in males and 60-86% in females) and erythrocyte (20-40% in males and <-22% in females) cholinesterase activities. Brain cholinesterase was not affected. No treatment-related effects were observed at the two lowest dose levels (0.05 and 0.1 mg/kg/d). There was no increase in tumour incidence of any type in any organ or tissue at any of the dose levels tested (0.05, 0.1, 1.0 and 10 mg/kg/d). The NOEL for this study, based on toxicologically (>20%) and statistically-significant inhibition of plasma ChE activity in both sexes at 1.0 mg/kg/d, was 0.1 mg/kg/d. The NOEL for erythrocyte ChE was 1.0 mg/kg/d, based on toxicologically (>20%) or statistically-significant inhibition in both sexes at 10 mg/kg/d. The NOEL for brain ChE was 1.0 mg/kg/d based on toxicologically (> 10%) and statistically-significant inhibition in both sexes at 10 mg/kg/d. There were no clinical signs of cholinesterase intoxication at the highest dose tested (10 mg/kg/d). No tumorigenic effects of chlorpyrifos treatment were evident in this study (Young & Grandjean, 1988).

Beagle dogs were administered chlorpyrifos in the diet at dose levels of 0, 0.01, 0.03, 0.1, 1.0 or 3.0 mg/kg/d for up to 2 years. This study had inadequacies in data collection and recording. The chronic

effects of dietary chlorpyrifos intake could not be evaluated in the absence of complete pathology and histopathology findings; however NOELs for cholinesterase inhibition (>20%) could be established. The NOEL for this study, based on plasma cholinesterase inhibition in both sexes was 0.01 mg/kg/d (LOEL 0.03 mg/kg/d). The NOEL for RBC cholinesterase for both sexes was 0.03 mg/kg/d (LOEL 0.1 mg/kg/d), and for inhibition of brain cholinesterase activity, the NOEL in both sexes was 1.0 mg/kg/d (LOEL 3.0 mg/kg/d) (McCollister et al, 1971b).

This supplement provides some of the data identified as lacking/inadequate in the original study report outlined above (McCollister et al, 1971b). However the "in life" individual animal observations were very limited and do not appear to consistently include basic observations. Individual body weight records, ophthalmology data, pretest and terminal physical examinations, and a tissue inventory for histopathology was included in this supplement. The conclusions of the evaluation of the original study remain unchanged by this supplement, with the additional observation that there was no effect of treatment detected by the ophthalmology examinations (Kociba et al, 1985).

Beagle dogs were fed the chlorpyrifos metabolite TCP (3,5,6-trichloro-2-pyridinol) at doses of 0, 3, 12 or 48 mg/kg/d for one year. No details of clinical observations were reported. In males, body weights were not statistically significantly different between groups. High-dose females consistently displayed lower body weights than controls, and statistically-significant lower body weight gains were recorded for these high-dose females). There were no changes in food consumption in treated groups, and no alterations in haematology or urinalysis parameters. Dose-related changes in clinical chemistry values attributed to TCP administration were increased serum ALP and ALT values in both males and females seen at 3, 6 and 12 months. These values were comparable to controls at 3 mg/kg/d, always elevated although generally not to a statistically significant level at 12 mg/kg/d, and at 48 mg/kg/d exhibited an increase over controls which was generally statistically significant at each assay time. There were no gross or histopathological lesions associated with treatment and no changes in absolute or relative organ weights. Based on the biologically significant increased levels of serum ALP and ALT values at 12 mg/kg/d, the NOEL for dietary intake for one year of TCP in male and female dogs was 3 mg/kg/d (Zempel et al, 1987).

Female chickens were fed chlorpyrifos in the diet at 0, 25, 50 and 200 ppm (approximately 0, 2.5, 5 and 20 mg/kg bw/d) for 52 weeks. Hen mortality was unaffected by treatment; there was 13, 3, 7 and 10% mortality for the 0, 25, 50 and 200 ppm groups, respectively. Plasma cholinesterase was assayed in three hens/dose at 1, 3, 7, 14, 22 and 29 days after treatment started, and monthly thereafter, as well as 1, 2 and 3 weeks after treatment finished. The onset of cholinesterase activity inhibition was rapid and dose-related (22, 45 and 76% inhibition at week-1 for 25, 50 and 200 ppm respectively), and persisted at similar levels throughout the study but returned to control levels during the recovery period. Overall feed consumption, body weight, egg production, feed efficiency, egg weight and shell thickness were not affected by treatment. There was no NOEL for plasma cholinesterase inhibition in this study based on significant plasma cholinesterase activity inhibition at the lowest dose tested, 2.5 mg/kg/d (25 ppm) (Sherman & Herrick, 1973).

Reproductive toxicity

In a dietary study, Sprague-Dawley rats were fed chlorpyrifos at doses of 0, 0.03, 0.1 and 0.3 mg/kg/d

for the first generation, and 0, 0.1, 0.3 and 1.0 mg/kg/d for the second and third generations. The F3B foetuses were used for teratological examination. Clinical signs of toxicity were not seen in any parents or offspring. Parental body weights were not significantly affected by treatment, and food consumption was variable but unaffected by treatment in either sex. The fertility, gestation and lactation indices were comparable between groups and generations. The viability index was decreased at 1.0 mg/kg/d. This effect on pup viability at 1.0 mg/kg/d was also seen when comparing mean litter size at day 21. In the dams producing the F3B pups, mean bodyweight gain showed a dose-related increase; at 0, 0.1, 0.3 and 1.0 mg/kg/d, body weight gains for gestation days 0-20 were 114, 120, 123 and 126 g respectively. In this generation, there were no treatment-related effects on fertility (% pregnant), mean number of corpora lutea or implantations, viable litter size or pup weight following caesarean section. Skeletal examination of pups revealed common minor variants such as incomplete ossification of sternabrae, and the occurrence of extra ribs. Visceral examination of pups revealed minor variants of the urogenital system (hydronephrosis or hydroureter left, right or bilateral) to be more common in the treated group. A single foetus with multiple abnormalities occurred in the 1.0 mg/kg/d group. Group-average plasma and erythrocyte cholinesterase activity was decreased (>20%) at 1.0 mg/kg/d in males and females of the F2 generation. Based on the reduction in pup viability seen in each generation at 0.3 and 1.0 mg/kg/d, the decreased plasma and RBC cholinesterase activity seen at 1.0 mg/kg/d in males and females, and the decreased RBC cholinesterase activity seen in 0.3 mg/kg/d females, the NOEL for this study was 0.1 mg/kg/d (Thompson et al, 1971).

To supplement the findings of the previous study (Thompson et al, 1971), which recorded an equivocal decrease in neonatal survival at 1.0 mg/kg/d, Sprague-Dawley rats were fed diets containing chlorpyrifos (purity 96.6 to 99.0%) at doses of 0, 0.5, 0.8 or 1.2 mg/kg/d for two generations. Parental animals displayed no significant clinical signs in either generation. Body weight and food intake were unaffected by treatment. The mean fertility index (number of females delivering a litter expressed as a percentage of the total number of females placed with a male) was reduced at 0.5 and 0.8 mg/kg/d, but not at 1.2 mg/kg/d, and a similar inverted dose-response was recorded for gestation length; individual animal data were not presented. Litter sizes (10-11) were comparable in all dose levels, as were survival indices (>91% in all groups on days 1, 4, 7, 14 and 21) during lactation. Pup weights (presumably litter weight/number of pups) were also comparable. In F1 adults, male body weight was reduced at 1.2 mg/kg/d on days 160-182 (post cohabitation), and female body weight showed slight sporadic increases compared to controls. Food intake was sporadically decreased in males, but not in females. There were no effects on female body weight during lactation. The fertility index in treated groups exceeded control values. Litter sizes were comparable in all groups, as were pup weights and pup survival during lactation. The adequacy of this study for regulatory purposes was limited by the dose selection. As no adverse effects were demonstrated at the highest dose, it was unclear whether the study clearly demonstrated the reproductive toxicity potential of the test material in rats. The lack of individual animal data and the inverted dose-response for some parameters did not allow for an unequivocal interpretation of the findings. However in the absence of clear adverse findings on reproductive parameters at any dose level, the NOEL for this study was 1.2 mg/kg/d (Dietz et al, 1983).

Technical chlorpyrifos (purity 95.8%) was given to groups of rats at dietary concentrations of 0 (control), 2, 10, or 50 ppm over two generations. These dietary levels corresponded to a dose range of 0.1-8.1 mg/kg/d. Food consumption was similar in control and treated groups, and no adverse, treatment-related effects on group mean bodyweights were observed at any of the dose levels, in either of the generations. Mating performance and duration of gestation were similar in control and treated

groups. Some slight intergroup variation was observed in the incidence of total prebirth loss in both the F0 and F1 generations but in the absence of any consistent relationship between dose and effect, these findings were not considered to be related to treatment. In the F0 generation, there were statistically-significant increases in litter size and litter weight and decreases in mean pup weight at a number of dose levels at most sample intervals, but these findings were generally only slightly different from controls, and there was no consistent relationship with dose. The decrease in mean pup weights was probably related to increased litter size. In the F1 generation, litter data were similar in control and treated groups, with the exception of a single statistically-significant decrease in litter weight at 50 ppm on day 21. This isolated finding was considered to be incidental to treatment. No consistent, dose-related effect on organ weights was reported during the study. Pathological examinations did not reveal any macro-or microscopic effects that were considered to be related to treatment. Pathological findings were confined to general effects seen in laboratory rats, with a low incidence for most findings, and no consistent relationship with dose for any findings. Under the conditions of this study, no adverse, treatment-related effects were observed at any dose, up to and including 50 ppm (equivalent to a dose of up to 8.1 mg/kg/d, depending on sex and age of animal). Slight increases in group mean body weights in high-dose F0 males were the only effect attributed to treatment. No adverse effects on reproductive performance were demonstrated (James et al, 1988).

In a 2-generation dietary reproduction study, four groups of SD rats were fed chlorpyrifos (purity 97-8-98.5%) at doses of 0, 0.1, 1.0 or 5.0 mg/kg/d. No significant effects of treatment were seen on clinical signs, food intake or body weight. Slight, but not statistically-significant, decreases in body weights were seen in F1 males at 5 mg/kg/d, and F1 females showed reduced body weight gain during lactation at 5 mg/kg/d. Plasma cholinesterase activity was inhibited in parental F0 and F1 rats in both sexes at 1 mg/kg/d and at 5.0 mg/kg/d, and plasma cholinesterase inhibition was also seen in both sexes of both generations at 0.1 mg/kg/d, as part of a dose-related trend, which generally failed to reach biological or statistically significance at this dose. Erythrocyte cholinesterase activity was strongly inhibited at 1.0 and 5.0 mg/kg/d , but brain cholinesterase inhibition was seen only at 5 mg/kg/d (both sexes, both generations). Gross pathological observations of F0 and F1 parents recorded no significant observations, and significant histopathological changes in the parental animals were limited to vacuolation, consistent with fatty change in the adrenal zone fasciculata. In both generations, treatment had no effect upon fertility, length of gestation, gestation survival, time to mating, sex ratio or litter size. F1 pups showed slightly decreased body weight gain during lactation at 1.0 mg/kg/d, and body weight gain and survival were decreased (statistically significant) at 5 mg/kg/d. No effect of treatment was seen during gross pathologic observations or daily observation of the F1 weanlings. F2 pups did not show dose-related decreases in body weight gain during lactation. In these F2 pups, survival was decreased during lactation in control and 5 mg/kg/d groups due to total loss of 3 and 5 litters respectively; this was stated to be due to maternal neglect since stomach of pups in these litters contained no milk. No effect of treatment was seen during gross pathological observations or daily observation of the F2 weanlings. The NOEL for maternal toxicity based on plasma cholinesterase inhibition in adult animals was 0.1 mg/kg/d, based on significant plasma cholinesterase inhibition at 1 mg/kg/d. The NOEL for RBC cholinesterase inhibition in adults was 0.1 mg/kg/d and for brain cholinesterase inhibition was 1.0 mg/kg/d, with reduced maternal weight gain during lactation seen at this dose also. The NOEL for neonatal effects was 1.0 mg/kg/d based on decreased body weight gain and survival at 5 mg/kg/d and the NOEL for fertility and reproductive effects was 5 mg/kg/d (Breslin et al, 1991).

Developmental toxicity (TGAC)

In a developmental study in CF-1 mice, animals were first given chlorpyrifos (96.8% purity) at 0, 1, 10, or 25 mg/kg/d by gavage. These doses were based on a range-finding study which used doses up to 60 mg/kg/d. Due to severe maternal toxicity observed at 25 mg/kg/d in this first main study, additional groups were dosed at 0, 0.1, 1 or 10 mg/kg/d (second study). The dose-ranging study found severe cholinergic signs and consequent reproductive failure at doses of 30 mg/kg/d and above. In the first main study, there was a dose-related incidence of clinical signs at 1, 10, and 25 mg/kg/d, including mortality, excessive salivation, tremors, urine-soaked coat, ataxia and lethargy. Body weight gain was reduced at 25 mg/kg/d, but no effects were observed on litter size, resorption incidence or sex ratio. Foetal body weight and crown rump length were significantly reduced at 25 mg/kg/d. Cholinesterase activity was depressed in a dose-related manner in all tissues examined. Exencephaly was reported in all groups, and the incidence of delayed ossification was increased at 25 mg/kg/d. Statistically-significant increases were only seen in the case of the delayed ossification. The second study did not record any cholinergic signs in the dams at any of the dose levels of 0, 0.1, 1 or 10 mg/kg/d. Body weight, food intake, maternal liver weights, gravid uterine weight and adjusted body weights were also unaffected. Cholinesterase levels were rapidly and significantly inhibited in plasma and erythrocytes at 1 and 10 mg/kg/d, but were depressed in foetal homogenates only at the high dose. No effects were observed on materna1 parameters or on litter size, incidence of resorptions, incidence of dead foetuses, sex ratio, foetal body weight or crown-rump measurements. Neither teratology study provided evidence for teratogenic activity at any dose level, but foetotoxicity was seen at 25 mg/kg/d (reduced pup weight, crown-rump length and delayed ossification), and foetal homogenate cholinesterase activity was depressed >20% at 10 mg/kg/d in both studies. On the basis of these findings, the NOEL for foetotoxicity was 1 mg/kg/d. Erythrocyte cholinesterase inhibition was seen at 1 mg/kg/d, and cholinergic signs were seen in one of the studies at 1 mg/kg/d; on the basis of these findings, the NOEL for maternotoxicity was 0.1 mg/kg/d (Deacon et al, 1980; Deacon et al, 1989).

In a range-finding developmental study, Fischer 344 rats were given chlorpyrifos (96.6% purity) by gavage at 0, 3, 10, 15, or 30 mg/kg/d during organogenesis. Slight maternal toxicity was observed at 15 mg/kg and severe toxicity was observed at 30 mg/kg, with typical cholinergic signs of excessive salivation, lacrimation, urination, defecation and body tremors, and general observations of unkempt appearance, decreased body size and matting of perineal/facial hair. Mortality was also seen at 30 mg/kg/d, and animals from this group also recorded decreased body weight gain, decreased food consumption, enlarged adrenal glands, decreased liver weight and shrunken thymus glands. Both plasma and erythrocyte cholinesterase activities were depressed at all dose levels. Maternotoxicity was observed at all doses (based on plasma and RBC cholinesterase inhibition in adult animals at 3.0 mg/kg/d), but foetotoxicity (increased resorptions) was seen at 30 mg/kg/d only (Ouelette et al, 1983a).

Fischer 344 rats were dosed with chlorpyrifos (96.6% purity) by gavage at doses of 0, 0.1, 3, or 15 mg/kg/d on days 6 through 15 of gestation. There were no unscheduled deaths. Clinical signs of maternal toxicity including excessive salivation, urine staining (perineal region), porphyrin deposits about the eyes, vaginal bleeding. Tremors were observed in the 15 mg/kg/d group only, and at this dose there was also decreased body weight gain, but no other maternal findings were reported at necropsy. Both plasma and RBC cholinesterase activity were depressed at the 3.0 and 15.0 mg/kg/d dose levels. No adverse effects were observed on reproductive parameters, and no teratogenicity was observed. Variations and malformations were randomly distributed through the dose groups, and no effect of treatment on skeletal development was seen. The NOEL for maternal toxicity was 0.1 mg/kg/d, based on plasma and RBC cholinesterase inhibition seen in all adult animals at 3.0 mg/kg/d and above. No foetotoxicity was observed at any dose (Ouelette et al, 1983b).

In a range-finding developmental study in rats, chlorpyrifos (95.5% purity) was given to mated females by gavage at doses ranging from 0.02 to 62.5 mg/kg/d on days 6-15 of gestation. Treatment-related mortality and clinical signs (tremors and staining of the periorbital, nasal and/or urogenital regions) was seen at 62.5 mg/kg/d, and all animals at this dose died or were killed *in extremis*. No mortality occurred at other doses, but at the next highest dose (12.5 mg/kg/d) a single animal displayed tremor, peri-orbital and urogenital staining between days 11 and 16 of gestation. Group mean body weight and body weight gain were markedly and statistically significantly reduced at 12.5 mg/kg/d. Statistically and biologically significant, dose-related decreases in plasma cholinesterase activity were observed at doses of 0.05, 2.5, and 12.5 mg/kg/d. Statistically-significant decreases in pre-implantation loss were observed in all treatment groups, suggesting that the pre-implantation loss in controls was higher than expected. A slight but statistically-significant increase in post-implantation loss was seen at 12.5 mg/kg, and this finding may be related to the maternotoxicity of the test material at this dose. Under the conditions of this study, no treatment-related effects were reported at doses of 0.1 mg/kg/d and below, but inhibition of plasma cholinesterase activity was observed at doses of 0.5 mg/kg/d and above. No evidence of maternotoxicity was observed at doses of 2.5 mg/kg/d and below, but clinical signs, decreased body weight and reduced food consumption were observed at 12.5 mg/kg/d (Rubin et al, 1987a).

Female CD (SD) rats were given chlorpyrifos (purity 96.1%) by oral gavage at doses of 0.5, 2.5, and 15 mg/kg/d, on days 6-15 post coitum. No treatment-related mortality was observed during the study, and clinical signs of intoxication were confined to tremors seen late in the study in three animals at 15 mg/kg/d. Slight but statistically-significant decreases in mean food consumption and body weight gains were seen at 15 mg/kg/d. Statistically-significant, dose-related decreases in plasma cholinesterase activity were observed at all test doses. Total live litter sizes were unaffected by treatment, but at 15 mg/kg/d the mean number of live male foetuses was statistically significantly decreased, and post-implantation loss was slightly elevated. Statistically-significant increases in pre-implantation loss were seen at all test doses, but there was no consistent dose-response relationship associated with this finding, the pre-implantation losses were within historical control values for this testing laboratory, and this finding was not considered to be related to treatment. At 15 mg/kg/d, slight but statistically-significant increases were seen in mean foetal weight and mean crown-rump length but as the increase in foetal weight did not correlate with a decrease in litter size, this finding was possibly associated with advanced foetal development, and was not considered to be of toxicological significance. Similarly, the statistically-significant decrease in the incidence of small foetuses (observed in all test groups) was not considered to be toxicologically significant. A range of minor structural variations were reported (included a single

incidence of anophthalmia (0.5 mg/kg/d) and single instances of microphthalmia at 0.5 and 2.5 mg/kg/d), but the incidence of such findings was very low, and/or there was no relationship with dose. As such, these findings were considered to be incidental to treatment. At skeletal examination, no adverse treatment-related findings were observed. An NOEL was not demonstrated in this study, based on the inhibition of plasma cholinesterase activity at doses of 0.5 mg/kg/d and above. The NOEL for frank maternotoxicity was 2.5 mg/kg/d, based on reduced body weights, tremors and transient reductions in food consumption at 15 mg/kg/d. The NOEL for foetotoxicity was 2.5 mg/kg/d, based on a slight increase in post-implantation loss at 15 mg/kg/d, probably associated with maternotoxicity at this dose level. No evidence of major malformations was observed at any dose (Rubin et al, 1987b).

In a range-finding developmental study, female New Zealand White rabbits were given chlorpyrifos (95.5% purity) by gavage on gestation days 7 to 19, at doses of 0 (vehicle control), 1, 3, 10, 30, 90, 270, and 800 mg/kg/d. Treatment-related mortality was reported at doses of 270 and 800 mg/kg/d, and at these doses the remaining animals were killed on humane grounds, and no further data were collected during the study. Diarrhoea was observed in animals at doses of 10 mg/kg/d and above. Statistically-significant reductions in group mean food consumption were observed at doses of 10, 30 and 90 mg/kg/d during treatment, and this decrease in food consumption persisted past the treatment period at 90 mg/kg/d. At all doses, body weight gains were reduced during the treatment period in a dose-dependent manner. Statistically-significant decreases in group mean body weights were seen at 90 mg/kg/d on days 19-25, with decreases of about 10% compared with controls, and this finding was considered to be related to treatment. At day 18/19 of gestation, statistically-significant, dose-related decreases in plasma cholinesterase activity were measured in all treatment groups compared with controls. Slight but statistically-significant decreases in mean foetal weight were observed at 10 and 90 mg/kg/d, but not at 30 mg/g/day, and the toxicological significance of this finding, and its relationship to treatment, was unclear. Statistically-significant decreases in pre- and post-implantation losses were seen in almost all treatment groups compared with controls, but this effect was not considered to be toxicologically significant, and probably reflects higher than normal control values for these endpoints. Decreased plasma cholinesterase activity was seen at all doses, and frank maternotoxicity (decreases in body weights) was seen at 90 mg/kg/d and above. No unequivocal foetal toxicity was demonstrated during this study, and there was no evidence of gross treatment-related malformations (Rubin et al, 1987c).

Female New Zealand White rabbits were mated and given chlorpyrifos (purity 96.1%) by gavage on days 7 to 19 of gestation at doses of 1, 9, 81, and 140 mg/kg/d. No treatment-related mortality was observed during the study, and no treatment-related clinical signs were reported. Maternal group-mean food consumption was unaffected by treatment, and group-mean body weights and body weight gains were not affected by treatment at doses up to 81 mg/kg/d, with similar growth seen in control and treated animals. At 140 mg/kg/d, body weight gain was inhibited for most of the treatment period. Statistically-significant, dose-related decreases in plasma cholinesterase activity were seen at all test doses after 10 days of dosing. Statistically-significant decreases in pre-implantation loss were observed at most doses, but this finding was not considered to be toxicologically significant. Statistically-significant increases in post-implantation loss were observed at 9 and 140 mg/kg/d, but no increase in this effect was observed at 81 mg/kg/d. In the absence of a consistent dose-response relationship, the toxicological significance of this finding was unclear, and no historical control data were supplied for this effect from the testing laboratory. A slight but statistically-significant decrease in mean foetal crown-rump length, and a decrease in mean foetal weight were observed in the 140 mg/kg/d group, and these effects were

considered to be related to chlorpyrifos administration. A number of foetal anomalies were observed, but these findings were not considered to be treatment-related, as the incidence was generally very low, and there was no relationship to dose. An increased incidence of foetuses with fifth sternebra and/or xiphisternum unossified at 140 mg/kg/d was considered to be related to slightly delayed development at this dose, as seen by reduced foetal weight and length in this group. However, given the variability in the incidence of other skeletal observations in treated groups, it was possible that this finding was also due to chance, and was unrelated to treatment. Under the conditions of this study, an NOEL was not demonstrated, based on the inhibition of plasma cholinesterase activity at doses of 1 mg/kg/d and above. The NOEL for frank maternotoxicity was 81 mg/kg/d, based on decreased body weight gain at 140 mg/kg/d. The NOEL for foetotoxicity was 81 mg/kg/d, based on a slight decrease in mean foetal crown-rump length, a decrease in mean foetal weight, and an increased incidence of foetuses with fifth sternebra and/or xiphisternum unossified, at 140 mg/kg/d. No major treatment-related malformations were observed in this study (Rubin et al, 1987d).

Developmental toxicity – Metabolite (TCP) (3,5,6-trichloro-2-pyridinol)

In a dose-ranging study in Fischer 344 rats, animals were given 0, 43, 75 and 127.5 mg/kg/d of TCP (3,5,6-trichloro-2-pyridinol; 99.7% pure) on days 6 through 15 of gestation, by oral gavage. Clinical signs (perineal soiling) were seen sporadically in all the 127.5 mg/kg/d and some of the 75 mg/kg/d dams. Food and water consumption were unaffected by treatment. Signs of maternal toxicity were limited to reduced body weight gain in the dams of the high-dose group. Relative and absolute liver and kidney weights were unaffected by treatment. There was no observable influence of treatment on reproductive parameters. The study indicated that maternal toxicity may be seen at doses exceeding 43 mg/kg/d (Scortichini et al, 1986a).

Groups of Fischer 344 rats were given 0, 50, 100 and 150 mg/kg/d of TCP (99.7% pure) on days 6 through 15 of gestation, by oral gavage. Clinical signs of toxicity were not seen in any group. Slight vaginal bleeding was seen in three animals at day 13 (1 at 50, and 2 at 150 mg/kg/d). Food consumption was slightly decreased in the 100 mg/kg/d group and decreased about 10% in the 150 mg/kg/d group. Dose-related and significant decreases in body weight gain over the dosing period (days 6-16) were recorded at 100 and 150 mg/kg/d. Changes in the relative and absolute liver and kidney weights were restricted to a significant increase (4%) in relative liver weight at 150 mg/kg/d, consequent to a significant decrease (5%) in body weight at this dose. There was a dose-related trend in implantation sites/dam which was reflected in the number of foetuses/litter and probably in gravid uterine weight; this latter trend may also reflect the trend to lower body weight with higher dose. Foetal examination revealed only a small number of malformations in each dose group including controls. Overall, there was no significant influence of treatment on reproductive parameters or on foetal observations. Based on decreased body weight gain during treatment, the NOEL for maternal effects was 50 mg/kg/d. The NOEL for foetotoxicity and teratogenic effects was 150 mg/kg/d, the highest dose tested (Hanley et al, 1987a).

In the first phase of a dose-ranging study in inseminated New Zealand White rabbits, animals were given TCP (99.7% pure) on days 6 through 18 of gestation, by oral gavage at 0, 48.5, 80 and 133.5 mg/kg/d. As there was uncertainty that the Maximum Tolerated Dose (MTD) had been reached in this phase, additional groups of animals were dosed at 128 and 212 mg/kg/d (phase 2). Maternal body weight gain during gestation was depressed, but this was not statistically significant and was highly variable at the

highest dose in both phases. Gross pathology examination of all animals did not record any significant findings nor any differences between groups. All litters at 212 mg/kg/d showed resorptions; the percentage of implantations resorbed and the percentage of litters with resorptions was statistically different from the comparable phase 2 control group. There was no observable influence of treatment on reproductive parameters at doses up to 133.5 mg/kg/d. The range-finding study indicated that maternal toxicity and foetal toxicity may be seen in rabbits at doses exceeding 133.5 mg/kg/d (Scortichini et al, 1986b).

Groups of New Zealand White rabbits were given TCP (99.7% pure) by oral gavage on days 7 through 19 of gestation at doses of 0, 25, 100 and 250 mg/kg bw/day. This study met GLP requirements. Female animals were injected with HCG to synchronise oestrus, and were inseminated 3 weeks later on the presumed day 0 of gestation. Clinical signs of toxicity were not seen in any group. Water and food consumption were not reported. There was a dose-related decrease in body weight gain over the dosing period (days 7-20) which reached statistical significance at the high dose level. There were no treatment-related changes in the relative and absolute liver and kidney weights. Foetal examination revealed only a small number of malformations in each dose group including controls. Overall, there was no statistically-significant influence of treatment on reproductive parameters or foetal observations. However, an increased incidence of CNS malformations at mid- and high doses was suggestive of a possible teratogenic effect of TCP at these doses. There was no strong dose relationship for these findings, and the incidence was not statistically significantly different to controls. However, the study authors concluded that these findings were evidence of teratogenic potential despite the lack of statistical significance. The malformations included severe dilation of the cerebral ventricles and hydrocephaly. There was no change in the incidence of minor alterations observed externally, viscerally or upon skeletal examination. Based on decreased body weight gain during treatment, the NOAEL for maternal effects was 100 mg/kg bw/day. The NOAEL for foetal toxicity and teratogenic effects was 25 mg/kg bw/day, based on increased CNS malformations at 100 mg/kg bw/day and above (Hanley et al., 1987b).

Genotoxicity

Chlorpyrifos was negative for genotoxicity in a range of *in vitro* and *in vivo* studies.

Neurotoxicity

No signs of delayed ataxia or paralysis were reported in hens (3/group) treated with single oral doses of chlorpyrifos (40, 75, 100, or 150 mg/kg). Doses of 100 and 150 mg/kg resulted in deaths and other clinical signs of toxicity (Stevenson, 1966). Similarly, hens (10/group) given single oral doses of chlorpyrifos at up to 100 mg/kg did not display any delayed neurotoxicity, and no neuropathological findings associated with treatment were detected (Rowe et al, 1978). In another study, single oral doses of chlorpyrifos at up to 5 times the oral LD50 in hens (up to 150 mg/kg) resulted in significant inhibition of NTE and ChE activity (Capodicasa et al, 1991), although extensive and aggressive antidote treatment, both prior to and throughout the treatment and recovery periods, was required for the birds' survival. The oral administration of a single dose of chlorpyrifos (110 mg/kg) to groups of ten hens, followed by a repeat dose after 21 days, did not produce any clinical signs of delayed neurotoxicity. This observation was supported by histopathological examination, which showed no treatment-related change in nerve tissues in the birds as a result of chlorpyrifos administration (Roberts et al, 1987).

When chlorpyrifos was given to chickens for 91 days in the diet at up to 10 mg/kg/d, no compound-related clinical signs or increases in histopathological lesions of the nerve tissues (characteristic of OPIDN) were seen. Concurrent positive controls exhibited both toxic signs and histopathological lesions of the nerve tissues typical of a delayed neurotoxicant (Barna-Lloyd et al, 1986).

The acute delayed-neurotoxicity potential of chlorpyrifos was tested in white leghorn hens, for 21 days at up to 12 mg/kg/d, dosed by oral gavage in a peanut oil vehicle. Dose- and time-related increases in the incidence and severity of ataxia were observed. Plasma and whole blood cholinesterase activities were reduced at 5 and 12 mg/kg/d. Ataxia was observed at doses of 2, 5, and 12 mg/kg/d. Necropsy examination did not reveal any findings associated with treatment. Histopathological examination of the brain and spinal cord revealed a number of findings at a low incidence, without any relationship with dose, and these findings were considered to be incidental to treatment. The ongoing administration of chlorpyrifos resulted in ataxia in test groups during the study, which would have made it difficult to observe any delayed neurological effects in treated birds, had any such effects been manifest. As such, the adequacy of this study for regulatory purposes is limited (Anon, 1996f).

In a rat study, single oral doses of chlorpyrifos at 50 to 100 mg/kg resulted in treatment-related effects and clinical signs for several days after dosing. No mortality occurred at any dose. At 10 mg/kg, effects were confined to isolated observations of perineal staining and/or decreased activity. No neuropathological lesions were reported in animals receiving chlorpyrifos at up to 100 mg/kg (Wilmer, 1992; Mattsson et al, 1996).

In a study in adult Long-Evans rats, the oral administration of chlorpyrifos at doses up to 10 mg/kg/d for 4 weeks resulted in dose-related inhibition of cholinesterase activity (1 mg/kg/d and above for plasma and RBC; 3 mg/kg/d and above for brain). These effects were accompanied by clinical signs of intoxication at 10 mg/kg/d. A cognitive behavioural study indicated some non-cognitive changes associated with impaired motor activity at 10 mg/kg/d, but no clear treatment-related effects on cognitive function were observed (Maurissen et al, 1996).

Following the oral administration of chlorpyrifos to rats at single doses up to 100 mg/kg, no treatment-related effects were observed on NTE activity at any dose. Plasma, RBC, and heart ChE activities were statistically significantly decreased compared with controls at doses of 5 mg/kg and above, and brain ChE was decreased at 50 mg/kg and above. No significant ChE inhibition was observed at 0.5 or 1 mg/kg/d (Dittenber, 1997).

The neurotoxicity potential of chlorpyrifos was assessed in male Long-Evans Hooded rats following single oral gavage dosing at up to 100 mg/kg. All animals were examined using a Functional Observational Battery (FOB). Cholinesterase activity was significantly inhibited in a range of tissues and tissue components, including brain, plasma and erythrocytes, at all doses of 10 mg/kg and above. No clinical or behavioural signs were observed at 10 mg/kg, but were observed at 30 mg/kg and above at the 4.5 h interval. The selection of doses in this study resulted in a skew of some correlation data, especially in the correlations for ChE inhibition (in blood and blood components) with behavioural and clinical findings. Marked ChE inhibition was observed at the lowest dose in this study in the absence of clinical findings. The use of a single dose in this study also limited the usefulness of the findings. No clinical signs were seen when brain ChE activity was inhibited by less than 60%, or when whole blood

ChE inhibition was less than 80% following a single dose of chlorpyrifos, but there were no data to suggest that these figures could be extrapolated to repeat-dose situations. It is possible that the apparent threshold ChE inhibition that is needed before clinical and/or behavioural signs were observed after repeat dosing would be lower than the 60% (in brain) or 80% (in blood) inhibitions that were observed after a single dose (Nostrandt et al, 1997).

In a 13-week neurotoxicity study in Fischer rats at doses up to 15 mg/kg/d, a low incidence of perineal staining was seen in females at 5 and 15 mg/kg/d. No decrease in body weight was recorded at any dose. A slight reduction in motor activity was observed at 15 mg/kg/d during week 4 only, but as the magnitude of this isolated effect was not great, and the effect was not observed at other doses or intervals, the relationship with treatment was unclear. The doses used in this study were based on another rat study (Szabo et al., 1988), and it is likely that the transient and reversible change in motor activity at 4 weeks, if related to treatment, occurred only in the presence of significant brain and erythrocyte cholinesterase inhibition. Cholinesterase activity was not measured in this study. No neuropathological findings related to treatment were observed at any dose. The NOEL for this neurotoxicological study was 1 mg/kg/d (Shankar et al, 1993).

In a study designed to investigate the effects of age on chlorpyrifos toxicity, rats received either corn oil or chlorpyrifos (adult dose: 80 mg/kg; young rat (post-natal day17 or PND 17) dose: 15 mg/kg); these doses were equally effective in inhibiting ChE. In adult rats, peak behavioural changes and ChE inhibition occurred in males at 3.5 h after dosing, while in females the onset of functional changes was sooner, the time course was more protracted and recovery was slower. In young rats (PND17), maximal behavioural effects and ChE inhibition occurred at 6.5 h after dosing, and there were no gender-related differences. Behavioral changes showed partial to full recovery at 24 to 72 h, whereas ChE inhibition recovered markedly slower. Blood and brain ChE activity in young rats had nearly recovered by 1 week after dosing, whereas brain ChE in adults had not recovered at 2 weeks. Muscarinic-receptor binding assays revealed apparent down-regulation in some brain areas, mostly at 24 and 72 h. PND17 rats generally showed more receptor down-regulation than adults, whereas only adult female rats showed receptor changes in striatal tissue that persisted for 2 weeks. Thus, compared to adults (1) young rats (post-natal day 17) showed similar behavioural changes and ChE inhibition although at a five-fold lower dose; (2) the onset of maximal effects was somewhat delayed in the young rats; (3) ChE activity tended to recover more quickly in the young rats; (4) young rats appeared to have more extensive muscarinic receptor down-regulation, and (5) young rats showed no gender-related differences in functional changes (Moser & Padilla, 1998).

Chlorpyrifos was administered subcutaneously to neonatal rats at 1 or 5 mg/kg/d from postnatal days 1 through 4 (PND 1-4) or daily at 5 or 25 mg/kg/d to neonates from days 11-14 (PND 11-14). The strain and number of animals assessed was not stated. These two groups of pups were examined at postnatal days 5 and 10 or 15 and 20 respectively. PND 1-4 animals showed significant mortality at 5 mg/kg/d (>50%) but not at 1 mg/kg/d. The body weights of these 5 mg/kg/d pups were decreased by 10-15% and the brain as a whole was significantly smaller compared to controls. The survivors exhibited cell loss in the brainstem and brainstem growth was maintained by enlargement of the remaining cells. This effect was not seen at 1 mg/kg, a dose that did not compromise survival or growth, nor was there any adverse effect at either dose in the forebrain, despite the fact that both brainstem and forebrain possess comparable cholinergic projections. There was no mortality at 5 mg/kg/d but significant mortality (>80%) at 25 mg/kg/d in PND 11-14 animals at day 15. On day 15 the body weights of the pups at

25 mg/kg/d were significantly decreased (>40%) and the major target for cell loss shifted from the brainstem to the forebrain. The pups treated with 5 mg/kg/d recorded a small transient loss in body weight and no significant effects on whole brain weight. However, these pups displayed a loss of forebrain cell number between 15 and 20 days of age. The total amount of DNA in the forebrain declined in the 5 mg/kg/d group when compared to the rise seen in the control pup forebrains. The brainstem also showed a smaller but not significant cell loss during this period. The cerebellum differed from the other regions in that it showed short-term elevations of DNA after chlorpyrifos exposure in either early or late postnatal periods; nevertheless, values then regressed to subnormal, in parallel with the loss of cells in other regions. Although regions rich in cholinergic projections, such as brainstem and forebrain, were apparently more affected than non-cholinergic regions (cerebellum), the maturational timetable of each region (brainstem earliest, forebrain intermediate, cerebellum last) may have been more relevant for the development of these effects. Brain region weights remained within normal limits despite severe reductions in cell number, possibly because reactive hypertrophy of the remaining cells masked the cell loss. These authors felt that these results indicated that, even when growth or survival are unaffected, chlorpyrifos produced cellular deficits in the developing brain that could contribute to behavioural abnormalities. However, no information on clinical effects was provided and no assessment of behaviour or cognitive function of the pups was made. The conclusions regarding the nature of the cellular deficits (ie. regarding cellular hypertrophy) appear to have been based solely on quantitative measurements of DNA and protein concentrations, and not on histopathological examination of the tissues. The usefulness of this study for regulatory purposes is limited by the route of administration (subcutaneous injection), which may have had a significant effect on the toxicokinetics of the test material, and the lack of information on the protocol, including the number of animals used (Campbell, Seidler & Slotkin, 1997).

Chlorpyrifos was administered subcutaneously to neonatal rats at doses of 1 or 5 mg/kg/d from postnatal days 1-4 (PND1-4) or at 5 mg/kg/d from PND11-14. These two groups of pups were examined at postnatal days 5 and 10 or 15 and 20 respectively. Investigations for signs of interference with the adenyl cyclase signalling cascade examined the heart and developing brain regions viz. brainstem, and cerebellum. PND 1-4 animals at 1 mg/kg/d showed no signs of toxicity, while there was significant mortality at 5 mg/kg/d (>50%). PND 11-14 animals at 5 mg/kg/d showed no mortality or clinical signs. There were no significant changes in brain region weights at the apparently non-toxic doses. Cholinesterase activity in the brainstem of the 1 mg/kg/d pups was inhibited by 25% when measured 24 h after the last dose and recovery was substantial (<10% inhibition) by PND10. Cholinesterase activity in the brainstem of the pups administered 5 mg/kg/d from PND11-14 was inhibited by >60% when measured 24 h after the last dose and recovery was again substantial (<30% inhibition) by PND20. Separate examinations of the forebrain, cerebellum and heart found that chlorpyrifos evoked deficits in multiple components of the adenyl cyclase cascade: expression and activity of adenyl cyclase itself, functioning of G-proteins that link neurotransmitter and hormone receptors to cyclase activity, and expression of neurotransmitter receptors that act through this cascade. Disruption of signalling function was not restricted to transduction of cholinergic signals but rather extended to adrenergic signals as well. In most cases, the adverse effects were not evident during the immediate period of chlorpyrifos administration, but appeared after a delay of several days. Results from this study were generally presented in summary form only (Song et al, 1997).

The acute effects (as determined by a functional observational battery (FOB) and motor activity) of two

carbamates (carbaryl, aldicarb) and five organophosphates (OP) (chlorpyrifos, diazinon, parathion, fenthion, and diisopropyl fluorophosphate, or DFP) were evaluated in 10-week old male Long-Evans rats on the day of dosing at the time of peak effect, at 1 and 3 days, and at 1 week after dosing. All doses (oral gavage, in corn oil) were administered at volumes of 1 ml/kg, except carbaryl (2 ml/kg). Weight loss (about 10%) was recorded for each substance at the high dose only. Generally all cholinesterase inhibitors produced autonomic signs of cholinergic over-stimulation (salivation, lacrimation, and miosis), hypothermia, mild tremors and mouth-smacking (chewing motions), lowered motor activity, decreased tail-pinch response, and altered neuromuscular function (gait changes and increased foot splay). The measures generally found to be most sensitive on the day of dosing were body temperature, motor activity, gait, and the presence of mouth-smacking and fine tremors. However, no single measure was the most sensitive across all compounds. For some measures, differences in the slopes of the dose-response curves were evident. Many effects were still observed at 24 h, but recovery was apparent for all compounds. Interestingly, residual effects at 72 h were obtained with the carbamates (carbaryl, aldicarb) as well as with the OP fenthion, but not with the other compounds; this observation may be explicable, at least in the case of the carbamates, by slow absorption from the GI tract. Thus, in summary: the overall clinical toxicity profile was similar for these cholinesterase inhibitors, but compound-specific differences emerged in terms of the individual measures, dose-response, and time course; behavioural effects produced by these compounds may be dependent on both cholinergic and non-cholinergic mechanisms. Only summary data were available for evaluation (Moser, 1995).

Chlorpyrifos was administered subcutaneously to neonatal rats at 2 mg/kg on postnatal day 1 (PND1) or at 11 mg/kg on PND 6-9. The developing brain regions (brainstem, forebrain and cerebellum) were examined for signs of interference with cell development using markers for cell division. One-day-old rats given 2 mg/kg of chlorpyrifos showed significant inhibition (about 15%) of DNA synthesis in all brain regions within 4 h of treatment. Equivalent results were obtained when a small dose (0.6 µg or 2 µg/g brain) was introduced directly into the brain via intracisternal injection, suggesting that the actions may not have been secondary to systemic toxicity. Comparable inhibition (>10%) of DNA synthesis was also seen at 8 days of age; however, at this point, there was regional selectivity, with sparing of the cerebellum (about 1% inhibition). In another phase of this study, 6-8 day-old animals were pretreated with mecamylamine, a nicotinic receptor antagonist, followed by chlorpyrifos (11 mg/kg). The pretreatment caused a decline in DNA synthesis by itself, and also prevented any further decrement in DNA synthesis due to chlorpyrifos. Chlorpyrifos administration at 1 day of age caused a large (>30%) inhibition of protein synthesis throughout the brain; the effect was distinct from that on DNA synthesis, as it diminished substantially by 8 days of age (<10%) and did not develop any regional selectivity. Assays of ornithine decarboxylase activity 4 and 48 h after chlorpyrifos treatment of 1 and 8-9 old rat pups found no significant alteration of activity in any brain region when compared to appropriate controls. The authors concluded that the effects of chlorpyrifos on DNA and protein synthesis were not secondary to generalised cell damage or suppression of cell metabolism, as evidenced by the maintenance of normal ornithine decarboxylase activities. However, the data do not necessarily support such a conclusion, as effects on DNA synthesis might arise following systemic effects (such as inhibition of acetylcholinesterase) leading to secondary effects such as regulation of receptor number and changes in secondary messenger pathways. In the absence of cholinesterase inhibition data, it is not possible to determine whether any effects on DNA synthesis, if such effects did occur, would occur at doses that do not cause other measurable findings. The use of subcutaneous and intracisternal routes of administration in this study make the results limited for regulatory purposes. Results from this study were presented in summary form only (Whitney et al, 1995).

Pregnant Sprague-Dawley rats were given chlorpyrifos at doses of 0, 0.3, 1 or 5 mg/kg/d in corn oil vehicle from day 6 of gestation through day 11 of lactation (GD6-LD11) before evaluation of neurobehavioural performance. An additional satellite group of mated females were similarly dosed every day from GD6 through to GD20, and used for blood and brain sample collection and cholinesterase analysis. There were no deaths and only a few clinical signs (fasciculations, hyperpnea, hyperreactivity and decreased body weight gain) in the dams at the high dose. Between LD 1-5, a total of 3 dams in the 5 mg/kg/d dosage group had total litter loss and an additional 57 pups died or were cannibalised; these losses led to a decreased viability index. Necropsy of many of these dead pups indicated treatment-related lack of maternal care (no milk in stomach). There were no similar effects of treatment in the other dose groups. In dams, both plasma and erythrocyte cholinesterase activity were significantly inhibited at all three dose levels (>40% inhibition at 0.3 mg/kg/d), while brain cholinesterase was significantly inhibited at the two higher doses only (18% inhibition at 1.0 mg/kg/d). Pups, which were exposed to chlorpyrifos *in vivo* and for a period post-partum, displayed signs of toxicity at the high dose. Male and female pup weight in the high dose group was significantly lower than the other dose groups, consistent with the decreased food consumption and lack of body weight gain in high dose dams. Morphometric measurements of the brain and brain sections of 48 pups (6/sex/dose group) found a pattern, strongest in male pups, of an increase in morphometric measurements at the low dose and a decrease at the high dose, paralleling the brain weight differences between the groups. Chlorpyrifos treatment induced maternal toxicity (clinical signs and decreased body weight gain) at the high dose only (NOEL 1.0 mg/kg/d). Plasma and RBC cholinesterase activity in dams were significantly inhibited at all three doses (LOEL 0.3 mg/kg/d), while brain AChE activity was significantly inhibited at the two highest doses (NOEL 0.3 mg/kg/d). Chlorpyrifos treatment induced toxicity in the pups (decreased: viability index; relative brain weight and delayed sexual maturity) at the high dose only (NOEL 1.0 mg/kg/d). Cognitive functions in the pups (learning, memory and habituation) were not affected by treatment (NOEL 5.0 mg/kg/d). The lack of a functional observational battery and analysis of reflex ontogeny, and the fact that cholinesterase activity was not measured in pups, means that this study was of limited regulatory value in determining the relative sensitivity of young and adult animals to the effects of chlorpyrifos (Hoberman, 1998).

In a study performed in parallel to the developmental neurotoxicity study by Hoberman (1998), pregnant rats (25/group) were given chlorpyrifos once a day from day 6 of gestation through to day 10 of lactation (GD6-LD10) orally via gavage at doses of 0, 0.3, 1 or 5 mg/kg/d. Clinical signs, bodyweights and reproductive parameters were recorded. Cholinesterase inhibition and the concentrations of chlorpyrifos and metabolites were measured in dams and developing pups. Chlorpyrifos and chlorpyrifos-oxon were determined in milk, and chlorpyrifos, chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol (TCP) were determined in blood samples from fetuses/pups and dams. Cholinesterase activity was determined in plasma, RBC, heart and brain samples from dams and pups. Dams showed a clear dose relationship for chlorpyrifos and TCP levels in blood and milk. Chlorpyrifos concentration in the blood of male and female pups showed a similar dose-related increase; these blood levels in pups declined rapidly and no chlorpyrifos was detected from PND5 onwards. Male pups recorded higher chlorpyrifos concentrations in blood than females. Oxon levels were rarely quantifiable in any pup- or dam-samples. Cholinesterase activity was significantly decreased in a clear dose-related manner in all compartments in the dams. Minimum plasma and RBC activity was recorded on PND1. Cholinesterase inhibition in the pups was less severe than in the dams and recovered more quickly. Pups sacrificed at PND65 showed no

cholinesterase activity depression compared to controls in any compartment or at any dose level. There was some protection of the fetuses by the dams. The data from GD20 showed a higher blood level of chlorpyrifos in dams than in the fetuses at 1.0 mg/kg/d (dams 2.6 vs 1.0 ng/g) and 5.0 mg/kg/d (dams 110 vs 40-50 ng/g). This was consistent with the lack of any cholinesterase inhibition in fetuses from the 0.3 and 1.0 mg/kg/d dose groups, whereas dams showed significant cholinesterase inhibition in all compartments at 1.0 mg/kg/d and in blood at 0.3 mg/kg/d. The NOEL for cholinesterase inhibition in pups was 1.0 mg/kg/d based on the significant inhibition seen in the fore- and hind-brain, heart, plasma and RBC compartments on gestation day 20 at 5.0 mg/kg/d. There was no NOEL for cholinesterase inhibition in dams as there was significant inhibition seen in the plasma and RBC compartments on gestation day 20 at 0.3 mg/kg/d and higher doses (Mattsson *et al*., 1998).

Adult male Long-Evans rats were trained to perform an appetitive test of memory and motor function and were then injected sc with single doses of 0, 60, 125 or 250 mg/kg of chlorpyrifos in peanut oil (2 ml/kg) and tested 5 days/week for 7 weeks. Unconditioned behaviour was also rated for signs of cholinergic toxicity. Cholinesterase activity was measured in whole blood and several brain regions, and muscarinic receptor density and the hypothermic effect of oxotremorine challenge were also measured. Clinical signs of toxicity were seen in one rat at 250 mg/kg. Whole blood cholinesterase activity was inhibited by 60-75% (dose-dependent) at the first sample (day 4), and recovered to near control values after day 53 (60 mg/kg) and day 74 (125 mg/kg). Brain cholinesterase activity was strongly inhibited in a dose-dependent manner, and at the first sample (day 7) was up to 95% inhibited at 250 mg/kg. By day 21, partial recovery of cholinesterase activity was seen in some brain regions at the 60 and 125 mg/kg dose levels, but no significant recovery was seen at 250 mg/kg. Muscarinic receptor density decreased with dose and time. At 250 mg/kg receptor density in the hippocampus, frontal cortex and striatum fell to 70-75% of control after 7 days and to 60% of control 21 days after dosing; hypothalamic receptors were much less affected. Compared to controls, the hypothermia induced by oxotremorine challenge was significantly less pronounced in each dose group on days 8 and 32, but was comparable to controls by day 52. Unconditioned behaviour was relatively unaffected by treatment, with a fine tremor in head and limbs peaking on day 9 and gone by 14 days after dosing the only sign of cholinergic overstimulation. Functional deficits (in working memory and motor function) appeared within 2 days after injection of chlorpyrifos and recovered within 3 weeks (Bushnell et al, 1993).

Pregnant Sprague-Dawley rats were injected sc with either the peanut oil vehicle or chlorpyrifos (98%) at 200 mg/kg as a single dose on gestation day 12 (GD12) and then sacrificed on either GD16, GD20, or postnatal day 3 (PND3) for measurement of maternal and developmental indicators of toxicity. The day of birth was PND0. While most treated dams exhibited no overt signs, 4/28 showed moderate to severe cholinergic signs at 2-3 days after treatment, and these rats were omitted from further studies. Maternal body weight was initially decreased (15% reduction, 3 days after treatment) after which the body weight increase was comparable to controls. Chlorpyrifos exposure did not significantly affect foetal body weights or brain weights when assayed on GD16 and GD20. AChE inhibition (82-88%) was noted in maternal brain at all three time points following acute exposures. Foetal brain cholinesterase activity was less inhibited; with 40% inhibition at GD16 and 58% inhibition at GD20. This compared to 19-25 % inhibition at PND3 in treated pups cross-fostered to control dams and in control pups cross-fostered to treated dams following repeated exposures (25 mg/kg per day). At GD16 and GD20, foetal brain AChE activity was inhibited 42-44%. While some degree of recovery in AChE activity was noted in pup brain by PND3, AChE activity was still inhibited (30%) in treated pups cross-fostered to control dams. *In vitro* inhibition of maternal and foetal (GD20) brain AChE activity by the active metabolite,

chlorpyrifos oxon, suggested that the prenatal brain AChE activity was somewhat more sensitive than adult brain AChE. Maternal brain muscarinic receptor binding was more extensively reduced (30-32%) at GD20 and PND3 as compared to the developing brain at GD20 (16%) and PND3 (11%). A simple postnatal reflex test (righting reflex) was transiently (present at PND1 but not PND3) altered by chlorpyrifos exposure. The results suggested that acute chlorpyrifos exposure of dams during gestation produces more extensive neurotoxicological effects in the dam than in the developing foetus. The use of subcutaneous administration of the test material limits the usefulness of this study for regulatory purposes (Chanda et al, 1995).

Pregnant Sprague-Dawley rats were injected sc with either peanut oil vehicle or chlorpyrifos (98%) at 25 mg/kg/d from gestation days 12-19 (GD12-19) and sacrificed either on GD20 or postnatal day three (PND3). In a separate dose-response study, rats were similarly exposed to chlorpyrifos at 6.25 or 12.5 mg/kg/d, from GD12-19 and sacrificed on GD 20 for analysis of various neurochemical markers. No clinical signs of maternal toxicity was seen at any dose. Treatment with 25 mg/kg/d of chlorpyrifos induced only an initial slight transient decrease in maternal body weight. Treatment commencing on GD12 did not affect foetal weight at GD16 or GD20, but pup weight on PND1 was significantly reduced by treatment at 25 mg/kg/d. Maternal brain cholinesterase activity measured at GD20 after treatment at 6.25, 12.5 and 25 mg/kg/d during GD12-19 was significantly inhibited in a dose-related manner. Foetal brain cholinesterase was less inhibited (40 and 60% inhibition at respective doses of 6.25 and 25 mg/kg/d). Extensive AChE inhibition (10-17% of control value) was noted in dams after treatment with 25 mg/kg/d from GD12 to GD 16, GD20 and PND3. A significant dose-related down-regulation of muscarinic receptors in maternal and foetal brain was noted at GD20 following the GD12-19 exposures at all doses. Righting reflex and cliff avoidance tests were markedly altered following repeated exposures. Generally, the neurochemistry parameters in dams were more severely affected than the developing nervous system of the foetus, despite the higher sensitivity of foetal brain cholinesterase to chlorpyrifos inhibition. Comparison of these results with those from an earlier study which used acute exposure to chlorpyrifos (Chanda et al, 1995) revealed that repeated exposures caused greater down-regulation of muscarinic receptors than the equivalent dose delivered acutely (Chanda & Pope, 1996).

Human/exposure studies

A number of reports address the potential exposure and/or risks associated with the use of chlorpyrifos insecticides in domestic and/or urban settings (Fenske et al, 1990; Currie et al, 1990; Lean & Cantrell, 1992; Cantrell, 1992; Vaccaro et al, 1987; Wright et al, 1991; Wright et al, 1994; Dow Chemical Company, 1990; Berteau et al, undated; Ishikura 1988; Ishikura, 1989; Kawikata, 1987; Vaccaro, 1983; Ludwig et al, 1970). These studies contain a range of monitoring data, and estimates of dermal and or inhalational exposure varied widely depending on the methods used for application and sample collection. Similarly, estimates of risk to humans were largely dependent upon the default assumptions and uncertainty factors used in the exposure models.

In a review of chlorpyrifos poisoning data (Blondell & Dobozy, 1997), the US EPA summarised the case reports, case series, statistical surveys, and epidemiological studies of acute and chronic health effects reported to be related to chlorpyrifos. The authors noted the limitations in preparing such a report, including inadequate documentation of exposure and effects, reporting biases, and absence of denominator information on the population at risk. However, where consistent patterns of risk factors

were identified, the report also made recommendations to mitigate such risk. The report concluded that chlorpyrifos was one of the leading causes of acute insecticide poisoning incidents in the United States. This finding was based largely on an examination of Poison Control Centre reports. Certain types of uses were considered to pose greater health risks. The main concern was associated with the use of chlorpyrifos liquid formulations used by homeowners or Pest Control Operators (PCOs) indoors or outdoors, termite treatments, and liquid sprays and dips applied to domestic animals. Most of the more serious poisonings were associated with misuse or inappropriate use (spills, inadvertent contamination) by a PCO.

In response to the above US EPA review on chlorpyrifos poisonings (Blondell & Dobozy, 1997), DowElanco (Shurdut et al, 1997) stated that there were deficiencies in the EPA report which invalidated its use for reaching any conclusions about the safety of chlorpyrifos. The review concluded that there has been a misinterpretation of Poison Control Centre data, and that data from state and national poison control centres supported the relative safety of products containing chlorpyrifos. The review also claimed that the US EPA report failed to respect the limitations of anecdotal information, failed to consider the extensive testing and long use history for chlorpyrifos products with regard to neurological injury, made an inappropriate use of anecdotes and studies of organophosphates to characterise the safety of chlorpyrifos, failed to consider the extensive database on exposures following use of chlorpyrifos products, and selectively presented the data.

In a medical case-report (Aiuto et al, 1993a), a previously well 3-year-old boy was found playing near an open, spilled bottle of insecticide containing chlorpyrifos (Dursban; Dow Chemicals; concentration not stated). The authors of this paper noted that clinical manifestations of organophosphate-induced polyneuropathy (OPIDN) usually begin 1-3 weeks after the acute cholinergic crisis, and that examinations were consistent with distal, symmetric, predominantly motor polyneuropathy. In this patient, the symptoms of cholinesterase inhibition, along with the manifestations of weakness and areflexia 11 days after ingestion and the electromyographic findings, confirmed the presence of an acute transient polyneuropathy, with a more proximal distribution than usually seen in adults. The acute, reversible bilateral vocal cord paralysis reported in this patient has not previously been seen as part of OPIDN, but the authors believed that the onset of stridor and vocal cord paralysis coincided with the onset of muscle weakness, and resolved at a similar time to the normalisation of peripheral neuropathy.

Gutmann and Bodensteiner (1993) responded to the Aiuto et al, 1993 article, and suggested that this case may not represent a clear example of OPIDN. They noted that chlorpyrifos has been associated with OPIDN only after severe intoxication, characterised by extensive cholinesterase inhibition. The inhibition of plasma cholinesterase activity in this case was instead suggestive of mild chlorpyrifos intoxication. It was also noted that the clinical findings associated with OPIDN were predominantly motor polyneuropathy, with flaccid weakness and atrophy primarily in the distal limb muscles, and recovery usually requires months to years, and is often incomplete. The diagnosis of the patient in this case implied that evoked compound muscle action potentials and sensory nerve action potentials had normal amplitude, which would run counter to a diagnosis of OPIDN.

In response to these comments, Aiuto et al (1993b) noted that the patient's symptoms were consistent with cholinesterase inhibition of >90%, and that the plasma cholinesterase levels did not necessarily correlate with the severity of the ingestion. The authors concluded that the patient suffered from a delayed, life-threatening neuropathy, but were unable to determine the mechanism of such a neuropathy.

In another case report, a 38-year-old man drank an undefined quantity of chlorpyrifos 25% solution. The patient was treated with atropine 3 mg iv every two h for six days (total 400 mg), but any attempt to remove atropine treatment resulted in reappearance of respiratory distress. After this time, the patient was stuporous, catatonic and suffering from respiratory distress, with white mucous secretions, and his serum cholinesterase activity was undetectable. Ipratropium, 0.5 mg, was administered endotracheally as an aerosol mist over a ten-minute period, and there was an improvement in the patient's clinical condition, though the salivary secretions continued. The patient continued to receive ipratropium (between 1 and 2 mg daily), and by the fifth day the respiratory support was discontinued. A significant amount of catatonia and coarse tremor were relieved following administration of dantrolene 10 mg iv, followed by 25 mg po, three times daily. Serum cholinesterase activity was still undetectable one month after discharge (Shemesh et al, 1988).

In a case where a 26-year-old man intentionally ingested approximately 360 ml of a 6.7% chlorpyrifos formulation, 360 ml of a 2,4-D (10.8%) and MCPP (11.6%) formulation, and a few granules of a warfarin (0.025%) concentrate, the patient's major clinical findings on admission were coma, myoclonus, miosis, cardiac arrythmias, and a progression into hypotension, oliguria and death after several episodes of asystole. The authors stated that such symptoms were consistent with those seen in other patients following ingestion of chlorofenoxy acetic acids such as 2,4-D and MCPP. The patient did not display many of the usual signs of organophosphate poisoning, such as lacrimation, salivation, respiratory paralysis, or muscle fasciculation, but the red blood cholinesterase activity was inhibited by about 70% at 13h, and by up to 90% by 26 h. Plasma cholinesterase activity was inhibited completely at all time intervals. Lymphocyte neurotoxic esterase (NTE) activity was inhibited by about 50% after 13 h but was within normal limits after 19 h. Cerebral cortex NTE activity was within normal limits, but peripheral nerve NTE activity was inhibited by about 70% compared to normal control levels. This inhibition of NTE activity was measured in the absence of any significant signs of organophosphate intoxication (Osterloh et al, 1983).

Four incidents of birth defects allegedly associated with exposure to chlorpyrifos were reported (Sherman, 1996). In this paper, the children were reported with a range of birth defects including ventricular, eye, and palate defects and growth retardation (all children), hydrocephaly, microcephaly, mental retardation, blindness, hypotonia, wide-spread nipples, and deformities of the teeth, ears and external genitalia. The mothers of the affected children were reportedly exposed to chlorpyrifos in either the workplace or the home during pregnancy. Two of the children were born to the same mother. The exposure of the mothers to chlorpyrifos is very poorly characterised, but the paper does not indicate that exposure was severe or sustained during organogenesis. The range of birth defects reported in the children is not consistent with results of studies in laboratory animals, where similar effects have not been demonstrated. On the basis of the findings in this report, and the poor characterisation of exposure, it is not possible to determine whether the reported effects were associated with chlorpyrifos exposure.

In a review of the previous paper by Sherman (1996), the authors disputed the association of the reported defects with chlorpyrifos exposure (Gibson, 1996). This review stated that the medical records of the cases indicated that the effects seen were not the same in all children, and that the effects in some of the children were consistent with a specific diagnosis of an autosomal recessive birth defect syndrome of the brain and eye known as cerebro-oculio-facio-skeletal syndrome (COFS), or a closely related

syndrome known as MICRO syndrome. On the basis on the information provided in this paper, and the lack of information in the original case report paper (Sherman 1996), it is not possible to ascribe an association between the cases reported and chlorpyrifos exposure.

In a study of the effects of a single oral dose of chlorpyrifos at 0, 0.5, 1.0 or 2.0 mg/kg bw (purity - 99.8%; lactose powder was the diluent/placebo) to fasted human males and females (6/sex/dose), the NOEL for clinical signs or symptoms in this study was 2.0 mg/kg, the highest dose tested. The NOEL for RBC cholinesterase inhibition was 1.0 mg/kg based on significant inhibition in 1/12 subjects exposed at 2.0 mg/kg. Plasma cholinesterase activity was not measured. There were no effects of treatment at any dose level on general health measures during the study nor on clinical chemistry parameters measured at 7 days after dosing. The study design was double-blind, randomised, placebo control and conducted in two phases separated by 14 days. Volunteers (18-55 years old) were screened for general good health according to set criteria, and instructed to refrain from alcohol, strenuous exercise and prescription medications before and during the study. Doses were taken by capsule after an overnight fast. The health status of subjects was closely monitored; vital signs (blood pressure, pulse, respiration and temperature) were assessed prior to dosing and at 1, 2, 4, 8, 12, 24, 48 and 168 hours post-treatment. Subjects were questioned regarding their well being at each sample time, and clinical evaluation of the symptomology was recorded. Subjects were aware of the signs and symptoms of cholinergic toxicity and were instructed to inform the study physician of any adverse effects experienced. The subjects were blind as to their treatment group and the assessment and treatment of these signs and symptoms was perfomed by a physician also blind to the treatment group of the subject. There were no significant deviations from the study protocol. One male (phase 1 control) and one female (subject 56, phase 2, 2.0 mg/kg) did not provide a complete series of blood and urine samples. The only treatment related effect was recorded for the female volunteer who withdrew from the study (subject 56, 2.0 mg/kg). This female had decreased RBC cholinesterase levels compared to her pre-treatment values at most of the sample times (98.4%, 77.2%, 71.8%, 74.1%, 81.4% and 79.5% of pre-treatment value at 4, 8, 12, 24, 36 and 48h post-dose). If the data for this subject were removed from the analysis, then the 2.0 mg/kg female group-means were indistinguishable from the concurrent control values (Kisicki et al., 1999).

Groups of four healthy adult male volunteers received chlorpyrifos in tablet form at dose levels of 0 (placebo control), 0.014, 0.03 and 0.10 mg/kg/d for up to 27 days. The period of dosing was different for each dose level, and ranged from 9 to 27 days (48 days for controls). A single subject in the 0.1 mg/kg/d group reportedly suffered from a runny nose, blurred vision and a feeling of faintness on the final day of dosing, and this subject's plasma cholinesterase activity was inhibited by 70% compared with pretest levels. No decrease in erythrocyte cholinesterase activity was noted in any test subjects during the study. With the exception of plasma cholinesterase inhibition, no other treatment-related effects were noted. Rapid and marked depression of plasma cholinesterase activity was observed in subjects at the high dose of 0.1 mg/kg/d chlorpyrifos. Within-day comparisons with placebo controls revealed that the group mean depression of activity was greater than 40% by day 6 and greater than 60% by day 9, at which time the treatment was suspended. Plasma cholinesterase activity did not return to control levels until about day 34 in these high dose subjects. Similar results were observed when the plasma cholinesterase activity in the high-dose subjects was compared with the baseline activity of the group prior to treatment. At 0.03 mg/kg/d, mean plasma cholinesterase activity was inhibited by greater than 20% compared with placebo controls on days 16-20 of treatment. When compared to baseline levels pre-treatment, the mean plasma cholinesterase activity in this group was reduced by up to 34% on day

18, and activity was still inhibited by more than 20% compared with baseline levels on day 34. Individual subject plasma cholinesterase inhibition (relative to each subject's mean predose activity) was as high as 53% at day 18 of administration at 0.03 mg/kg/d. Plasma cholinesterase activity did not return to control levels for several weeks after cessation of treatment at this dose. At 0.014 mg/kg/d, plasma cholinesterase activity was inhibited by 20% at a single sample interval only (day 13), and plasma cholinesterase levels were similar to baseline activity from day 20 to day 27, even with continued chlorpyrifos administration. A separate statistical analysis was conducted by the investigators to determine the significance of plasma cholinesterase activity at the higher dose levels. At 0.03 and 0.1 mg/kg/d, two-way analysis of variance by the investigators indicated that the inhibition of mean plasma cholinesterase activity was significant ($p<0.05$) compared with controls at the high dose level (0.1 mg/kg/d) only. The NOEL for inhibition of erythrocyte cholinesterase activity was 0.1 mg/kg/d, with no effect on activity at any dose level. As the statistical significance of the inhibition at 0.03 mg/kg/d was equivocal, and depended upon the statistical tests used, the NOEL for this study was 0.03 mg/kg/d, based on the inhibition of plasma cholinesterase activity at 0.1 mg/kg/d (Coulston et al, 1972).

Six male Caucasian volunteers, aged 27-50 years, were used in this study. A single volunteer (Volunteer A) was first given a single oral dose (0.5 mg/kg) of chlorpyrifos in the form of a lactose capsule, administered approximately 30 minutes after food. This dose was administered approximately one month prior to the treatment of other volunteers. Volunteer A was also given a dermal dose of chlorpyrifos (0.5 mg/kg, dissolved in methylene chloride) when the other volunteers were given their oral dose. Two weeks after the first dermal dose, Volunteer A was given a further dermal dose (0.5 mg/kg) in dipropylene glycol methyl ether (DPGME). The remaining volunteers were given a dermal dose of 5.0 mg/kg chlorpyrifos in DPGME four weeks after the administration of their oral dose. All urine was collected from volunteers from 24-48 h prior to dosing, through 120 h post-dosing. No signs or symptoms of chlorpyrifos toxicity were reported in any volunteers during the study. Following the oral administration of 0.5 mg/kg chlorpyrifos, plasma cholinesterase was inhibited by 71% (compared with predose levels) in volunteer A, and by 85% (mean) in the other volunteers within 12-24 h after treatment. The range of individual values was not provided. Plasma cholinesterase activity had returned to >80% of the mean predose value by day 30 after oral treatment. Following dermal application at day 30, plasma cholinesterase activity decreased to approximately 70% of predose levels, and returned to about 80-90% of these levels by day 40 of the study. The intra-group variation was stated to be considerable by the authors of the study, but these figures were not provided in the report. Erythrocyte cholinesterase inhibition was not significantly inhibited following either oral or dermal doses of chlorpyrifos (Nolan et al, 1982, 1984).

A monitored field test was used to determine the effect that chlorpyrifos had on spraymen applying treatment at the rates used for programs for eradication of mosquitoes. This study was conducted following two earlier studies which were of limited success. These studies were conducted in 1966, 1967 and 1968, respectively. Cholinesterase activity measurements were conducted, and individual exposure was terminated if ChE inhibition reached 50% of baseline values. In the first study, three spraymen showed a marked depression in plasma ChE activity, with reductions of 68 to 82% compared with baseline levels. In the two remaining spraymen, where baseline values were not available, there was a reduction in plasma ChE activity of 52-56% over the 9-day period. The study was discontinued after two weeks due to the significant decrease in ChE activity. In the second study, there was reportedly no treatment-related effect on cholinesterase activity during the short study period. In the third study, RBC

ChE activity was not affected by treatment. Plasma ChE activity was reduced by 50-80% in spraymen using a suspension formulation at the first sample interval. All workers in this group had plasma ChE activity reduced by 50% or more, and activity had generally returned to pre-exposure levels within 2 months following cessation of use. The four spraymen using an emulsion formulation did not display a reduction in plasma ChE activity during the exposure period (Eliason et al, 1969; Kenaga, 1967b).

In another paper, the authors reported 8 case studies where chlorpyrifos exposure was claimed to cause sensory neuropathy. The cases each presented with a range of symptoms, and exposure characterisation was poor. Only 1 patient (case 3) reported signs of cholinesterase inhibition, including lacrimation, muscle twitching and diarrhea, and this individual was a pesticide applicator who was reportedly exposed to chlorpyrifos in a closed environment for 6 months. The patient's RBC cholinesterase activity was reportedly "low" initially, but recovered to "normal" levels within two months. No neurological evaluations were conducted until 6 weeks after the other symptoms were reported, and at this time the evaluation reported sensory loss of all modalities in a stocking-glove distribution test, mild distal weakness and areflexia in the lower extremities. Nerve conduction studies and quantitative sensory threshold studies reportedly revealed changes consistent with peripheral neuropathy of the distal anonopathy type. The decreased reflexes and mild distal weakness were consistent with polyneuropathy. Follow-up examination at one year revealed remission of all symptoms. In the other 7 patients (4 from the same family), symptoms were non-specific, and the absence of symptoms of cholinesterase inhibition suggested that these patients were not acutely exposed to chlorpyrifos to the extent of case 3. The lack of immediate electrodiagnostic testing, the poor exposure characterisation, and the variability in the reported clinical findings makes interpretation of the case studies generally difficult. Additionally, the method of questioning used to obtain information from the patients was subjective. No follow up examinations were conducted for a range of parameters on a number of patients, and not all patients were subjected to the same initial testing regimen. Based on the information provided in this report, there was evidence that one patient (case 3) was exposed to chlorpyrifos occupationally, with exposure sufficient to decrease cholinesterase activity and cause cholinergic symptoms. This patient also presented evidence of mild, reversible polyneuropathy, possible in the presence of ongoing decreased RBC cholinesterase activity. Two other patients (cases 2 and 8) had limited evidence of mild polyneuropathy (Kaplan et al, 1993).

Discussion

An extensive database of toxicology studies has been evaluated as a component of the ECRP review of this compound; no significant data deficiencies were identified in this review.

Metabolism and kinetics: In studies in humans and laboratory animals, chlorpyrifos was rapidly absorbed when swallowed, but did not persist for long periods in the tissues or organs of animals, and passed relatively quickly from the body. Absorption through the skin was relatively poor (about 2% in humans).

Acute toxicity: The signs of acute chlorpyrifos intoxication were consistent with cholinesterase inhibition, and included inactivity, salivation, dyspnoea, flaccid paralysis, vomiting, piloerection, exophthalmia and diarrhoea, and female animals were generally more sensitive to the acute effects of chlorpyrifos exposure than male animals. The acute dermal toxicity of chlorpyrifos was consistently lower than similar oral or inhalational exposures, and was indicative of the relatively low dermal absorption of chlorpyrifos

compared with absorption following oral exposure to chlorpyrifos.

Repeat-dose toxicity: The toxicity of chlorpyrifos was generally related to the inhibition of cholinesterase activity, and in most repeat-dose studies with chlorpyrifos the inhibition of plasma cholinesterase activity was the most sensitive toxicological parameter, followed by inhibition of erythrocyte cholinesterase activity, then inhibition of brain cholinesterase activity, and clinical signs of intoxication at higher doses. A summary of the no-observed effect levels, including those for inhibition of cholinesterase activity, is provided in the table below. Based on the limited data available, humans were of similar sensitivity to the cholinesterase-inhibiting effects of chlorpyrifos as laboratory animals. Chlorpyrifos was not carcinogenic in long-term animal studies, and was not genotoxic in a wide range of assays.

Neurotoxicity: A range of studies were performed to investigate the neurotoxicity potential of chlorpyrifos in hens and rats, both after acute and repeated exposures. These studies did not reveal any delayed or irreversible neurotoxicological sequelae to chlorpyrifos exposure in animals. As expected, dose-related, reversible inhibition of cholinesterase activity was observed, but this effect was not accompanied by any microscopic changes in nerve tissues, even in those animals that displayed clinical signs of intoxication. On occasion, some impairment of motor activity was reported at higher doses, but this effect was transient and reversible.

Reproduction and development: Chlorpyrifos did not induce major malformations or significant effects on most reproductive parameters in experimental animals. In some studies, decreases in survival of offspring were seen at high doses in the presence of frank maternotoxicity, sometimes associated with maternal neglect of the offspring. In developmental studies with chlorpyrifos, no treatment-related structural malformations were seen in mice, rats, or rabbits, and signs of delayed development (including decreases in foetal weight and crown-rump lengths) were only observed at maternally toxic doses. Doses of chlorpyrifos that caused maternal toxicity (clinical signs and decreased weight gain), cholinesterase inhibition (in dams and pups) and perinatal toxicity in pups (decreased viability index, relative brain weight and delayed sexual maturity) did not affect cognitive functions (learning, memory and habituation) in pups (Hoberman, 1998).

In developmental studies with the chlorpyrifos metabolite TCP, there was no statistically-significant influence of treatment on reproductive parameters or foetal observations in rats or rabbits. However, an increased incidence of CNS malformations at doses of 100 mg/kg/d and above in rabbits was noted. There was no strong dose relationship for these findings, which included severe dilation of the cerebral ventricles and hydrocephaly, and the incidence was not statistically significantly different to controls. As these effects were only reported at high doses, and there was no change in the incidence of minor alterations observed externally, viscerally or upon skeletal examination, TCP was not considered to pose a teratogenic risk to humans (Hanley et al., 1987b).

Toxicity in young animals: A number of studies have been conducted to determine whether neonates and young animals are more sensitive to chlorpyrifos than adult animals. In one of these studies, it has been reported that brain cholinesterase activity in foetuses was somewhat more sensitive to inhibition by chlorpyrifos than adult brain cholinesterase, while another study reported that acute chlorpyrifos exposure of dams during gestation produced more extensive neurotoxicological effects in dams than in developing foetuses (Chanda et al., 1995; Chanda & Pope, 1996). In general, the usefulness of these

studies for regulatory purposes has been restricted by the study design and protocols employed. For example, many of these studies have used routes of administration such as subcutaneous injection, and/or at high doses that cause clinical signs and/or extensive inhibition of cholinesterase activity, and so the relevance of findings in these studies in determining the public health risks associated with exposure to chlorpyrifos from food residues and/or non-agricultural uses is limited. In addition, many of these studies have not been conducted according to established test guidelines or according to Good Laboratory Practice guidelines. The reliability and validity of the results obtained from such studies is not clear.

In several studies designed to investigate treatment-related effects on brain DNA synthesis, neonatal rats were exposed to chlorpyrifos by intracisternal and/or subcutaneous injection. In these studies, the study authors reported inhibition of DNA synthesis and reductions in DNA amounts in brain regions. However, the usefulness of these studies for regulatory purposes was limited by the routes of administration (which are not relevant for assessing public risks for chlorpyrifos uses in agricultural and/or residential uses), study protocol deficiencies (including the lack of information on animal numbers used), and the use of high doses (1 to 2 orders of magnitude higher than the NOELs for inhibition of plasma and/or erythrocyte cholinesterase activity). In addition, the conclusions regarding the nature of the reported cellular deficits in brain regions appear to have been based solely on quantitative measurements of DNA and protein concentrations, and not on histopathological examination of the tissues. As no information on clinical effects or extent of cholinesterase activity was provided, and no assessment was made on cognitive or behavioural functions of the pups, it is not possible to correlate any reported changes in DNA or protein levels with any physiological manifestations of chlorpyrifos toxicity (Campbell et al., 1997; Whitney et al., 1995).

In rat studies in which chlorpyrifos was administered to adult and young and/or neonatal rats by oral gavage, there were apparent differences between young and old rats following acute exposure. These include findings that young rats (post-natal day 17) displayed behavioural and cholinesterase changes at a lower dose than adults, but also included observations that the onset of maximal toxicity was slower and recovery was more rapid. The limited dose selection in this particular study (Moser & Padilla, 1998; 80 mg/kg bw in adults compared with 15 mg/kg bw in young rats) makes the usefulness of these findings limited for regulatory purposes.

In a recently published study[1], neonatal (7 days old) and juvenile (21 days old) rats were reportedly more sensitive to the acute toxicity of chlorpyrifos. The oral LD50 values for chlorpyrifos were estimated to be approximately 40, 80, and 120 mg/kg bw, for neonates, juveniles and adults, respectively.[2] Oral LD50 values for chlorpyrifos vary widely, even in studies in adult rats, and range from 96 to 475 mg/kg bw, and the lower of these two figures has already been used to establish the Poisons Schedule for chlorpyrifos in Australia.

In the same study (Zheng et al, 2000), following a single oral dose of chlorpyrifos of between 0.15 and 15 mg/kg bw, neonates were more sensitive to the cholinesterase inhibiting effects in plasma and brain than adult rats. However, when exposure was extended over a 14-day period, at the same dose levels, the neonatal animals were usually no more sensitive, if not less sensitive, to the effects of chlorpyrifos than the adults, based on the inhibition of cholinesterase activity. The authors concluded that while immature

1 Zheng et al., Toxicological Sciences 55, 124-132 (2000). Comparative cholinergic neurotoxicity of oral chlorpyrifos exposures in preweanling and adult rats.

2 Based on the curves presented in the paper. The results were given in the study as LD10 values.

animals can be markedly more sensitive to the lethal effects of high doses of chlorpyrifos, lesser or no age-related differences were apparent for non-lethal endpoints, particularly after repeated exposures.

Based on these findings, it is considered that the normal safety factors for individual variability, used in combination with the NOEL in humans for the most sensitive toxicological endpoint (inhibition of plasma cholinesterase activity), are adequate for setting public health standards for the repeated exposure of humans to low levels of chlorpyrifos.

Effects in humans: Mild distal axonopathy consistent with organophosphate-induced delayed polyneuropathy (OPIDN) has been reported some weeks after poisoning incidents, but only in those cases in which severe cholinergic signs and significant inhibition of neuropathy target esterase and cholinesterase activities required treatment with aggressive, extensive antidotal therapy and artificial ventilation. Such effects are only expected after the ingestion of large doses of chlorpyrifos, intentionally or otherwise. There is also evidence that individuals, if exposed to chlorpyrifos occupationally (at doses sufficient to decrease cholinesterase activity and cause cholinergic signs) may present with mild, reversible polyneuropathy, possibly in the presence of continual inhibition of erythrocyte cholinesterase activity.

In supervised studies, when volunteers have received oral doses of chlorpyrifos for up to 27 days, inhibition of plasma cholinesterase activity (at doses of 0.1 mg/kg bw/day and above) and erythrocyte cholinesterase activity (at doses above 0.1 mg/kg bw/day) were observed. No other signs of toxicity have been observed at these doses.

NOEL considerations

To determine the lowest NOEL for the establishment of health standards for chlorpyrifos, a summary of the NOELs determined in those studies deemed adequate for regulatory purposes is shown in the Table below.

Species	Duration	NOEL (mg/kg/d)	LOEL (mg/kg/d)	Effect	Reference
mouse	78 weeks	-	0.7	plasma ChE	Gur et al (1991)
		0.7	6.1	RBC ChE	
		0.7	6.1	brain ChE	
mouse*	2 years	1.5[#]		carcinogenicity	Warner et al (1980)
rat	2 years	0.1	1.0	plasma ChE	McCollister et al (1971)
		0.1	1.0	RBC ChE	
		1.0	3.0	brain ChE	
rat	2 years	0.012	0.3	plasma ChE	Crown et al (1988)
		0.3	6	brain ChE	
rat	2 years	0.1	1.0	plasma ChE	Salminen & Ma (1992)
		1.0	10.0	RBC ChE	
		1.0	10.0	brain ChE	
dog	2 years	0.01	0.03	plasma ChE	McCollister et al (1971b)
		0.03	0.1	RBC ChE	
		1.0	3.0	brain ChE	
chicken	1 year	<2.5		plasma ChE	Sherman & Herrick (1973)

Species	Duration	NOEL (mg/kg/d)	LOEL (mg/kg/d)	Effect	Reference
human	9-28 days	0.03	0.1	plasma ChE	Coulston et al (1972)
		0.1	-	RBC ChE	

* Study suitable for oncogenicity determination only. # highest dose tested

Determination of Public Health Standards

Acceptable Daily Intake

In Australia, the current Acceptable Daily Intake (ADI) for chlorpyrifos is 0.003 mg/kg/day, based on the NOEL for plasma cholinesterase inhibition of 0.03 mg/kg/day in a human volunteer study (Coulston et al, 1972), and using a 10-fold safety factor.

Following the considerations of the Advisory Committee on Pesticides and Health (ACPH) and the Scientific Director of the Chemicals and Non-Prescription Drug Branch (CNPDB) of the Therapeutic Goods Administration (TGA), the current ADI for chlorpyrifos was affirmed.

Considerations of the Advisory Committee for Pesticides and Health (ACPH)

The Committee provided advice on the selection of an appropriate No-Observed Effect Level (NOEL) on which to base the Acceptable Daily Intake (ADI) for chlorpyrifos.

Four issues were identified for the Committee to examine and resolve. Firstly, whether the Committee wished to maintain its current policy on the use of plasma ChE inhibition as a toxicological endpoint for certain anticholinesterase pesticides. Secondly, whether plasma ChE inhibition was an appropriate toxicological endpoint for establishing the chlorpyrifos ADI. Thirdly, if the plasma ChE inhibition endpoint was maintained for chlorpyrifos, whether the NOEL from the human (Coulston et al, 1972) study was 0.01 or 0.03 mg/kg/day, and lastly, what safety factor should be selected when estimating the chlorpyrifos ADI.

The Committee acknowledged that the NOEL is based on the depression of plasma ChE activity, and that the toxicological significance of this effect remains unclear but, nevertheless, maintained the view that it is a chemically induced response that cannot be dismissed. Accordingly, the Committee agreed that it would be prudent to continue to base ADIs for anticholinesterase pesticides on the most sensitive ChE inhibition endpoint which in the majority of cases, including chlorpyrifos, is plasma ChE inhibition.

It was agreed that whilst the Coulston study may be inadequate by contemporary standards, its results were considered to be most appropriate for basing the chlorpyrifos ADI, as the use of a NOEL from a human study removed the extra uncertainty associated with extrapolating from a NOEL in animals studies. The Committee noted that the statistical significance of plasma ChE inhibition at 0.03 mg/kg/day in humans was considered to be equivocal and agreed that the ADI should remain unchanged. Accordingly, the existing chlorpyrifos ADI of 0.003 mg/kg/day was affirmed based on a NOEL of 0.03 mg/kg bw/day and using a ten-fold safety factor. It was highlighted that this value is consistent with the NOELs for a number of ChE inhibition endpoints identified in toxicology studies in animals.

Acute Reference Dose

To reflect safe/acceptable exposure from a single or short exposure to chlorpyrifos, an acute reference dose (acute RfD) may be derived using appropriate data. In a human study (Coulston et al, 1972), the oral administration of chlorpyrifos at doses up to 0.1 mg/kg/d for up to 3 days did not result in any significant inhibition of plasma or erythrocyte cholinesterase activity. The acute RfD based on this study is 0.01 mg/kg/d, derived from the NOEL and using a 10-fold safety factor for individual variability.

Public exposure

In Australia, chlorpyrifos is registered for a range of uses, including use on crops, as a termiticide for use by licensed Pest Control Operators, and in some home-garden insecticide products. Chlorpyrifos MRLs have been established in a wide range of foods, including fruits and vegetables; the current Australian MRL list is outlined in Chapter 1 of the main assessment document.

Dietary Exposure Considerations

An estimate of chlorpyrifos intake was derived from the Australian Total Diet Survey (formerly the Australian Market Basket Survey), based on food consumption in Australia. The estimated consumption of chlorpyrifos residues in food varied considerably between surveys. This variability may be a reflection of changes in use patterns, or of the sampling protocols used in the surveys. In the 1996 survey, the highest dietary exposure to chlorpyrifos in the groups studied, based on the 95th percentile energy intake, was 0.0904 µg/kg bw/day in infants aged 9 months, and was estimated to be 3% of the Acceptable Daily Intake (ADI). The lowest dietary exposure was 0.0311 µg/kg bw/day in girls aged 12, with an intake estimated to be 1% of the ADI. The ADI is derived from toxicological data obtained from humans, and using a safety factor to account for inter-individual variation.

In 1990, the highest estimated intake was calculated for boys (aged 12 years, approximately 40 kg bodyweight), with 0.014 µg/kg/day. In 1992 the highest estimated intake was 0.544 µg/kg/day in children aged 2 years (12.3 kg bodyweight), and in 1994 the highest estimated intake was 0.1584 µg/kg/day, also in children aged 2 years (12.3 kg bodyweight). In the group with the highest consumption (children aged two, 1992), the estimated intake accounted for approximately 18% of the ADI, giving an additional 5.5-fold safety factor between the ADI and maximum estimated dietary intake.

Non-Dietary Exposure Considerations

Termiticide use: The issue of potential public hazard of inhalational exposure to chlorpyrifos arising from its use as a termiticide has been considered previously by the NHMRC Standing Committee on Toxicity (SCOT). The Committee noted that chlorpyrifos levels in Australian treated buildings did not exceed 1 µg/m^3 (approximating to an exposure of 0.2 µg/kg bw/day for a 24-hour period), and that results from animal toxicology studies indicated a wide margin of safety based on this potential human exposure. The Committee concluded that "exposure of occupants of dwellings treated … with chlorpyrifos was not considered to pose a significant risk".

Data on indoor air concentrations of chlorpyrifos following a soil barrier treatment against termites in the sub-floor space of seven dwellings have been re-assessed as part of the ECRP review. Mean indoor

air concentrations did not exceed 1 μg/m^3. At this level of exposure, it was not considered that there was a significant public health risk associated with the use of chlorpyrifos as a sub-floor termiticide, and under-slab treatment with chlorpyrifos would be unlikely to give rise to levels higher than those in sub-floor studies.

Based on the estimated daily air intakes in population subgroups (using factors from the International Programme on Chemical Safety Environmental Health Criteria document #210, 1999), a concentration of 1 μg/m^3 equates to a maximum equivalent dose of 0.44 μg/kg bw/d (in 5-11 year olds), equivalent to approximately 14.6% of the Acceptable Daily Intake (ADI). Intake estimates for other subgroups were 0.38 μg/kg bw/d in children aged 7 months to 4 years (12.7% of the ADI), 0.36 μg/kg bw/d in 12-19 year olds (12 % of the ADI), 0.32 μg/kg bw/d in adults aged 20 years and above (10.7% of the ADI), and 0.28 μg/kg bw/d in infants aged less than 6 months (9.3% of the ADI).

Indoor broadcast use: The public health issues associated with the indoor broadcast use of chlorpyrifos, including use on floors, furnishings and carpets, has been considered on a number of occasions during the 1990s by National Health and Medical Research Council (NHMRC) Committees, including the Standing Committee on Toxicity (SCOT) and the National Drugs and Poisons Scheduling Committee (NDPSC), or their predecessors, the Committee on Toxicity (COT) and the Drugs and Poisons Scheduling Standing Committee (DPSSC). These Committees expressed concerns over the potential public health risks associated with such uses. SCOT concluded that chlorpyrifos posed a potential human health hazard following indoor application, and advised the DPSSC of its concerns, with the recommendation that appropriate labelling instructions be prescribed for chlorpyrifos products intended for indoor broadcast application. The DPSSC agreed, as a general rule, that organophosphorous pesticides (including chlorpyrifos) should not be available in domestic products for use by householders, particularly on floor coverings. SCOT also recommended that, in considering chlorpyrifos products intended for indoor use, efforts should be made to reduce air and surface levels. Adequate ventilation should be provided for at least 24 hours after application to aid in the dissipation of airborne chlorpyrifos. Adults should not re-occupy rooms until treated areas are dry and that children should avoid contact with treated surfaces for 48 hours after application.

The exposure data on broadcast indoor use have been reassessed as part of the ECRP review. It has been concluded that there is a public health issue associated with the indoor broadcast use of chlorpyrifos, based on the hazard and potential exposure to chlorpyrifos associated with this use. In the absence of data on the residual concentrations of chlorpyrifos following, such uses should not be supported.

The TGA has been advised by the NRA that labels on chlorpyrifos products do not currently support indoor broadcast use. However, consideration should be given by the NRA to review chlorpyrifos labels, and to amending existing labels (where appropriate) to clearly specify that chlorpyrifos products should not be used for indoor broadcast use.

Pet collars: The toxicity and release properties of flea collars that contain chlorpyrifos was not considered specifically in the ECRP review, but previously has been considered in some detail by the TGA. It was felt that the public health risk associated with the use of chlorpyrifos flea collars was minimal in adults but could be somewhat higher in children. However, based on the following information, it was considered that this risk was not significant.

- The active material is impregnated in a tough plastic resin, making the removal of pieces by biting extremely difficult, and exposure by this route was not considered likely.
- The absorption of chlorpyrifos through the skin is very low, and thus exposure by the dermal route would not be significant.
- A rate-of-release study indicated that the maximum release of chlorpyrifos from the collars worn by cats and dogs was estimated to be 1.1 and 8.6 mg/day, respectively. The data did not indicate any build-up of chlorpyrifos on the surface of the collar while it was being worn by the animal.
- A dose of 8 mg (dog collar) represents approximately three hundredths of the median lethal dose for a 15 kg child; this safety margin is much greater for a cat collar.
- Chlorpyrifos is not a volatile compound and there is negligible exposure potential from inhalation. The safety directions relating to skin and eye contact are a precaution against excessive or deliberate skin or eye contact with the very low levels of active ingredient on the surface of the collar.

Based on these earlier considerations, the TGA does not recommend that the NRA make changes to the registration status of flea collars containing chlorpyrifos.

Other residential uses: The NRA "Guidelines for pesticides used by householders" (Ag Requirements Series, Part 3, Toxicology, Appendix 3-1) indicate that pesticides for household, home garden or domestic use should be relatively harmless or capable of causing only mild illness if poisoning occurs. They should not cause irreversible toxicity on repeated exposure, nor require the use of safety/personal protective equipment that is not readily available to householders. Based on the toxicity of chlorpyrifos, products containing chlorpyrifos at 50g/L or less are generally acceptable for use by householders in terms of compliance with the NRA guidelines, and to minimise the risk to the public from use of such products.

The ECRP toxicology assessment has identified that there are a number of emulsifiable concentrate (EC) and/or liquid concentrate (LC) formulations registered for use in domestic, home garden and/or lawn areas. Most of these formulations contain chlorpyrifos at concentrations between 240 and 500 g/L, and are available in home garden pack sizes (1 litre or less). The toxicity of these formulations is such that they do not comply with NRA Guidelines for pesticides used by householders. The TGA has recommended that risk-mitigation measures be made to reduce public health risks from the use of these products, and such measures might include changes in availability, registration status, labelling information and or packaging.

Aggregate exposure estimates

An estimate of the highest intake of chlorpyrifos from dietary and residential exposures to chlorpyrifos resulting from termiticide use has been tabulated below. A number of assumptions have been made in these estimates, namely: 1) Termiticide use in Australia accounts for the highest potential for residential exposure, 2) other residential chlorpyrifos uses do not present a significant exposure for the public, and 3) the highest air concentration from chlorpyrifos in Australian residences following termiticide application was 1$\mu g/m^3$ (see 'Termiticide use' section above). The agricultural report prepared as part of the ECRP review indicated that termiticide use in Australia accounted for almost 30% of the usage of chlorpyrifos, while turf, home garden and other non-agricultural uses accounted for only 2.4% of chlorpyrifos usage

in Australia.

The dietary intake figures from Australian Market Basket Surveys was highest for all age categories in the 1992 survey, where estimates of intakes at the 95th percentile were some 10-fold higher than dietary intakes in the 1996 survey. [It is not clear whether such differences in intake estimates arose from differing survey methodologies or significant changes in the agricultural uses of chlorpyrifos in those years.]

Aggregate maximum exposure estimate for dietary and termiticide uses of chlorpyrifos

Population Group*	Dietary exposure estimate[1]	Residential exposure estimate[2] (termiticide use only)	Total exposure estimate (µg/kg bw/day)	Percentage of the ADI[3]
Adults	0.49[4]	0.32	0.81	27%
Children aged 12 years	0.5[4]	0.44	0.94	31%
Toddlers aged 2 years	0.63	0.38	1.01	34%
Infants aged 9 months	0.52	0.28	0.80	27%

* Based on groups used in the Australian Market Basket Surveys (AMBS).
1 Highest intake estimate (95th percentile) from AMBS 1992 in µg/kg bw/day
2 Based on a maximum exposure of 1 µg/m^3, and using air intake estimates from IPCS EHC 210, 1999. Population subgroups are: adults aged 20 years +, children aged 5-11 years, children aged 7 months –4 years, and infants aged less than 6 months. Expressed as µg/kg bw/day
3 Expressed as a percentage of the Acceptable Daily Intake (ADI) of 0.003 mg/kg bw/day
4 Females

Based on the above assumptions and exposure estimates, there are no public health concerns associated with exposure to chlorpyrifos from food residues and indoor air levels following termiticide use.

TOXICOLOGY MAIN REPORT

1. INTRODUCTION

1.1 Regulatory History of Health Considerations in Australia

Chlorpyrifos is a broad-spectrum organophosphorus insecticide that has been used in Australia for over 30 years. The current Acceptable Daily Intake (ADI) for chlorpyrifos is 0.003 mg/kg/d, based on the no-observed effect level (NOEL) for plasma cholinesterase inhibition of 0.03 mg/kg/d in a human volunteer study, and using a 10-fold safety factor. The current poisons schedule classification for chlorpyrifos is Schedule 6, with a cutoff to Schedule 5 when used in preparations at concentrations of 5% or less of chlorpyrifos, when in aqueous preparations containing 20 % or less of micrencapsulated chlorpyrifos, or in controlled release granular preparations containing 10% or less of chlorpyrifos. Potting or soil mixes containing 100g per cubic metre or less of chlorpyrifos are exempt from poisons scheduling.

Regulation

In Australia, public health standards for agricultural and veterinary chemicals, such as the poison schedule, first aid and safety directions and an acceptable daily intake (ADI), are set by the Department of Health and Aged Care (hereafter called the Department). Poisons schedules are set by the National Drugs and Poisons Schedule Committee (NDPSC) of the Australian Health Ministers Advisory Council [formerly the Drugs and Poisons Schedule Committee (DPSC) of the National Health and Medical Research Council (NHMRC)]. Maximum residue limits (MRLs) were formerly established by the Pesticide and Agricultural Chemicals Standing Committee (PACSC) of the NHMRC. In 1992, the Department became directly responsible for establishing MRLs, a function subsequently transferred to the National Registration Authority for Agricultural and Veterinary Chemicals (NRA) in June 1994. In 1993 an independent expert committee, the Advisory Committee on Pesticides and Health (ACPH), was established by the Department to provide advice and peer review on toxicology and public health issues.

An extensive toxicological database of studies has been submitted and assessed since 1985, with clearance of the technical grade active constituent (TGAC) granted in 1990. Chlorpyrifos has been used as a grain protectant since 1985 and as an under-slab termiticide since 1993.

Other names for the chlorpyrifos compounds are Brodan, DOWCO 179, Eradex, Lorsban, and Pyrinex. Since most of the experimental work on chlorpyrifos was carried out in the 1960s and the initial developmental name was DOWCO 179, most of the early studies use the name DOWCO 179 (97% chlorpyrifos). The first US registration was in September 1965 and a patent was assigned to DOW in 1966 under the trade names of Dursban for domestic use products and Lorsban for agricultural uses.

The chemical has a very wide range of uses. It is used to control insect pests on a wide range of food and feed crops. It is registered for use as a direct application to pets and various meat-producing animals. It is also used for treatment of animal quarters, domestic dwellings, storage bins, ornamentals, lawns and turf, and terrestrial structures (termiticide).

Chlorpyrifos has been formulated with various other insecticides including pyrethrins and pyrethroids, parathion-methyl, diazinon, dichlorvos and others. The applications to food/feed crops may be dormant, delayed dormant, foliar, seed treatments, or direct at-planting soil applications.

Toxicity studies have also been carried out using TCP (3,5,6-trichloro-2-pyridinol) which is the major metabolite (degradation product) of chlorpyrifos.

Existing Chemicals Review Program

Chlorpyrifos is one of some 80 agricultural and veterinary chemicals identified as candidates for priority review under the ECRP. Following data call-in processes, a number of additional data submissions on the toxicology of chlorpyrifos have been received from industry and the public. These data, together with all previously submitted data have been evaluated and are detailed in the report below.

1.2 International Toxicology Assessments

WHO/FAO

The toxicology of chlorpyrifos has been reviewed internationally by the Joint WHO/FAO Meeting on Pesticide Residues (JMPR).

The JMPR originally established the ADI in 1973 at 0.0015 mg/kg bw/day, based on the NOAEL (no observed adverse effect level) of 0.015 mg/kg bw/day observed for plasma cholinesterase in a human study. Subsequent statistical analysis of the results of this study by the sponsors indicated a revised NOAEL for plasma cholinesterase inhibition to be at 0.03 mg/kg bw/day which forms the basis for the establishment of the current Australian ADI.

At the 1982 JMPR the use of plasma cholinesterase inhibition as an end-point for toxicity was discontinued. The Meeting noted that the NOAELs for red-cell erythrocyte cholinesterase inhibition were without significant effects on mortality, behaviour, food consumption or growth, haematology, clinical chemistry, urinalysis, or gross and microscopic examination of tissues (where determined). As a consequence, the 1982 JMPR revised its ADI to 0-0.01 mg/kg bw/day based on the NOAEL for red-cell erythrocyte cholinesterase inhibition in the human study.

The JMPR reviewed the toxicology of chlorpyrifos in 1999, and affirmed this ADI on the basis of the NOAEL of 1 mg/kg bw/day for inhibition of brain cholinesterase activity in studies in rats, mice and dogs using a 100-fold safety factor, and on the NOAEL of 0.1 mg/kg bw/day for inhibition of erythrocyte cholinesterase activity in the study of human subjects exposed for nine days using a 10-fold safety factor. The Meeting also allocated an acute reference dose of 0.1 mg/bw on the basis of the NOAEL of 1 mg/kg bw for inhibition of erythrocyte cholinesterase activity in a study in which volunteers received a single oral dose of chlorpyrifos and with a safety factor of 10.

US EPA

In June 1997, the USA registrants of chlorpyrifos agreed to implement measures designed to reduce

household exposure to chlorpyrifos, as part of a negotiated risk reduction plan. This plan involved deletion of indoor broadcast use, use as an additive to paint, direct application to pets (sprays, shampoos and dips), and indoor total-release foggers.

In addition, the registrants have implemented the following measures:

- revised labels for safer termiticide and pet care products;
- accelerated education and training for pest control operators (PCO's) to reduce risk
- and exposure,
- label improvements;
- undertaken epidemiological research and established a Blue Ribbon Panel to provide scientific direction for study design for chlorpyrifos; and
- continued the Poison Control Center Stewardship Project (University of Minnesota) for chlorpyrifos to monitor incident reporting related to chlorpyrifos. This includes follow-up on the identity of products and the circumstances responsible for exposure.

In October 1999, the US EPA released a revised preliminary health effects assessment and a preliminary ecological risk assessment on chlorpyrifos for public comment.

The preliminary health effects assessment for chlorpyrifos proposed an acute dietary reference dose (ARfD) of 0.005 mg/kg/d based on a no-observed adverse effect level (NOAEL) of 0.5 mg/kg/d from an acute oral rat blood time-course study, and using a 100-fold safety factor. The chronic RfD (analogous to the ADI) was proposed to be 0.0003 mg/kg bw/d based on an oral NOAEL of 0.03 mg/kg/d from a 2-year dog study, and using a 100-fold safety factor.

These figures are considerably lower than the corresponding values recommended in the ECRP report, due in part to the US EPA decision not to use the available human studies to derive these dietary intake standards.

In June 2000, the EPA released its revised risk assessment document for chlorpyrifos. In this revised assessment, the Agency decided to use an additional 10x safety factor due to concerns for increased sensitivity of infants and children, as part of their Food Quality Protection Act (FQPA) evaluation (instead of the aditional 3-fold factor used in their 1999 assessment). As a result, the chronic RfD remains at 0.0003 mg/kg bw/d and the acute RfD remains at 0.005 mg/kg bw/d, each based on NOAELs from animal studies and using a 100x safety factor for inter-species extrapolation and intra-species variability. However, the chronic Population Adjusted Dose (cPAD) for infants, children and women aged 13-50 years is 0.00003 mg/kg bw/day, and the acute PAD (aPAD) is 0.0005 mg/kg bw/d, as a result of this additional FQPA safety factor.

To reduce the risks from chlorpyrifos, the registrants in the USA have negotiated with the EPA and are withdrawing most residential and recreational uses of chlorpyrifos, and a number of changes in agricultural usage are being modified or removed to mitigate acute dietary risk concerns.

Other countries

In the United Kingdom, the Advisory Committee on Pesticides (ACP) considered the human health

review of chlorpyrifos in July 2000. The Committee recommended that agricultural uses of chlorpyrifos should continue whilst the following data were obtained: Information on the pattern of usage and work rates for both contractor and farm operators, and a four-week dog study measuring cholinesterase in peripheral tissues as well as in erythrocytes and the brain.

The Committee recommended that off-label approval for use as a bulb dip should be revoked unless a suitable protocol for an operator exposure study was provided within 8 weeks and that the amateur home-garden uses of chlorpyrifos should be revoked because sufficient data on the exposures of users had not been provided. The revocation would be to normal Pesticide Safety Directorate (PSD) timescales. The uses of chlorpyrifos that are regulated by the Health and Safety Executive (HSE) will be examined at a later date.

The European Union (EU) selected chlorpyrifos as one of about 90 compounds to be reviewed on a priority basis in 1994. Spain is the rapporteur country for this assessment, but it is not clear when the EU review will become available.

1.3 Chemistry

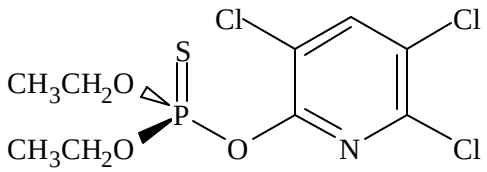
Chemical Name: 0,0-diethyl-0-3,5,6-trichloro -2-pyridyl phosphorothioate (IUPAC)
0,0-diethyl 0-(3,5,6-trichloro-2-pyridinyl) phosphorothioate (CA)

Common Name: chlorpyrifos

Trade Names: Dursban FM, Dursban R, Dursban XP, Dursban FE

CAS No: 2921-88-2

Structural Formula:



Empirical Formula:	$C_9H_{11}Cl_3NO_3PS$
Molecular Weight:	350.6
Physical State:	White crystalline solid
Odour:	Mild mercaptan
Water Solubility:	0.00012g/100g
MP	42-43.5°C
VP	2.7 mPa (1.87×10^{-5} mm Hg) (25°C)
Kow	50000-129000
Solubility mg/L (25°C):	water: 1.4
	benzene: 7900
	acetone: 6500

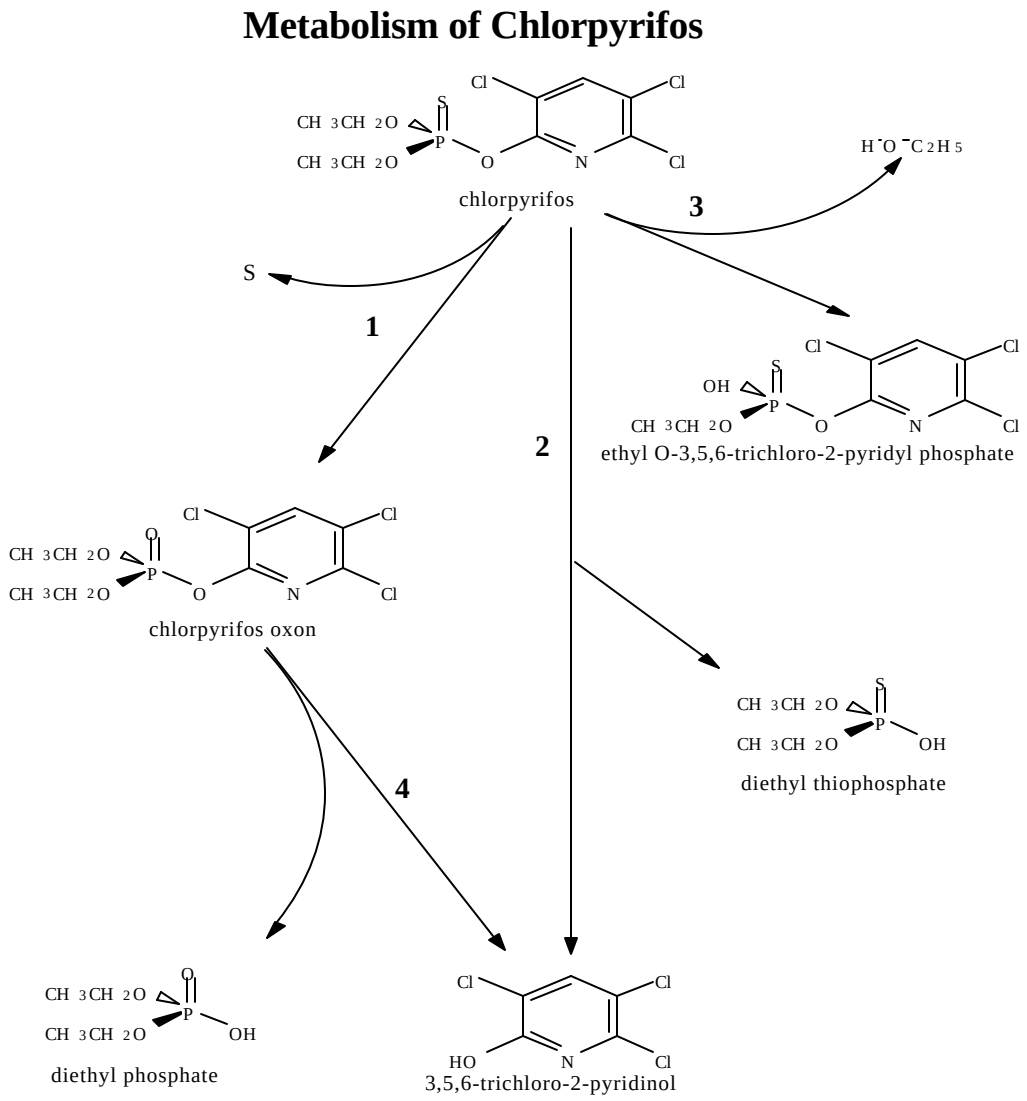
chloroform: 6300
carbon disulfide: 5900
diethyl ether: 5100
xylene: 5000
isooctanol: 790
methanol: 450

Density: 1.4 g/cm³ at 43°C.

2. METABOLISM AND TOXICOKINETICS

Studies reviewed in this section include investigations of 3,5,6-trichloro-2-pyridinol (3,5,6-TCP) which is the major metabolite and degradation product of chlorpyrifos, chlorpyrifos-methyl and triclopyr.

A generalised metabolic pathway for chlorpyrifos is shown below.



Overview

Introduction: Chlorpyrifos, like many of the most commonly used organophosphorus (OP) insecticides is a phosphorothionate, and as such, is a relatively weak anticholinesterase. Phosphorothionate insecticides are bioactivated by the microsomal cytochrome P-450 system(s) within the bodies of vertebrates and insects to their active oxon (phosphate ester) metabolite, which are about three orders of magnitude more potent as anticholinesterases. The bulk of bioactivation takes place in the liver, while

detoxification takes place in the liver and plasma. TCP has insignificant anti-cholinesterase activity and is not regarded as toxicologically significant, whereas chlorpyrifos oxon is a potent cholinesterase inhibitor.

Absorption, Distribution, Metabolism: A fourth of the cardiac output passes through the liver (Cahalan & Mangano, 1982), which, combined with the high activities of hepatic biotransformation enzymes, ensures the primacy of the liver as the major controller of the systemic level of chlorpyrifos and other toxicants. In oral exposures, essentially all of the dose passes through the liver before entering the systemic circulation (Nakatsugawa, 1992). Chlorpyrifos molecules are mostly bound to macromolecules in the blood and as the blood enters the liver there is an equilibrium established between the free and bound molecules in the blood and in the parenchymal liver cells. Extrapolating from Nakatsugawa (1992), it is estimated that the chlorpyrifos molecules may have up to 4 min of interaction with the hepatocytes before reaching the centrilobular vein. An incoming dose may well be totally consumed by biotransformation processes before leaving the liver circulation, as the chlorpyrifos oxon generated by the activation pathway is rapidly sequestered by the hepatic aliesterases. It is when the dose is sufficiently high to allow chlorpyrifos or its oxon to appear in the post-hepatic circulation that toxicity symptoms first appear. Recent work in perfused rat liver has found that chlorpyrifos oxon may exit the liver in sufficient quantity to reach important extra-hepatic sites (Sultatos, 1991). Other routes of administration may lead to systemic circulation of chlorpyrifos and its bioactivation in a variety of target tissues.

Chlorpyrifos is rapidly metabolised by mixed function oxidases to the highly reactive chlorpyrifos oxon via oxidative desulphuration (step 1). The oxidative metabolism involving concurrent activation and a degradation via a common intermediate, appears to be a general scheme for P=S esters (Nakatsugawa et al, 1968; Wolcott et al, 1972; Yang et al, 1971). The oxidative desulfuration is believed to proceed via an electrophilic phosphooxathiiran intermediate (Kamataki et al, 1976). This reactive intermediate can "suicidally" inactivate cytochrome P450 and possibly, covalently bind to other tissue nucleophiles. (Halpert & Neal, 1980). The degradation step is conversion directly to TCP and diethyl thiophosphate (step 2). The highly reactive oxon can be deactivated by hydrolysis of the oxon to diethylphosphate and 3,5,6-trichlorophenol (step 4) (Ma & Chambers, 1994; Sultatos & Murphy, 1983). A minor reaction pathway is hydrolysis to monethyl 3,5,6-trichloro-2-pyridinol phosphorothioate (step 3).

Hydrolysis is the most important route of detoxification of organophosphorus esters. Hydrolytic esterases are distributed ubiquitously in the blood and tissues of virtually all organisms, and catalyse the hydrolysis of a variety of esters including organophosphorus esters, but have little activity on the OP itself. Esterases which interact with OPs have been characterised as A- or B-esterases according to their sensitivity to inhibition by organophosphorus compounds. Plasma and tissues of mammals have significant levels of the calcium activated A-esterases, arylesterase (EC 3.1.1.2) and the high density lipoprotein associated paroxonase (EC 3.1.8.1), which are not inhibited by the substrate. The B-esterases, including aliesterases (EC 3.1.1.1) (also called carboxylesterases) and cholinesterases (eg. butyryl cholinesterase BuChE, EC 3.1.1.8) are inhibited by OP substrates such as chlorpyrifos oxon, and while binding them, will not hydrolyse them (Derelanko & Hollinger, 1995).

In human serum, BuChE is the predominant cholinesterase (>99%), while in the rat there is an approximately equal distribution of AChE and BuChE (Wilson et al; 1995 cited in Nolan, 1997). Detoxification via these enzymes is via the sequestration of OPs by binding or phosphorylation to/by

blood proteins, such as albumin, serum ChE (BuChE), or erythrocyte (RBC) acetyl cholinesterase, which stoichiometrically degrades them (Aldridge, 1953). Such protein bound organophosphorus esters are secreted into the bile. The RBC AChE (EC 3.1.1.7) is similar to the AChE in nervous systems and can bind and sequester, or hydrolyse OPs. The enzyme activities attributable to A-esterases, B-esterases and cholinesterases vary widely within populations, and are influenced by genetic and environmental factors and disease states (Derelanko & Hollinger, 1995).

Excretion: Chlorpyrifos is rapidly cleared from the tissues and eliminated primarily in the urine as TCP and its glucuronide. These hydrolytic products are much more soluble than chlorpyrifos and are rapidly eliminated; this supports the observed low bioaccumulative ability of the parent compound and its products. Oral doses have plasma clearance half-lives of <24h in rats.

Species and sex differences: Rabbits have serum levels of paraoxonase some 40 times greater than the rat and a substantially higher LD50 (2000 mg/kg vs 118-245 mg/kg) following equivalent dosing with chlorpyrifos (McCollister et al., 1974). Most avians including hens have relatively low levels of A-esterases, and a concomitant lower LD50 (15 mg/kg) for chlorpyrifos (Capodicasa et al., 1991). Humans have a mean value of serum chlorpyrifos oxonase activity 10 times that of rat serum (Furlong et al., 1989).

In humans, a substrate dependent polymorphism of serum paraoxonase is observed, where one isoform of paraoxonase has a high turnover number for paraoxon and the other a low turnover number (Furlong et al, 1989; Smolen et al., 1991). The polymorphism is also observed with the oxons of methyl parathion, chlothion, and EPN. However both isoforms appear to hydrolyse chlorpyrifos-oxon and phenylacetate at the same rate. Cloning and sequencing of the human paraoxonase cDNAs has elucidated the molecular basis of the polymorphism; arginine at position 192 determines high paraoxonase activity, and glutamine at this position, low paraoxonase activity (Humbert et al, 1993). In addition to this polymorphism, a 13-fold variation in serum enzyme levels within a given genetic class is seen in humans (Furlong et al, 1989).

Toxicity and Anticholinesterase Activity: The major determinant of OP toxicity appears to be the sensitivity of AChE to inhibition by the active compounds or their metabolites, such that there is a general correlation between the inhibition of AChE and species sensitivity to the toxic effects of OPs. The inhibitor binds with a serine residue of AChE to form a reversible complex. The ensuing phosphorylation of the enzyme occurs rapidly and is followed either by rapid hydrolysis and dephosphorylation, which restores the intact enzyme, or "ageing" of the phosphorylated site by O-dealkylation, which markedly inhibits this reactivating hydrolysis (Wallace, 1992). The balance between reactivation and ageing is an important determinant of species and individual sensitivity to OPs.

Chlorpyrifos is only moderately toxic when compared to some other phosphorothionates such as parathion. This may relate to the hydrolytic detoxification of the oxon by A-esterases such as paraoxonase such that high serum levels of paraoxonase may be protective against poisoning by those organophosphorus insecticides whose active metabolites are paraoxonase substrates (LaDu and Eckerson, 1984; Omenn, 1987; Geldmacher-von Mallinckrodt and Diepgen, 1988). This has been directly demonstrated in rats, where injection with purified paraoxonase prior to dosing with chlorpyrifos oxon greatly reduced brain AChE inhibition in these animals compared to controls (Costa et al., 1990).

Initial work has correlated the oral LD50 values of chlorpyrifos in rats with the activation rates measured in brain rather than liver (Chambers, 1992), which suggests that local biotransformation in target tissues may be an important determinant of the toxicity profile. However, a more recent review by Chambers & Carr (1995) compared published LD50 or LC50 levels for a variety of insecticides in several vertebrate species. Studies in rats indicated that brain AChE sensitivity to inhibition by various phosphorothionate oxons did not correlate with acute toxicity levels. Chlorpyrifos oxon has a greater affinity for rat brain AChE than paraoxon (I_{50}s of 4.0 and 22.5 nM respectively), but a lower LD50. This data is however consistent with the greater affinity (I_{50}s 0.75 nM) of chlorpyrifos oxon for plasma aliesterases than for rat brain AChE, indicating that aliesterases provide protection against chlorpyrifos oxon.

Further work by Chambers and Carr (1995) indicated a more prolonged inhibition of esterases following chlorpyrifos exposure when compared to parathion. This fact was attributed to the high lipophilicity of chlorpyrifos compared to parathion (Hexane:acetonitrile partition coefficients 0.285 and 0.062 respectively), and the assumption that a substantial fraction of the chlorpyrifos dose would have been sequestered by fat, and released gradually for later bioactivation. Studies of hepatic microsomal metabolism of parathion and chlorpyrifos indicated that desulfuration of parathion was favoured over dearylation (activation vs deactivation), whereas the reverse was seen for chlorpyrifos (Ma & Chambers, 1994). However, in the channel catfish, the acetylcholinesterase sensitivity to oxon inhibition reflects the acute toxicity level of these same insecticides, and may be largely responsible for determining the acute toxicity level in this species. Thus, metabolism of insecticides appears to be far more influential in some species than others in determining the toxicity elicited.

2.1 Rat

Smith GN, Watson BS & Fischer FS (1967) Investigations on Dursban insecticide. Metabolism of [^{36}Cl] O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothiate in rats. Agric Food Chem 15: 132-138, 1967.

Male Wistar rats (ca. 4 months old, bw. 200 g) were given single oral doses of ^{36}Cl-chlorpyrifos (activity 0.029 mCi/mmole, labelled at the 3- and 5-position) by stomach tube. Chlorpyrifos was dissolved in refined corn oil to give a solution containing approximately 10 mg/ml of the labelled insecticide. Each animal was given 1 ml of the corn oil solution. At various time intervals (4, 24, 48, 96, 168, 240 h post-dosing), the animals were sacrificed in groups of two and the blood and tissues analysed.

Results

Initially (at 4 h) liver and kidney had the highest level of radioactivity (respectively ca. 0.069 and 0.092 mmoles radioactive compound/kg of tissue). Other organs to show measurable amounts of radioactivity were (in ppm in descending order of concentration) lung (0.041), fat (0.032), heart (0.029), skin (0.024), spleen (0.021), testes (0.016), bone (0.010) and muscle (0.009). At 72 h post-dosing, fat tissue contained the highest level of radioactivity (0.005), which was approximately 3 times the kidney and 4 times the liver concentrations at that time. The biological half-life in fat was the longest (62 h) for all the tissues studied, with liver, kidney and muscle showing half-lives for radioactivity of 10, 12 and 16 h, respectively. The majority of the activity was eliminated in the urine (90%) and faeces (10%) indicating

that the compound was readily absorbed from the gastrointestinal tract. Data indicates that three compounds were present in the urine and faeces: 3,5,6-TCP phosphate (75-80% of the recovered radiolabel), 3,5,6-TCP (about 20-25%) and chlorpyrifos (trace quantities).

Branson DR & Litchfield NH (1971a) Absorption, excretion and distribution of O, O-diethyl O-3,5,6-trichloro-(2, 6-C^{14})-2-pyridyl phosphorothioate in rats. Dow Chemical USA. Report No.: NBA-9. Report dated April 23, 1971 [Dow; Submission 939 (1988) Part 4, pps 4.24-4.35. Dow; Submission 238 (1987) Part 4, pps 4.29-4.39]

Two male Sprague-Dawley rats (bw. 200 g) were given single oral (stomach tube) doses of ring-labelled 0,0-diethyl 0-3,5,6 (2,6-^{14}C)-trichloro-2-pyridyl phosphorothioate (chlorpyrifos), administered in 0.3 ml (19 mg/kg) of corn oil. Samples of blood (1, 3, 5, 7, 9, 11, 13, 24, 48, 72 hrs), urine (13, 24, 48, 72 h) and faeces (24, 48, 72 h) were collected at intervals (indicated above) after treatment commenced. The CO_2 in respired air was collected daily. At necropsy, selected tissues and the carcass were analysed for radioactivity by the acid combustion method for total combustible $^{14}CO_2$.

Results

The analyses of eliminated radioactivity were reported as equivalents of the radiolabelled parent compound and not as metabolites. The total radioactivity recovered was 85-89% of the administered dose. Radioactivity peaked in the blood at 3 h (3% of total dose) and declined steadily to 24 h. By 72 h, 83-87% of the total radioactive dose had been eliminated, mainly in the urine (68-70%, mainly TCP), faeces (14-15%) and respired air (0.15-0.39%). Residues at 72 h were ca. 1.7%. These 72 h tissue residues were low (less than 1 ppm) especially in brain (0.007 ppm) and highest in the fat (0.75 ppm).

Nolan RJ, Streeter CM & Kastl PE (1986) Chlorpyrifos: Absorption by female Fischer 344 rats exposed for 6 hours to vapours of chlorpyrifos in a nose-only or a whole-body inhalation chamber. Dow Chemical Company. Laboratory Report Code HET K-044793-82, dated 12 November, 1986.

Segment 1: In the first segment of this study, four groups of female Fischer 344 rats (four/group; Charles River Breeding Laboratories, USA; 6-8 weeks of age prior to laboratory acclimatisation) were used. Two groups were administered single doses of chlorpyrifos (Dow Chemical Company, USA; purity 100%; batch not stated) via oral gavage, at doses of 0.084 or 4.48 mg/kg body weight. Prior to dosing, the chlorpyrifos was dissolved in USP corn oil to produce concentrations of 0.045 and 2.31 mg chlorpyrifos/ml, and a dosage volume of 2 ml/kg body weight was used. The remaining two groups of rats were exposed to chlorpyrifos by inhalation for six hours, either by whole-body (6.6 ppb/94.5 µg/m^3) or by nose-only (23.5 ppb/338µg/m^3) exposure. The nose-only exposures were conducted in a commercially-supplied apparatus, while the whole body exposures were conducted in a 1 m^3 stainless-steel/glass chamber. Animals were placed in metabolism cages following exposure, and urine was collected for 48 h post-treatment for analysis.

Segment 2: A group of four female rats was exposed for six hours to 14 ppb chlorpyrifos (202 µg/m^3) in a nose-only exposure chamber. A second group of four female rats was given a single oral gavage dose of ^{14}C-chlorpyrifos at a dose of 0.93 mg/kg body weight, using a dose volume of 2 ml/kg of a corn oil solution containing 0.50 mg and 10.2 µCi of radiolabelled chlorpyrifos. Animals were placed in glass

metabolism cages after treatment, and urine, faeces and cage wash was collected for 48 h for analysis.

Results

No clinical signs of intoxication were observed during the study. Following the oral administration of non-radiolabelled chlorpyrifos to female rats in Segment 1 of this study, the mean recovery of 3,5,6-trichloro-2-pyridinol (3,5,6-TCP) from the urine after 48 h ranged from 29% of the administered dose of test material when 0.084 mg/kg chlorpyrifos was administered, to 33% of the administered dose when 4.48 mg/kg of chlorpyrifos was administered. The intra-group variation was considerable at the lower dose, and metabolite recovery for individual animals ranged from 15-46% of the administered dose. In Segment 2 of the study, the recovery of radiolabel from the urine was 79% of the administered dose following oral administration. The total combined recovery of radiolabel from urine, faeces, and cage-wash was 99.8% of the administered oral dose.

Analysis of urine from rats exposed to chlorpyrifos in Segment 1 of the study indicated that the average urinary excretion of 3,5,6-TCP following the whole-body and nose-only inhalation exposures was 3.86 and 10.3 µg of the metabolite, respectively, which are equivalent to 0.58 and 0.48 µg of 3,5,6-TCP per ppb of chlorpyrifos in air, on an exposure-adjusted basis. For the Segment 2 nose-only exposure, urinary excretion was 3.85 µg of 3,5,6-TCP, or 0.28 µg of 3,5,6-TCP per ppb of chlorpyrifos in air, on an exposure-adjusted basis.

Whole-body exposed rats (Segment 1) excreted similar amounts of 3,5,6-TCP in their urine (approximately 3.85 µg) when compared to nose-only exposed rats (Segment 2), but the whole-body exposed animals were exposed to half the chlorpyrifos concentration (6.6 ppb vs 14.0 ppb). The whole-body-exposed rats absorbed more chlorpyrifos than would be expected to occur by the inhalation route alone. Grooming activity by rats probably contributed to the larger oral intake of chlorpyrifos in animals exposed in whole-body chambers.

Branson DR & Litchfield NH (1970) Comparative absorption, elimination and distribution of DOWCO 179, its methyl analog DOWCO 214 and their major metabolite, 3,5,6-trichloro-2-pyridinol. Dow Chemical USA, CRI No.: 70 4479. Report dated 30 September, 1970. [Dow; Submission 939 (1988) Part 4, 4.11-4.23]

Branson DR & Litchfield NH (1971b) Absorption, excretion and distribution of 3,5,6-trichloro-2, 6-C^{14}-2-pyridinol in rats. Dow Chemical USA. Report No.: NBA-10. Report dated April 23, 1971 [Dow; Submission 939 (1988) Part 4, pps 4.24-4.35. Dow; Submission 238 (1987) Part 4, pps 4.40-4.51]

In this study, ^{14}C-ring labelled chemicals were administered orally to rats, and the radioactivity in each set of samples was used to compare the relative rates of absorption, and the proportion of the dose eliminated and retained as a residue, as well as the distribution of the residue in selected tissues. The labelled chemicals were chlorpyrifos (0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate or DOWCO 179), methyl chlorpyrifos (0,0-dimethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate or DOWCO 214) and the common metabolite of these compounds, 3,5,6-trichloro-2-pyridinol or 3,5,6-TCP.

Labelled ^{14}C-3,5,6-TCP (specific activity 10.2 mCi/mmole, purity >99%) was used to synthesise both the chlorpyrifos and methyl chlorpyrifos used in these tests. The test compounds were administered in corn oil (0.3 ml gavage doses) to groups of 2 male Sprague-Dawley rats as single oral doses which amounted to a total dose of 3.8 mg for chlorpyrifos, 3.2 mg for methyl chlorpyrifos and 1.4 mg for ^{14}C-3,5,6-TCP. After dosing, the pre-acclimatised rats were placed in metabolism cages for 72 h to enable separate collection of urine, faeces and expired air. Tail-tip blood samples were taken at 1, 3, 5, 7, 9, 11, 13, 24, 48 and 72 h post treatment. Expired air CO_2 and faecal samples were collected at 24-h intervals, while urine samples were collected at 13, 24, 48 and 72 h post treatment. At 72 h, the rats were necropsied and radioactivity determined in brain, heart, lungs, diaphragm, stomach, intestines, spleen, liver, kidneys, adrenals, testes, fat, thymus, bone, muscle, skin and carcass. Skin radioactivity was apportioned by measurement into the dermal, subcutaneous fat and hair compartments.

Results

The blood-level peaks for 3,5,6-TCP, chlorpyrifos and methyl chlorpyrifos occurred at 1, 3 and 6 h respectively. At 72 h, most of the radioactivity in the blood was associated with the serum rather than the erythrocytes. Urinary metabolites of 3,5,6-TCP, chlorpyrifos and methyl chlorpyrifos were almost exclusively 3,5,6-TCP. The biological half-life of each chemical was estimated as between 8-17 h. Residue levels were uniformly low in the brain. The highest residue levels occurred in the bone and intestine following 3,5,6-TCP administration, in fat and intestine following chlorpyrifos administration, and in bone, fat and carcass following methyl chlorpyrifos administration. Generally, the residue levels of methyl chlorpyrifos were lower than the chlorpyrifos levels.

Chlorpyrifos and derivatives: elimination and residues in the rat

	Chlorpyrifos	**Methyl chlorpyrifos**	**3,5,6-TCP**
Total dose (mg)	3.8	3.2	1.4
total eliminated at 72 h	83-87%	90-94%	77-81%
eliminated as urine	68-70%	83-85%	73-76%
eliminated as faeces	14-15%	7-9%	6-7%
eliminated as CO_2	0.14-0.89%	0.23-0.43%	0.34-0.63%
maximum residue remaining at 72 h	1.7%	1.0%	1.8%

Administration of a single oral dose of ^{14}C- chlorpyrifos, 3,5,6-TCP, or methyl chlorpyrifos did not indicate accumulation of these compounds in rat tissues.

Nolan RJ, Dryzga MD, Landenberger BD & Kastl PE (1987) Chlorpyrifos: Tissue distribution and metabolism of orally administered ^{14}C labelled chlorpyrifos in Fischer 344. Dow Chemical Company. Laboratory Report Code HET K-044793-(76), dated 23 December, 1987.

An expert panel convened by the Sponsor Company reviewed this study. The report of that expert panel is reproduced here, with minimal alteration.

Groups of 5 Fischer 344 rats/sex (food withdrawn for 14-18 h pre-dosing) were administered ^{14}C-labelled chlorpyrifos (>99% radiochemical purity) as a single oral dose of 0.5 or 25 mg/kg, or as 15 consecutive daily doses of 0.5 mg/kg/d of unlabelled chlorpyrifos, followed on day 16 by 0.5 mg

^{14}C-chlorpyrifos/kg. Urine and faeces were collected post dosing for 3 days from males, and for 6 days from females. Rats were sacrificed after collection of excreta was completed and radiolabel levels were measured in the bone, brain, perirenal fat, gonads, heart, kidney, liver, lung, blood, skeletal muscle, spleen, skin and carcass.

Radioactivity recovery was 96.8-98.5% of the administered dose, mainly in the urine (83.9-91.7% of administered dose), with 5.5-11.5% in faeces. At sacrifice, 0.01% of the 0.5 mg/kg dose (single or repeated doses, both sexes) and 0.2% of the 25 mg/kg dose remained in the tissues or the carcass.

The only difference in elimination of radioactivity, regardless of sex or dosage, was a slight increase (6-7%) in urinary excretion from multiple doses as compared to a single dose at the 0.5 mg/kg/d dose level.

Tissue residues of radioactivity occurred in perirenal fat of all test group males (≤0.14% of administered dose/g) and in liver of males and in the ovaries and fat of females administered 25 mg/kg bw. In all other tissues residues were not detected in sufficient quantities to be quantified (≤0.01% dose/g).

During the 3-day post-exposure period after single and multiple doses of 0.5 mg/kg, the half-life for ^{14}C excretion was 8-9 h compared to 12.4 h for males and 23.2 h for females at 25 mg/kg. These discrepancies probably reflect differences in absorption rates, since there were no indicators of saturation kinetics (eg. dose-dependent changes in metabolites or in routes of excretion). Females continued to excrete 1.2-1.4% of the 0.5 mg/kg dose and 4.7% of the 25 mg/kg dose over the 3-6 day post-dosing period. Most radioactivity excretion (both sexes) occurred in the 24 post-dosing period, the lower dose being more rapidly excreted.

Analysis of urine for metabolites failed to reveal any unchanged chlorpyrifos. The major metabolites were 3,5,6-trichloro-pyridinol (12%), its glucuronide conjugate (80%) and tentatively, a sulphate conjugate.

2.2 Poultry

Dishburger HJ, McKellar RL & Wetters JH (1972) Residues of chlorpyrifos and 3,5,6-trichloro-2-pyridinol in tissues and eggs from chickens fed chlorpyrifos. The Dow Chemical Co. Report No. GH-C 555, dated May 31, 1972 [Dow; submission 939, November 1988, part 4, vol 1, pp 4.250-4.284. Dow; Submission 238 (1987) Part 4, 4.408-4.442]

Laying hens (Hyline) were divided into 8 groups of 36 birds each. The hens were fed chlorpyrifos (Lot CP523-CD235C, 97.2% pure) at 0, 0, 0.3, 1, 3 10, 10 and 10 ppm in their diet for 30 days, at which time all the hens in the two control groups and 24 hens from each of the dose groups were sacrificed. Two groups fed 10 ppm for 30 days were sacrificed after 7 and 21 days on untreated diet. For residue analysis, eggs were collected from twelve hens fed treated diet for a total of 45 days at each dose level. Residues of chlorpyrifos and 3,5,6-trichloro-2-pyridinol were measured in the eggs and samples of muscle (with fat and skin), liver, kidney and peritoneal fat from all sacrificed hens by gas chromatographic methods, with a validated lower level of sensitivity of 0.01 ppm for chlorpyrifos and 0.05 ppm for 3,5,6-trichloro-2-pyridinol. The efficiency of the extraction method was determined from spiked samples of both residues. The extraction procedure showed average recoveries of >83% and tissue values were corrected for this efficiency. None of the animals displayed clinical signs of toxicity

during the treatment period.

Chlorpyrifos residues were not detected in any tissue except fat, where the levels were <0.1, 0.03 ppm and non-detectable, at feed levels of 3 ppm, 10 ppm and 10 ppm plus 7 days recovery, respectively. 3,5,6-TCP residues were recorded in liver (0.15 ppm) and kidney (0.33 ppm) at the 10 ppm feed-level; again, there were no residues detectable at 7 days withdrawal. The only residues recorded in eggs (<0.05 ppm) were seen at 10 ppm in the feed.

McKellar RL & Dishburger HJ (1974) Determination of residues of 3,5,6-trichloro-2-pyridinol in tissues of cattle, swine, chicken and eggs following alkaline hydrolysis. The Dow Chemical Co. Report No. GH-C 733, dated April 2, 1974 [Dow; submission 939, November 1988, part 4, vol 1, pp 4.285-4.298. Dow; submission 238, part 4 vol 1, pp 4.394-4.407]

Residues of chlorpyrifos and 3,5,6-TCP were determined in muscle, liver, kidney and fat of cattle, swine, chicken and eggs both with and without alkaline hydrolysis during the extraction procedures. The data showed no significant increase in residues of chlorpyrifos or 3,5,6-TCP after alkaline hydrolysis. It was concluded that chlorpyrifos and 3,5,6-TCP were not bound in animal tissues, and that the standard methanol extraction method was sufficient to remove all residues from tissues for analysis.

The results reported by the authors of this study on extraction technique are reproduced without alteration, and no detailed evaluation was reported.

2.3 Goat

Glas RD (1981a) The metabolic fate of ^{14}C-chlorpyrifos fed to lactating goats. The Dow Chemical Co. Study No.: GH-C 1408; dated February 12, 1981. [Dow; submission 238, part 4, vol 1, pp 4.127- 4.176]

Two goats were fed ^{14}C-ring labelled (positions 2 and 6) chlorpyrifos orally by capsule for 10 days. The doses were administered twice daily at a level equivalent to 15 to 19 ppm in the feed. Recovery of administered radioactivity averaged 85.6%. The majority (80.3%) of the total ^{14}C activity was recovered in the urine with smaller amounts in faeces (3.6%), gut (0.9%), tissues (0.8%), and milk (0.1%).

Analysis of the urine showed that most of the radioactivity was excreted as the ß-glucuronide conjugate of 3,5,6-trichloro-2-pyridinol with smaller amounts of free 3,5,6-trichloro-2-pyridinol and a minor component tentatively identified as S-ethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioic acid.

Analysis of the fat showed approximately 75% of the ^{14}C activity to be chlorpyrifos (0.12 ppm) with most of the remaining ^{14}C activity hydrolysable to 3,5,6-TCP

Liver and kidney tissues showed 3,5,6-trichloro-2-pyridinol to be the major metabolic product with minor amounts of chlorpyrifos and very low levels of unidentified metabolites. Greater than 94% of the ^{14}C-labelled residue in these tissues could be hydrolysed to 3,5,6-trichloro-2-pyridinol. No evidence was found of any metabolites with alterations in the pyridinol ring.

The results presented by the authors of this study in this summary report have been verified as

accurate, and the report was reproduced with only minor alterations. No detailed evaluation was reported.

Glas RD (1981b) Identification of ^{14}C-labelled residues in milk from goats fed ^{14}C-chlorpyrifos. The Dow Chemical Co. Study No.: GH-C 1470; dated October 9,1981. [Dow; Submission 238, part 4, vol 1, pp 4.177- 4.190]

Two goats were fed ^{14}C-ring labelled chlorpyrifos orally via capsule for 10 days. The doses were administered, after milking, twice daily at a level equivalent to 16 to 21 ppm in the feed. Recovery of administered radioactivity averaged 85.6%. Very little ^{14}C activity was recovered in milk (0.05 to 0.14%); larger amounts were recovered in tissues (0.8%), gut (0.9%), and faeces (3.6%), with the majority of the radioactivity in urine (80.3%).

Residues of ^{14}C-labelled materials in milk were low, averaging <0.03 ppm (0.002 to 0.046 ppm) over the course of the feeding study. Analysis of selected milk samples showed greater than two thirds of the ^{14}C activity to be chlorpyrifos. The balance of the activity was shown to be 3,5,6-trichloro-2-pyridinol or materials hydrolysable to 3,5,6-trichloro-2-pyridinol. No evidence was found of any metabolises with alterations in the pyridinol ring.

The results presented by the authors of this study in this summary report have been verified as accurate, and the report was reproduced with only minor alterations. No detailed evaluation was reported.

2.4 Pigs

Bauriedel WR & Miller JH (1981). The metabolic fate of the sodium salt of ^{14}C-labelled 3,5,6-trichloro-2-pyridinol orally administered to swine. The Dow Chemical Co. Report no: GH-C 1427, dated April 27, 1981. [Dow; submission 939, November 1988, part 4 vol 1, pp 4.151-4.204]

Twelve weanling pigs of mixed sex were given daily oral doses of the sodium salt of ^{14}C-labelled 3,5,6-trichloro-2-pyridinol for 7 days at the equivalent rate of 75 ppm in the total diet. On the final day of treatment, urine samples were collected from the six male animals. The animals were sacrificed in groups of three and tissues were obtained for analysis at withdrawal periods of 8 h, and 1, 3 and 7 days. Urine and tissue samples were examined for total ^{14}C activity and identification of the ^{14}C-labelled residues.

Results

The principal urinary excretion product was 3,5,6-trichloro-2-pyridinol, accompanied by a small amount of a polar metabolite characterised as the glucuronide conjugate of the pyridinol. The liver and kidney contained the highest initial residues, and the liver exhibited the slowest rate of clearance. The ^{14}C-containing residue in the liver, kidney, skeletal muscle, heart muscle and fat tissues consisted primarily of 3,5,6-trichloro-2-pyridinol. These residues decreased rapidly after withdrawal, falling to below 0.1 ppm sodium 3,5,6-trichloro-2-pyridinate equivalent by 3 days. The liver tissue contained the highest residue at the later time periods.

Comment: The above study summary was provided by the registrant; it is in accord with the reported data and was presented with only minor changes.

McKellar RL, Wetters JH & Dishburger HJ (1972) Residues of chlorpyrifos and 3,5,6-trichloro-2-pyridinol in tissues of swine fed chlorpyrifos. The Dow Chemical Company. Report No.: GH-C549, dated April 28, 1972 [Dow; submission 939, November 1988, part 4 vol 1, pp 4.205-4.229; Dow; submission 238, part 4 vol 1, pp 4.191-4.215]

Groups of 3 pigs (2 male, 1 female; Landrace, ca. 23 kg bw.) were fed basal rations containing 0, 1, 3 or 10 ppm chlorpyrifos (Lot CP523-CD235C; 97.2% pure) for 30 days and sacrificed with no withdrawal period. Six other animals fed the 10 ppm level for 30 days were put on untreated feed and 3 animals each were slaughtered after 7 or 21 days withdrawal period. Samples of muscle, liver, kidney, omental fat, renal fat and subcutaneous fat tissues from each animal were analysed for residues of chlorpyrifos and 3, 5,6-trichloro-2-pyridinol by gas chromatographic methods with a validated lower level of sensitivity of 0.01 ppm for chlorpyrifos and 0.05 ppm for 3,5,6-trichloro-2-pyridinol. The efficiency of the extraction method was determined from spiked samples of both residues.

Results

The extraction procedure showed average recoveries of >83% and tissue values were corrected for this efficiency. None of the animals displayed clinical signs of toxicity during the treatment period.

At the end of 30 days feeding with chlorpyrifos, residues were predominantly in the fat tissues, with maximum values of 0.02, 0.04 and 0.22 ppm for 1, 3 and 10 ppm feed levels, respectively; these values declined to 0.03 ppm and non-detectable at 7 and 21 days withdrawal, respectively. Maximum values detectable in other tissues at the 10 ppm feed level were 0.03, <0.01 ppm and non-detectable, for muscle, liver and kidney respectively.

At the end of 30 days feeding with chlorpyrifos, 3,5,6-TCP residues were predominantly in the liver and kidney and maximum values for the 10 ppm feed levels were 0.32, 0.16, 0.07 ppm and non-detectable for liver, kidney, fat and muscle respectively; these values had all declined to non-detectable by 7 days withdrawal.

2.5 Cattle

Glas RD (1977a) Residues of 3,5,6-trichloro-2-pyridinol and 2-methoxy-3,5,6-trichloropyridine in tissues from calves fed 3,5,6-trichloro-2-pyridinol. The Dow Chemical Co. Study No.: GH-C 1053; dated December 1, 1977. [Dow; Submission 939 (1988) Part 4, pps 4.36-4.55]

Five groups of three calves (sex not stated, 180 kg bw.) were fed commercial cattle feed containing 3,5,6-trichloro-2-pyridinol (AGR 143197, 99.7%) *ad libitum* for 28 days, at concentrations of 0, 3, 10, 30 and 100 ppm, and then slaughtered. Three additional groups of calves fed 100 ppm for 28 days were maintained on control feed for an additional 3, 7, or 21 days before slaughter. Samples of muscle, liver, kidney, and fat were analysed for residues of 3,5,6-trichloro-2-pyridinol and 2-methoxy-3,5,6-trichloropyridine using gas chromatographic methods validated to concentrations as low as 0.05 ppm.

The storage stability of the tissue residues and the efficiency of the extraction method were determined by the use of spiked samples. Food consumption figures were not provided.

Results

The efficiency of the extraction methods for the residues ranged from 80-88%. The loss of detectable residues during storage and before analysis ranged from 0-35%. No residues of 2-methoxy-3,5,6-trichloropyridine were found in any tissues from calves fed 100 ppm of 3,5,6-trichloro-2-pyridinol.

Residue levels of 3,5,6-TCP in liver and kidney showed a clear dose relationship, with the average residue level found at the lowest dose of 3 ppm being 0.21 ppm and 0.08 ppm respectively. At the 100-ppm feeding level, 3,5,6-TCP residues averaged 0.06 ppm in muscle, 0.15 ppm in fat, 3.1 ppm in liver, and 2.0 ppm in kidney. These residue levels showed a rapid decline to < 0.05 ppm in muscle and fat at 3 days, and in liver and kidney at 7 days after the return to untreated feed.

Glas RD (1977b) Residues of 3,5,6-trichloro-2-pyridinol and 2-methoxy-3,5,6-trichloropyridine in tissues from a calf fed 2-methoxy-3,5,6-trichloro-2-pyridine. The Dow Chemical Co. Study No.: GH-C 1052; dated November 30, 1977. [Dow; Submission 939 (1988) Part 4, pps 4.56-4.75]

A single calf (sex not stated, 180 kg bw) was fed 2-methoxy-3,5,6-trichloropyridine at the rate of 1000 ppm in the diet for 4 days , during which time the food consumption was 2.3 kg/d. The diet was then remixed to 500 ppm and the calf consumed 5.5 kg/d for 24 days, which approximated a dose of 15 mg/kg/d. Samples of muscle, liver, kidney and fat were collected at the end of the feeding period and analysed for 2-methoxy-3,5,6-trichloropyridine and 3,5,6-trichloro-2-pyridinol using methods validated to concentrations as low as 0.05 ppm. The efficiency of the extraction method was determined by the use of spiked samples.

Results

The efficiency of the extraction methods for the residues ranged from 79-88%. Residues of 2-methoxy-3,5,6-trichloropyridine were very low in all tissues. The following residues were found: < 0.05 ppm in muscle, <0.05 ppm in liver, 0.05 ppm in kidney, and 0.30 ppm in fat. Residues of 3,5,6-trichloro-2-pyridinol were much higher, reaching 0.75 ppm in muscle, 8.1 ppm in liver, 8.8 ppm in kidney, and 2.0 ppm in fat.

Glas RD (1977c) Residues of triclopyr, 3,5,6-trichloro-2-pyridinol and 2-methoxy-3,5,6-trichloropyridine in bovine tissues from calves fed trichlopyr. The Dow Chemical Co. Study No.: GH-C 1047; dated November 17, 1977. [Dow; Submission 939 (1988) Part 4, 4.76-4.110]

Six groups of three calves (sex not stated, 214 kg bw.) were fed commercial cattle feed containing triclopyr *ad libitum* for 28 days at concentrations of 0, 10, 30, 100, 300 and 1000 ppm, and then slaughtered. Three additional groups of calves fed 1000 ppm for 28 days were maintained on control feed for an additional 3, 7, or 21 days before slaughter. Samples of muscle, liver, kidney, and fat were analysed for residues of triclopyr, 3,5,6-trichloro-2-pyridinol and 2-methoxy-3,5,6-trichloropyridine

using gas chromatographic methods validated to concentrations as low as 0.05 ppm. The storage stability of the tissue residues and the efficiency of the extraction method were determined by the use of spiked samples. Food consumption figures were not provided.

Results

The efficiency of recovery of the three residues in all tissues exceeded 70%, and these values were used to correct all residue values reported.

Very low levels of triclopyr were found in muscle, liver and fat. At the 1000 ppm feeding level the average residues were <0.05 ppm for muscle, 0.09 ppm for fat and 0.14 ppm for liver. Triclopyr residues in kidney from the 1000 ppm feeding level averaged 4.3 ppm. Triclopyr residues in tissues of calves returned to control feed declined rapidly, reaching a level of <0.05 ppm within 3 days for muscle, liver and fat and within 21 days for kidney.

In contrast to triclopyr, residues of 3,5,6-trichloro-2-pyridinol were much higher, averaging 0.4 ppm in muscle, 1.0 ppm in fat, 5.9 ppm in liver and 11.7 ppm in kidney at the 1000 ppm feeding level. Residues of 3,5,6-trichloro-2-pyridinol showed a rapid decline in muscle and fat, reaching <0.05 ppm at 3 days after the return to untreated feed. Residues in liver kidney tissues declined more slowly and reached a level of <0.05 ppm at 21 days after the return to untreated feed. No residues of 2-methoxy-3,5,6-trichloropyridine were found in any tissue from calves fed the highest level, 1000 ppm triclopyr.

Dishburger HJ, McKellar RL, Pennington JY & Rice JR (1977) Determination of residues of chlorpyrifos, its oxygen analogue, and 3,5,6-trichloro-2-pyridinol in tissues of cattle fed chlorpyrifos. J Agric Food Chem 25(6):1325-1329 [Public Domain. Dow; submission 238, part 4, vol 1, pp 4.223- 4.242.]

Dishburger HJ, Rice JR, McKellar RL & Pennington JY (1972) Determination of residues of chlorpyrifos, its oxygen analogue, and 3,5,6-trichloro-2-pyridinol in tissues of cattle fed chlorpyrifos. The Dow Chemical Co. Report No. GH-C 566, dated June 30, 1972 [Dow; submission 939, November 1988, part 4, vol 1, pp 4.111-4.150]

Eighteen Hereford crossbred heifers (160-240 kg bw., 3/dose) were fed for a period of 30 days with chlorpyrifos (Lot No. CP523-CD235C, 97.2% pure), administered once each day by capsule, at levels of 0, 3, 10, 30 or 100 ppm on a daily dry matter intake basis and sacrificed with no withdrawal period. Samples of muscle, liver, kidney, omental fat, renal fat and subcutaneous fat were collected for residue analysis. Three other animals were fed 100 ppm chlorpyrifos for 30 days. At this time the chlorpyrifos was withdrawn. Omental fat samples were collected from these animals by surgical biopsy at weekly intervals for 5 weeks.

All samples were analysed for residues of chlorpyrifos, its oxygen analogue and 3,5,6-trichloro-2-pyridinol by gas chromatographic methods with validated lower levels of sensitivity of 0.01 ppm for chlorpyrifos and the oxygen analogue and 0.05 ppm for 3,5,6-trichloro-2-pyridinol. The storage stability of the tissue residues and the efficiency of the extraction method were determined by the use of spiked samples.

Results

The average efficiency of recovery of the three residues in all tissues exceeded 74%, and these values were used to correct all residue values reported.

In the cattle fed chlorpyrifos for 30 days with no withdrawal period, there was a dose related increase in chlorpyrifos residues in all tissues, but especially in fatty tissues. Samples of renal, omental and subcutaneous fat each had average chlorpyrifos residues ranging from 0.02 ppm at the 3 ppm dose, through to 3.3 ppm residue at the 100 ppm dose level. The average residue in fat declined to 0.93 ppm at 7 days, and 0.02 ppm at 35 days after withdrawal of the 100 ppm dose. Muscle, liver and kidney samples from the 100 ppm dose level had average residue levels of chlorpyrifos of 0.24, 0.02 and 0.02 ppm respectively.

3,5,6-trichloro-2-pyridinol was found predominantly in the liver and kidney. Average 3,5,6-TCP residues ranged from 0.20 ppm in liver and 0.11 ppm in kidney at the 3 ppm dose level, to 2.41 ppm in liver and 1.75 ppm in kidney when 100 ppm chlorpyrifos was fed. The levels in fat and muscle found at the 100 ppm dose level were 0.14 and 0.26 ppm respectively.

2.6 Humans

Drevenkar V, Vasilic Z, Stengl B, Frobe Z & Rumenjak V (1993) Chlorpyrifos metabolites in serum and urine of poisoned persons. Chem. Biol. Interact. 87(1-3): 315-22. Luxembourg Industries Dossier file no. 5.1/02 [David Gray; submission no. 11475]

Urinary and serum metabolites of chlorpyrifos were investigated from subjects poisoned with formulations containing chlorpyrifos. The subjects were a 25-year old man (subject A) and a 28-year old woman (subject C) who drank 30-60 ml of Chromorel D (a liquid formulation containing 50% chlorpyrifos), and a 40-year old woman (subject B) who ingested two spoonfuls of Zlatica-Pirifos (a powder containing 4% chlorpyrifos). The subjects were admitted to hospital 2-5 h after they ingested the formulations, and were initially treated with atropine and then with 4x500 mg of the oximes pralidoxime and/or HI-6 (identity not provided) daily.

The first serum and urine samples were obtained on the day of admission, and then daily during the hospitalisation period. Serum and RBC cholinesterase activities were measured, and serum and urine samples analysed for chlorpyrifos and chlorpyrifos metabolites.

In all patients, significant inhibition of serum and RBC cholinesterase activities were noted at hospital admission, with serum BChE activity reduced by 80-90% of baseline reference values (3.45 kU/L) and RBC AChE activity reduced by 70-80%, compared with the activity measured at the end of hospitalisation. After treatment with oximes, AChE activity recovered quickly, and within 24 h had reached levels of 66-87% of the estimated baseline activity (after 15 days of hospitalisation). The BChE activity did not return to reference values in any subject during the hospitalisation period. The BChE activity did begin to increase in subjects B and C within 3 days of initiation of treatment, but in subject A the BChE activity only began to increase after 27 days, and by 37 days had only reached 33% of the reference baseline.

Chlorpyrifos was detected in all patients in serum samples only. The concentration of total diethylphosphorous metabolites [diethylphosphate (DEP) plus diethylphosphorothioate (DETP)] was 1-2 orders of magnitude higher than the concentration of chlorpyrifos in serum, and the concentration of the metabolites in urine was significantly higher than in serum.

The decrease in concentrations followed first-order kinetics. In the initial elimination phase chlorpyrifos was eliminated from serum twice as fast ($t_{1/2}$ = 1.1-3.3 h) as the total diethylphosphorous metabolites ($t_{1/2}$ = 2.2-5. 5 h). The authors stated that the total urinary diethylphosphorous metabolites in six chlorpyrifos poisoned persons (3 from this study, and 3 from a previous study) were excreted with an average elimination half-life of 6.10 $\pm$ 2.25 h in the fastest and of 80.35 $\pm$ 25.8 h in the slowest elimination phase.

Nolan RJ, Rick DL, Freshour NL & Saunders JH (1982) Chlorpyrifos: pharmacokinetics in human volunteers following single oral and dermal doses. The Dow Chemical Company. Study No.: HEB-DR-0043-4946-4, dated August 1982 [Dow; submission 939, November 1988, part 4, vol 1, pp 4.299-4.313. Dow; Submission 238 (1987) Part 4, pps 4.228-4.242]

Nolan RJ, Rick DL, Freshour NL & Saunders JH (1984) Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol Appl Pharmacol 73:8-15 (1984) [Dow; Submission 1080. Dow; Submission 1053, part 3, pp 3.213-3.220. Dow; Submission 11462, reference 114]

Six male Caucasian volunteers, aged 27-50 years, were used in this study. In an initial pilot phase, Volunteer A was given a single oral dose (0.5 mg/kg) of chlorpyrifos (Dow; 99.8% purity; Lot AGR 166043) in the form of a lactose capsule, administered approximately 30 minutes after food. This dose was administered approximately one month prior to the treatment of other volunteers. Volunteer A was also given a dermal dose of chlorpyrifos (0.5 mg/kg, dissolved in methylene chloride) when the other volunteers were given their oral dose. Two weeks after the first dermal dose, Volunteer A was given a further dermal dose (0.5 mg/kg in dipropylene glycol methyl ether, or DPGME). The remaining volunteers were given a dermal dose of 5.0 mg/kg chlorpyrifos in DPGME four weeks after the administration of their oral dose.

Dermal applications were made to sites on the forearms of volunteers (approximately 100 cm^2), and the test material was not covered or occluded. The volunteers were encouraged to follow normal routine, and each took a bath or shower 12 to 20 h after application of the dermal dose. All urine was collected from volunteers from 24-48 h prior to dosing, through 120 h post dosing. Separate collections were made at 0, 6, 12, 24, 36, 48, 60, 72, and 96 h postdosing. Two additional 12-h urine collections were made starting 156 and 180 h post dosing, following the 5.0 mg/kg dermal treatment. Blood samples were collected prior to and at discrete intervals following treatment, for determination of chlorpyrifos, and its principal metabolite 3,5,6-trichloro-2-pyrinidol (3,5,6-TCP), and for the determination of cholinesterase activity.

Results

No signs or symptoms of chlorpyrifos toxicity were reported in any volunteers during the study. Following the oral administration of 0.5 mg/kg chlorpyrifos, plasma cholinesterase was inhibited by 71%

(compared with predose levels) in volunteer A and by 85% (mean) in the other volunteers, within 12-24 h after treatment. The range of individual values was not provided. Plasma cholinesterase activity had returned to >80% of the mean predose value by day 30 after oral treatment.

Following dermal application at day 30, plasma cholinesterase activity decreased to approximately 70% of predose levels, and returned to about 80-90% of these levels by day 40 of the study. The study authors stated that the intra-group variation was considerable, but these figures were not provided in the report. Erythrocyte cholinesterase inhibition was not significantly inhibited following either oral or dermal doses of chlorpyrifos. No unchanged chlorpyrifos was detected in the urine (analytical limit 10 ng/ml).

Following the 0.5 mg/kg oral dose of chlorpyrifos, 3,5,6-TCP was first detected in the blood after 1-2 h, after which time the concentration increased rapidly (half life of 0.5 h), reaching a mean maximum concentration of 0.93 µg/ml, six hours after ingestion of the dose. Following the 5.0 mg/kg dermal dose of chlorpyrifos, the mean half-life for appearance of 3,5,6-TCP in the blood was 22.5 h, with the highest mean concentration of 0.063 µg/ml observed 24 h after dosing. The mean predicted absorption following oral administration was 72 $\pm$ 11%, and following dermal administration was 1.35 $\pm$ 1.0%. This was consistent with the percentage of the administered dose recovered from the urine following oral (70%) and dermal (1.28%) administration.

2.7 Other Studies

Mortensen SR, Chanda SM, Hooper MJ & Padilla S (1996) Maturational differences in chlorpyrifos-oxonase activity may contribute to age-related sensitivity to chlorpyrifos. J Biochem Toxicol 11(6): 279-287

This paper investigated the increased sensitivity of younger animals to the toxicity of chlorpyrifos. The authors hypothesized that young rats have less chlorpyrifos-oxonase (CPFOase) activity than adults. CPFOase activity was measured in the brain, plasma, and liver of male, postnatal day 4 (PND4) and adult (PND90) Long-Evans rats (CRL:(LE)BR). CPFOase is biochemically defined as a Ca(2+)-dependent A-esterase that hydrolyzes chlorpyrifos-oxon (CPFO), the active metabolite of chlorpyrifos; this activity may be related to paraoxonase, and was determined spectrophotometrically by measuring generation of 3,5,6-trichloro-2-pyridinol (3,5,6-TCP) (Furlong CE *et al.*, 1989).

No brain CPFOase activity (activity/g wet weight) was detected at either age. Plasma and liver CPFOase activities were markedly lower at PND4 compared to adult: PND4 plasma and liver CPFOase activities were 1/11 and 1/2 the adult plasma and liver activities, respectively.

Because the Michaelis-Menten constant (Km) of CPFOase activity was high (ie. 210-380 µM), experiments were done to determine if this CPFOase activity could hydrolyze physiologically relevant concentrations (i.e. nM to low µM) of CPFO. Reasoning that tissue AChE inhibition may provide a sensitive bioassay for the concentration of chlorpyrifos oxon, these experiments compared the shifts in the tissue acetylcholinesterase (AChE) IC50 for CPFO in the presence or absence (inhibited by EGTA) of CPFOase activity. Brain cholinesterase activity was determined spectrophotometrically, while plasma and liver acetylcholinesterase activities were determined using a radiometric method with a [^{3}H]acetylcholine iodide substrate. An increase in the "apparent" IC50 indicates that CPFOase

hydrolyses substantial amounts of CPFO during the 30 minutes the tissue was preincubated with the CPFO. In the adult, both plasma and liver AChE apparent IC50 values were higher in the presence of CPFOase activity, suggesting that the CPFOase in those tissues was capable of hydrolysing physiologically relevant concentrations of CPFO within 30 minutes. In young animals, however, there was less of a shift in the IC50 curves compared to the adult, confirming that the young animal has less capacity than the adult to detoxify physiologically relevant concentrations of CPFO via CPFOase.

Sultatos LG (1991) Metabolic activation of the organophosphorus insecticides chlorpyrifos and fenitrothion by perfused rat liver. Toxicology 68(1): 1-9

Male and female SD rats were used in a series of experiments undertaken to characterise the metabolic activation of chlorpyrifos and fenitrothion by intact rat liver. A crude measure of mortality recorded as percentage survival was obtained from treating groups of rats (10/sex) with a dose of 150 mg/kg chlorpyrifos and 700 mg/kg fenitrothion. Chlorpyrifos was administered IP in 10% DMSO in corn oil at a volume of 1.0 ml/kg, while fenitrothion was administered IP without dilution.

Single-pass liver perfusions were performed *in situ* (Sies, 1978). Liver weights averaged 11.0 g. Chlorpyrifos and fenitrothion were dissolved in less than 2.0 ml of ethanol and added to the perfusate reservoir to give final concentrations between 10-80 µM, with a perfusate flow rate of 20 ml/min. The concentration of the test substances and their oxons in the perfusate was determined by HPLC analysis. Blood collected by cardiac puncture was used to determine *in vitro* detoxification of chlorpyrifos and fenitrothion oxons (Sultatos *et al*, 1985).

Result

Single-pass perfusions of rat livers with chlorpyrifos or fenitrothion achieved steady state conditions in about 18 minutes for chlorpyrifos and 20 minutes for fenitrothion. For both chlorpyrifos and fenitrothion, the perfused livers from males and females at steady state extracted about 30% and 20% of the incoming substances respectively, over the perfusate concentration range of 10-80 µM. The effluent from the liver perfusate contained chlorpyrifos oxon and fenitrooxon at concentrations of less than 1.0% of the perfusate chlorpyrifos and fenitrothion concentration, with oxon concentrations from male livers ca. twice that from female livers.

Detoxification of the oxons by rat blood (sex not stated) was observed with half-lives of 4-6 min. for chlorpyrifos oxon and 7-8 min. for fenitrooxon. This rate was not rapid enough to prevent passage of at least some of these chemicals from liver to extrahepatic tissues, suggesting that *in vivo*, hepatic biotransformation of chlorpyrifos and fenitrothion by rat liver results in their net activation.

The IP injection of 150 mg/kg chlorpyrifos produced 10% and 90% mortality for male and female rats respectively, while the equivalent figures after 700 mg/kg fenitrothion were 30% and 100%. Thus although male rat livers produced more chlorpyrifos oxon and fenitrooxon from the parent OPs than did livers from female rats, the acute toxicities of chlorpyrifos and fenitrothion were greater in females than in males. It appears unlikely that differences in hepatic activation of chlorpyrifos and fenitrothion can account for the sex differences in their acute toxicities in SD rats.

Chiappa S, Padilla S, Koenigsberger C, Moser V & Brimijoin S (1995) Slow accumulation of acetylcholinesterase in rat brain during enzyme inhibition by repeated dosing with chlorpyrifos. Biochem Pharmacol 49(7): 955-963

This study was designed to investigate the effects of chlorpyrifos exposure on the activity of brain acetylcholinesterase in rats.

Male, Long-Evans rats maintained at 350 +/- 5 g, were dosed (sc) weekly for 4 weeks with 0, 15, 30, or 60 mg/kg chlorpyrifos in peanut oil. Animals were sacrificed at 1, 3, 5, 7 and 9 weeks after treatment began for the processing of brain and other tissues. AChE activity was measured by a spectrophotometric method, and immunoreactive AChE protein (AChE-IR) was estimated by two-site ELISA using 2 monoclonal antibodies to rat brain AChE. Northern blots were carried out with mouse AChE-derived cDNA probes. Control experiments included *in vitro* tests which determined that chlorpyrifos oxon did not interfere with the AChE ELISA, and that chlorpyrifos oxon could gain ready access to both intra- and extra-cellular compartments of brain tissue when added to the *in vitro* system. The AChE assays incorporated ethopropazine to inhibit butyrylcholinesterase activity.

AChE activity fell significantly in a dose- and time-related manner at all times and doses, reaching a maximum inhibition of 80% in global brain samples (brainstem, diencepahalon and parts of telencephalon) after 4 weeks at the 60 mg/kg level. The inhibition was greatest in the forebrain, less in hippocampus and substantially less in cerebellum, diaphragm and liver. During the recovery phase, brain AChE activity steadily recovered, reaching control levels after 9 weeks at 15 mg/kg, but remaining 25-30% inhibited after 9 weeks at the 30 and 60 mg/kg dose levels. In the global brain samples, the AChE protein was not decreased by dosage at any time; in fact, AChE-IR increased at 3 and 5 weeks in the two higher dosage groups. However, no significant changes in AChE-IR were seen in the hippocampus, forebrain, cerebellum, diaphragm or liver. Northern blots were consistent with stable levels of AChE mRNA throughout the dosing and recovery periods.

The authors argue that chronically reduced brain AChE activity after chlorpyrifos reflects sustained enzyme inhibition, not loss of enzyme protein or suppression of AChE message. They suggest that this results from the propensity of chlorpyrifos for depot storage and gradual bioactivation. The pattern of residual AChE activity in the brain was consistent with the idea that new enzyme biosynthesis was sufficiently rapid to stabilise brain AChE activity at a new equilibrium between 10 and 20% of control levels. The possibility that a longer dosing period would lead to a lower equilibrium level of AChE activity was not addressed by this study.

Coulston F, Golberg L, Abraham R & Benitz FK (1971) Final report on safety evaluations and metabolic studies on Dowco 179 (IN 151) Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York. Report K-44793-(48)A dated March 18, 1971. Sponsor: Dow Chemical Company, USA. [Dow; submission 238, part 4: pp 4.52-4.126]

This report contained studies of the excretion of ^{14}C-labelled Dowco-179 and 3,5,6-TCP after IP injection in rats, and reports on analysis of urinary levels of Dowco-179 and metabolites after subchronic feeding studies in rats and monkeys.

Male (4) and female (3) albino rats were fed chlorpyrifos (DOWCO179) in their diet at an average daily intake of 0.75 mg/kg for a period of 6 months. Urine samples were collected at 2, 4 and 6 months and analysed for chlorpyrifos and its metabolites. At the 2-month collection, the amount (µg/ml) of chlorpyrifos in the urine was 300 times less than the concentration of 3,5,6-trichloro-2-pyridinol. The level of 3,5,6-trichloro-2-pyridinol in the urine at two months (mean 3.35 µg/ml) was similar to the level at the four-month (mean 3.03 µg/ml) analysis. It was apparent that there would not be any accumulation of chlorpyrifos or its metabolites.

These metabolic and toxicokinetic studies contain insufficient detail to be deemed adequate for regulatory purposes and no detailed evaluation was reported.

Bakke JE & Struble C (1981) Metabolism of 3,5,6-trichloro-2-pyridinol [Na+] in pigs. The Dow Chemical Company, report No.: GH-C1428. [Dow; submission 939, November 1988, part 4 vol 1, pp 4.230-4.235]

The authors reported the following: - Three weanling pigs were given single oral doses of the sodium salt of ^{14}C-3,5,6-trichloro-2-pyridinol at 6 mg/kg body weight. Urine, faeces and blood were monitored for ^{14}C activity. The animals were killed at 1, 2 and 3 days after dosing and the tissues examined for ^{14}C activity. It was reported that:
. total accounting of activity was excellent,
. that most of the ^{14}C activity was eliminated in the urine,
. the urinary excretion products were the free 3,5,6-trichloro-2-pyridinol and the glucuronide conjugate of the pyridinol, and
. tissue residues of ^{14}C activity decreased rapidly with no indication of retention in any tissue.

This metabolic study contained insufficient detail to be deemed adequate for regulatory purposes and no detailed evaluation was reported.

Bakke JE & Price CE (1976) Metabolism of O,O-dimethyl-O-(3,5,6-trichloro-2-pyridyl) phosphorothioate in sheep and rats and of 3,5,6-trichloro-2-pyridinol in sheep. J Environ Sci Health B11(1), 9-22 (1976). [Dow; submission 939, November 1988, part 4 vol 1, 4.236-4.249]

Two ewes were given ^{14}C-labelled 3,5,6-TCP orally (100 mg/kg, gelatine capsule). The sheep excreted it unchanged in the faeces and as the glucuronide of 3,5,6-TCP in the urine.

This metabolic study contained insufficient detail to be deemed adequate for regulatory purposes and no detailed evaluation was reported.

Gutenmann WH, St John LE Jr & Lisk DJ (1968) Metabolic studies with O,O-diethyl 0-(3,5,6-trichloro-2-pyridyl) phosphorothioate (Dursban) insecticide in a lactating cow. [Public domain. Dow; submission 238, part 4, vol 1, pp 4.216- 4.218]

A lactating cow which ingested 5 ppm of chlorpyrifos in the feed and excreted 1.7% of the consumed dose unchanged in the faeces, but none in the urine or milk. The bulk of the compound (ca. 63%) was metabolised and excreted in the urine as methyl esters of the diethylthiophosphate and diethyl phosphate.

This metabolic study contained insufficient detail to be deemed adequate for regulatory purposes and no detailed evaluation was reported.

McKellar RL, Dishburger HJ, Rice JR, Craig LF & Pennington J (1976) Residues of chlorpyrifos, its oxygen analogue, and 3,5,6-trichloro-2-pyridinol in milk and cream from cows fed chlorpyrifos. Agric Food Chem 24(2): 283-286. [Dow; submission 238, part 4 vol 1, 4.219-4.222]

Cows were fed a complete ration containing chlorpyrifos at 5 levels from 0.3 to 30 ppm for 2 weeks at each level. Milk and cream samples were collected at predetermined intervals during the feeding of the chemical and for 14 days following withdrawal of the highest feeding level. Residues of chlorpyrifos and its oxygen analogue (O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphate) were determined. The methods were validated to 0.01 ppm for the three compounds in milk and 0.01 ppm for chlorpyrifos and its oxygen analogue and 0.025 ppm for 3,5,6-TCP in cream with overall average recoveries of greater than 80%. The average residues found were 0.01 ppm of chlorpyrifos, <0.01 ppm of chlorpyrifos oxygen analogue, and 0.01 ppm of 3,5,6-TCP in milk and 0.10 ppm of chlorpyrifos, <0.01 ppm of chlorpyrifos oxygen analogue, and <0.025 ppm of 3,5,6-TCP in cream at the highest feeding level. Residues of all chemicals decreased rapidly upon removal of chlorpyrifos from the feed.

This metabolic study contained insufficient detail to be deemed adequate for regulatory purposes and no detailed evaluation was reported.

Lores EM, Sovocool GW, Harless RL, Wilson NK & Moseman RF (1978) A new metabolite of Chlorpyrifos: Isolation and identification. J Agric Food Chem 26(1): 118-122, 1978. [Dow; submission 238, part 4: pp 4.243-4.247]

A new metabolite of chlorpyrifos was discovered in a human poisoning case in which a lethal quantity of the pesticide was ingested. The metabolite was isolated from a human liver extract. After extensive cleanup, the metabolite was subjected to various instrumental analyses such as gas chromatography, mass spectrometry, and nuclear magnetic resonance. The metabolite was identified as a compound similar to chlorpyrifos with a methylthio (-SCH3) group substituted for a chlorine on the pyridinol ring.

This metabolic study contained insufficient detail to be deemed adequate for regulatory purposes and no detailed evaluation was reported.

3. ACUTE TOXICITY

3.1 Technical-Grade Active Constituent

Summary of Median Lethal Dose Studies

Numerous median lethal dose studies have been carried out using chlorpyrifos technical. The available lethal dose studies using chlorpyrifos technical are summarised below. Many studies have been evaluated previously, and are thus only presented in tabular form below. Acute toxicity studies submitted in response to the ECRP data call-in have been tabulated, and are also reported in detail (below). In general, the signs of acute chlorpyrifos intoxication are consistent with cholinesterase inhibition, and include inactivity, salivation, dyspnoea, flaccid paralysis, vomiting, piloerection, exothalmia and diarrhea.

Summary of acute oral toxicity studies with chlorpyrifos technical.

Species	Strain	Sex	Vehicle	LD50 mg/kg (95% CI or range)	Reference
Mouse	Smith Webster	M	1% aqueous gum tragacanth	102 (94-110)	Coulston et al (1971)
Mouse	NAMRU	F	soya bean oil	152 (143-162)	Berteau & Deen (1978)
Mouse	Swiss albino	M+F	vegetable oil	109 (93-127)	Anon (1995a)
Rat	ns	M F	5% corn oil	163 (97-276) 135 (97-188)	Taylor & Olson (1963)
Rat	SD	F	soya bean oil	169 (146-196)	Berteau & Deen (1978)

Summary of acute oral toxicity studies with chlorpyrifos technical (continued)

Species	Strain	Sex	Vehicle	LD50 mg/kg (95% CI or range)	Reference
Rat	SD	M F M+F	arachis oil	276 (167-455) 350 (285-429) 320 (260-393)	Dreher (1994a)
Rat	Wistar	M+F	vegetable oil	134 (102-163)	Anon (1995b)
Rat	SD	M F M+F	maize oil	264 141 192	Wilson & McBeth (1994)
Rat	SD	M F	maize oil	475 (311-727) 337 (220-515)	Buch & Gardner (1981)
Rat	SD	M F	corn oil	221 (181-269) 144 (105-200)	Nissimov & Nyska (1984b)
Rat	SD	M F M F M F	ns	205 (134-299 mg/kg) 96 (72-140 mg/kg) 248 (170-379 mg/kg) 97 (80-112 mg/kg) 270 (188-426 mg/kg) 174 (130-244 mg/kg)	Henck & Kociba (1980)
Guinea	ns	M	corn oil	504 (300-850)	Lackenby (1985b)

pig					
Rabbit	ns	M	corn oil	1000-2000	Lackenby (1985b)
Chicken	ns	M	capsule	32 (14-72)	Lackenby (1985b)
Chicken	Leghorn	M	diet	25 (21-31)	Sherman et al (1967)
Chicken	Leghorn	M	capsule	32	Stevenson (1963)
Chicken	White Rock	M&F	capsule	50-63	Stevenson (1966)
Chicken	ns	M&F	capsule	20-50	Ross & Roberts (1974)
Chicken	ns	M&F	gavage/corn oil	102 (64-169)	Ross & Roberts (1974)
Chicken	ns	M	capsule-undiluted	32 (14-72)	Taylor & Olson (1963)
Turkey	Beltsville Small Whites	ns	ns	32-63	Stevenson (1967)
Cavy	ns	M	10% corn oil	504 (299-850)	Taylor & Olson (1963)

ns = not stated
SD = Sprague-Dawley

Summary of acute dermal toxicity studies with chlorpyrifos technical

Species	**Strain**	**Sex**	**Vehicle**	**LD50* mg/kg (95%CI or range)**	**Reference**
Rat	SD	M+F	PEG	>2000	Lackenby (1985a)
Rat	SD	M+F	undiluted	>2000	Jackson & Ogilvie (1994a)
Rat	ns	M+F	undiluted	>2000	Nissimov & Nyska (1984a)
Rat	Fischer	M+F	undiluted	>2000	Jeffrey et al (1986)

Summary of acute dermal toxicity studies with chlorpyrifos technical (continued)

Species	**Strain**	**Sex**	**Vehicle**	**LD50* mg/kg (95%CI or range)**	**Reference**
Rat	SD	M+F	arachis oil	>2000	Dreher (1994b)
Rat	SD	M+F	saline	>5000 (Intact and abraded)	Buch et al (1980)
Rabbit	Himalayan	M+F	aqueous	1233 (993-1531)	Anon (1995c)
Rabbit	NZW	M+F M+F M+F	undiluted	1580 (828-2606) 1598 (1243-1919) 1801 (1023-3152)	Henck & Kociba (1980)
Rat (sub-cutaneous)	CFY	M+F	Tween 20/DMSO/ water 2:3:5	147 (120-179)	Davies & Kynoch (1970)

ns = not stated
SD = Sprague-Dawley

Summary of acute inhalation toxicity studies with chlorpyrifos technical

Species	Strain	Sex	Vehicle	LC50* mg/m^3 (95%CI or range)	Reference
Mouse	NAMRU	F	65% xylene	94 (83-106)	Berteau & Deen (1978)
Rat	Albino (HC/CFHB)	M+F	vapour	>200	Hardy & Jackson (1984)
Rat	Fischer 344	M+F	1% aqueous	>32	Phillips & Lomax (1989)
Rat	SD	F	65% xylene	78 (57-108)	Berteau & Deen (1978)
Rat	SD	M+F	Undiluted	>230 (4 h)	Anderson et al (1995)
Rat	SD	M F	40% xylene	>4070 (4 h) 2890 (2010-4160) (4 h)	Buch (1980)
Rat	Wistar	M+F	Undiluted	>1020 (4 h)	Kenny et al (1987)
Rat	Fischer 344	M+F	1% aqueous solution	>3200 (4 h)	Phillips & Lomax (1989)
Rat	SD	M+F	vapour	>36 (4 h)	Blagden (1994)
Rat	Wistar	M+F	not stated	560 (360-950) (4 h)	Anon (1996a)

SD = Sprague-Dawley

3.1.1 Acute oral toxicity studies

Wilmer JW, Berdasco NM & Crissman JW (1992) Chlorpyrifos: Acute oral toxicity (range-finding) study in Fischer 344 rats. The Dow Chemical Company Study No. K-044793-093A, dated 16 September 1992. GLP certificate. QA. [Dow; Submission 11462, reference 4]

A range-finding study with chlorpyrifos (98.1 %, Dursban F, Lot No. MM-890115-661) was performed with Fischer 344 rats (CrL, 9 weeks old) to determine a dose and time of peak effect. Fasted rats (2/sex/dose) were administered 50, 100, 150 or 200 mg/kg of the test material by single oral gavage as a 5% solution in corn oil. Animals were observed for clinical signs frequently during the first 9 h post-dose, and then daily for two weeks. Body weights were recorded on days 0, 2, 8 and 15 prior to sacrifice.

There were no deaths prior to scheduled sacrifice. Clinical signs were seen at 1.5 h after dosing, peaked at 6 h post-dose, were more severe in females and had resolved by day 5 post-dose. Only minor signs were seen at 50 mg/kg, and signs at higher doses included decreased activity, faecal and/or urine perineal staining, lacrimation, salivation, laboured respiration (females only), tremors and incoordination. All animals except females at 50 mg/kg lost weight during the initial parts of the study, with this effect also more pronounced in females. The authors concluded that 100 mg/kg was the highest dose suitable for an acute neurotoxicity study.

Wilson JA & MacBeth D (1994) Chlorpyrifos Tech: Acute oral toxicity (LD50) test in rats. Inveresk Research International, Scotland. Project. No: 556031; Report No: 10521; 31 August 1994. [Lerace Pty Ltd; Submission 11204; A3162/31, Box 31]

This study was conducted in accordance with UK Department of Health GLP regulations (as accepted by the US EPA and FDA), and was conducted in accordance with EPA (FIFRA) and OECD guidelines for acute oral toxicity testing in rats. Rats (Sprague-Dawley; Harlan Olac Ltd, UK; 5/sex/group) were given chlorpyrifos (Chlorpyrifos Tech; Luxembourg Industries, Israel; batch 007/94; 99% purity) in

maize oil (dose volume 10 ml/kg) by oral gavage at doses of 50, 200, 350, or 500 mg/kg, and observed for up to 14 days after treatment for mortality and/or signs of toxicity. All rats at 350 and 500 mg/kg died, or were killed *in extremis*, within 24 h of dosing, and mortality was also reported in female rats given 200 mg/kg (3/5 animals). Clinical observations preceding death included piloerection, increased salivation, ataxia, subdued behaviour, tremors, hunched appearance and laboured breathing. No significant signs of toxicity were reported at 50 mg/kg, and at 200 mg/kg all surviving animals had recovered by day 9 post treatment. There were no significant pathological findings reported. Under the conditions of this study, the LD50 for the test material was 141 mg/kg for females, 264 mg/kg for males, and 192 mg/kg for males and females combined. The 95% confidence limits associated with these LD50 values were not reported.

Dreher DM (1994a) Chlorpyrifos technical: Acute oral toxicity test in the rat. Safepharm Laboratories Ltd, UK Project no: 545/53. Report dated 23 November 1994. Cheminova Agro A/S report CHA Doc No: 8-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-1, TSCA Subpart B, Section 798.1175). The study was conducted between 29 June 1994 and 21 July 1994.

The test material (chlorpyrifos technical; Cheminova Agro A/S; Batch 341-HRR-153; certified purity 99.3%) was given once to male and female Sprague-Dawley rats (Harlan UK Ltd; approximately 5-8 weeks old at the start of the main study) by oral gavage (in arachis oil BP; dose volume 10 ml/kg). In a range-finding study, one animal/sex/dose was tested at doses of 50, 100, 200, 500, 1000, or 2000 mg/kg, and deaths and overt signs of toxicity were recorded 30 minutes, and 1, 2, and 4 h after dosing and then daily for five days. Based on the results of the range-finding study, the main study consisted of three groups of animals (5/sex/dose) given doses of 180, 300, or 500 mg/kg, in the same manner as the range-finding study. Deaths and overt signs of toxicity were recorded 30 minutes, and 1, 2, and 4 h after dosing and then daily for 14 days. Individual bodyweights were recorded prior to dosing on Day 0 and on days 7 and 14, or at death. At the end of the dosing period, all surviving animals were killed, and all animals, including those that died during the study, were subjected to gross pathological examination, including opening of the abdominal and thoracic cavities. No tissues were retained.

Results

In the range-finding study, animals died at 500 (both the male and the female), 1000 (the male only), and 2000 mg/kg (both the male and the female) within two days of treatment. Signs of intoxication included ataxia, fasciculations, hunched posture, lethargy, increased salivation, laboured respiration, occasional body tremors, splayed gait, and less frequently, diarrhoea, dehydration, clonic convulsions, exophthalmos, and increased lacrimation. At 50 and 100 mg/kg, clinical signs were confined to hunched posture, and occasional tremors.

In the main study, 2/5 males died at 180 mg/kg (between 4 h and 1 day), and 2/5 males and 1/5 females died at 300 mg/kg (between 1 and 3 days). At 500 mg/kg, all animals died before day 3 (two females

killed *in extremis*). Common clinical signs of intoxication were similar to those reported in the range-finding study, and surviving animals recovered two to seven days after dosing. In animals killed *in extremis* or that died during the study, common necropsy findings were haemorrhagic lungs, dark liver, pale or dark kidneys, and slight haemorrhage and/or sloughing of the gastric mucosa. No abnormalities were noted at necropsy in animals killed at the end of the study.

Under the conditions of this study, the acute oral median LD50 and 95% confidence limits for the test material in Sprague-Dawley rats were 276 (167-455) mg/kg in males, 350 (285-429) mg/kg in females, and 320 (260-393) mg/kg in all animals.

Anon (1995a) Acute oral toxicity study of chlorpyrifos technical in Swiss albino mice. Fredrick Institute of Plant Protection and Toxicology, India. Report no. 2739, dated 15 August 1995. Project no. 05-159-95. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

Technical chlorpyrifos (Ficom Organics, India; stated purity 96.2%; batch no. C503085) was suspended in refined vegetable oil and given to groups of Swiss albino mice (Fredrick Institute, India; aged 6-8 weeks at start of experiment; 5 animals/sex/dose) by oral intubation (dose volume 10 ml/kg). Doses were 75, 100, 125, and 150 mg/kg. Control animals received vegetable oil only. All animals had access to food and water within 2-3 h after dosing. All animals were observed for 14 days for mortality and/or signs associated with toxicity. All surviving animals were necropsied for gross pathological observations at the end of the observation period.

Results

The incidence of mortality was 0/10, 1/10, 4/10, 6/10, and 9/10, at 0, 75, 100, 125, and 150 mg/kg, respectively, and all animals that died did so in the first 48 h after treatment. The mortality data for the separate sexes was not presented.

Clinical signs of intoxication were recorded in the first 48 h after treatment, and these included dullness, lethargy, tremor, urinary incontinence and paralysis from 4 h onwards. The incidence of these findings in each of the test groups was not provided. No gross abnormalities were reported following necropsy of the animals that survived treatment.

Under the conditions of this study, the acute oral LD50 of the test material in mice was stated to be 109.58 mg/kg (95% confidence limits: 93.25 - 127.55 mg/kg).

Anon (1995b) Acute oral toxicity study of chlorpyrifos technical in Wistar rats. Fredrick Institute of Plant Protection and Toxicology, India. Report no. 2738, dated 18 August 1995. Project no. 05-158-95. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this

study.

Technical chlorpyrifos (Ficom Organics, India; stated purity 96.2%; batch no. C503085) was suspended in refined vegetable oil and given to groups of Wistar rats (Fredrick Institute, India; aged 6-8 weeks at start of experiment; 5 animals/sex/dose) by oral intubation (dose volume 10 ml/kg). Dose levels were 100, 150 and 200 mg/kg. Control animals received vegetable oil only. All animals had access to food and water within 2-3 h after dosing. All animals were observed for 14 days for mortality and/or signs associated with toxicity. All surviving animals were necropsied for gross pathological observations at the end of the observation period.

Results

The incidence of mortality was 0/10, 2/10, 6/10, and 9/10, at 0, 100, 150 and 200 mg/kg, respectively, and all animals that died did so in the first 48 h after treatment. The mortality data for the separate sexes was not presented.

Clinical signs of intoxication were recorded in the first 72 h after treatment, and these included dullness, lethargy, tremor, urinary incontinence and paralysis from 4 h onwards. The incidence of these findings in each of the test groups was not provided. No gross abnormalities were reported following necropsy of the animals that survived treatment.

Under the conditions of this study, the acute oral LD50 of the test material in rats was stated to be 134.18 mg/kg (95% confidence limits: 102.46 - 163.06 mg/kg).

Buch SA & Gardner JR (1981) Pyrinex Technical: Acute oral toxicity in the rat. Life Science Research, UK. LSR Report: 81/MAK035/104, 5 March 1981. [Makteshim; Submission 11471]

The acute oral toxicity of Pyrinex technical (chlorpyrifos concentration not stated) was studied in young adult CD (SD) rats (Charles River, UK), with the test material administered via oral gavage as a suspension in maize oil. The doses used were 133, 231, 400, 693, and 1200 mg/kg body weight, with 5 animals/sex/dose and a dosage volume of 20 ml/kg. These doses were selected after a preliminary test was conducted using 2 animals/sex/dose, at doses of 25, 50, 100, 200, and 400 mg/kg body weight, in which 1/2 males, and 0/2 females died at a dose of 400 mg/kg. A Quality Assurance Statement was issued for this study.

In the main study, mortality was 0/5, 0/5, 2/5, 4/5, and 5/5 in males, and 0/5, 1/5, 3/5, 5/5, and 5/5 in females, at doses of 133, 231, 400, 693, and 1200 mg/kg, respectively. The oral median lethal dose LD50 (95% confidence limits) was 475 mg/kg in males (311-727 mg/kg) and 337 mg/kg in females (220-515 mg/kg), with deaths occurring during days 2-4. Clinical signs of intoxication included diarrhoea, decreased motor activity, muscle tremors and fasciculations, lethargy, hunching, and respiratory distress. These signs had resolved in surviving animals by day 8 of the study.

Nissimov S & Nyska A (1984b) Pyrinex Tech: Acute oral toxicity in the rat. Life Science Research Israel, Study conducted 9-23 April, 1984. LSRI Report MAK/056/PYR, 12 May 1984. [Makteshim; Submission 11471; Amalgamated Chemicals; Submission 1396]

To test the acute oral toxicity of Pyrinex Tech (95.5% chlorpyrifos; Makteshim Chemical Works), the test material was prepared in corn oil, and administered by oral gavage to groups of young adult rats (CD (SD) strain; Charles River UK; 5/sex/dose) at doses of 90, 164, 298, 543, and 987 mg/kg body weight, with a dose volume of 5 ml/kg. This study was conducted in accordance with OECD and US EPA Guidelines, and was subject to Quality Assurance inspection.

Mortality in males was 0/5, 0/5, 5/5, 5/5, and 5/5, and in females was 0/5, 4/5, 5/5, 5/5, and 5/5, at doses of 90, 164, 298, 543, and 987 mg/kg, respectively. Deaths occurred between one and three days after dosing. Clinical signs of intoxication included tremors, salivation, diarrhoea, hunching, decreased motor activity, urogenital staining, and ataxia, in both decedent and surviving animals, and lacrimation in surviving animals only.

Under the conditions of this study, the oral LD50 (95% confidence intervals) was 221 mg/kg (181-269 mg/kg) in males, and 144 mg/kg (105-200 mg/kg) in females.

Lackenby F (1985b) Dursban F and Dursban F (OP1): Acute oral toxicity studies in the rat. Hazleton Laboratories Europe Report No.: 4703-50/390 & 591, dated September 1985. GLP. OECD Guidelines 401 [Dow; Submission 11462, reference 25]

Studies were performed to determine the acute oral LD50 of Dursban F (described as Dursban Technical, pale beige crystals, batch # EK830516110; purity not stated) and Dursban F (OP1) (described as off-white crystalline solid, batch #830516110) in rats. Following several screening studies, fasted rats (6-10 weeks, Sprague-Dawley Crl:CD(SD)BR, Charles River, UK, 5/sex/dose) were administered Dursban F in polyethylene glycol by single oral gavage at 25, 50, 71, 100, 141 and 200 mg/kg. Similarly, groups of 5/sex/dose were administered Dursban F (OP1) in corn oil by single oral gavage at 180, 255, 360 and 500 mg/kg.

Common clinical signs following administration of both formulations included: lethargy, prostration, hunched posture, piloerection, tremors and salivation; animals which survived appeared normal by days 3-5.

The Dursban F formulation exhibited acute oral LD50's (mg/kg, 95% limits) of 74 (59-89) for all animals, 84 (56-117) for males only and 66 (no limits calculable) for females only,.

The Dursban F (OP1) formulation exhibited acute oral LD50's (mg/kg, 95% limits) of 229 (156-282) for all animals, 287 (201-394) for males only and 181 (no limits calculable) for females only.

Henck JW & Kociba RJ (1980) Three samples of Dursban insecticide: Acute oral toxicity and acute percutaneous absorption potential. Dow Report A1A-130, undated. [Dow; Submission 11462; reference 16]

To test and compare the acute oral toxicity potential of three different batches of technical chlorpyrifos (Dursban; Dow; batches 6289-610, 693-76, 693-80; purity 97.1-100%), the test material was administered by oral gavage (vehicle and dose volume not stated) to groups of male and female Sprague-Dawley rats (6/sex/dose; Spartan Research Animals, USA). The dose levels were as follows:

* females only

batch 6289-610: 40*, 80, 160, 320, 630 mg/kg;
batch 693-76: 20*, 40*, 80, 100*, 160, 320, 630 mg/kg;
batch 693-80: 80, 160, 320, 630 mg/kg.

Animals were observed for 14 days following treatment.

All samples of test material resulted in essentially the same signs of toxicity, including lethargy, total body tremors, and diarrhoea. The LD50 values (95% confidence intervals) were as follows:

Sample 6289-610:	males 205 mg/kg (134-299 mg/kg)
	females 96 mg/kg (72-140 mg/kg)
Sample 693-76:	males 248 mg/kg (170-379 mg/kg)
	females 97 mg/kg (80-112 mg/kg)
Sample 693-80:	males 270 mg/kg (188-426 mg/kg)
	females 174 mg/kg (130-244 mg/kg)

Stevenson GT (1966) A single oral dose LD50 toxicity study of Dursban in broiler type chickens. Report label A1A-364, dated July 12 1966. [Dow; Submission 11462, reference 11]

Summary data only provided. This study was not suitable for regulatory purposes.

Stevenson GT (1967) A single oral dose LD50 toxicity study of Dursban in turkey/poultry. Dow Report A1A-362, 23 Jun 1967. [Dow; Submission 11462, reference 19]

The single oral dose LD50 of Dursban (Dow; purity not stated) was established in Turkey Poults (Beltsville Small Whites; 6-7 weeks of age; 5 birds/dose), with doses of 0 (control), 15.8, 25.2, 31.6, 63, and 126 mg/kg. Deaths were seen at 63 and 126 mg/kg (3/group). The LD50 was between 32-63 mg/kg.

Tucker RK (1967) Denver Wildlife Research Center, Dursban (Supplement #3): The median lethal dose for female domestic goats. Dow report A1A-55, Jun 1967. [Dow; Submission 11462, reference 18]

To determine the acute oral LD50 of Dursban (Dow; 94.5% chlorpyrifos) in goats, the test material was administered to female domestic goats (1/dose) at doses of 200, 500, and 1000 mg/kg. At 200 mg/kg, the test animal displayed symptoms including hunched hindquarters, neck shaking, and jerkiness, between approximately 1-5 h after administration. No effects were seen at 500 mg/kg, while at 1000 mg/kg, clinical signs were confined to uneasiness and diarrhoea during the first two days after treatment. After two days, the animal at 1000 mg/kg appeared weak, and died on the fifth day after administration.

3.1.2 Acute dermal toxicity studies

Jeffrey MM, Battjes JE & Eisenbrandt DL (1986) Dursban F (Tech): Acute Dermal Toxicity Study in Fischer 344 Rats. The Dow Chemical Company Report No: HET-K 044793-083, dated 30 June, 1986 [Dow; Submission 11462, reference 30]

Rats (Fischer 344; 5/sex; 9 weeks; Charles River, NY) were administered a single dermal application of Dursban F (98.5%, batch AGR 214637, solid) and observed for 2 weeks before necropsy. The test substance was applied to the clipped back skin under an occlusive dressing for 24 h (no details provided as to physical state of applied test substance). The application site was then washed clean of the test substance and scored for skin irritancy. The animals were observed daily for clinical signs and weighed weekly. This study was conducted in accordance with the intent of GLP.

All animals survived until scheduled necropsy at 2 weeks post treatment. Clinical signs were restricted to red soiling around the eyes in 3 M and 1 F. There were no signs of irritation at the application site, weight gains were unaffected by treatment, and all animals were within normal limits at gross necropsy. Under the conditions of this study the acute dermal toxicity of Dursban F was > 2000 mg/kg in rats.

Dreher DM (1994b) Chlorpyrifos technical: Acute dermal toxicity (limit test) in the rat. Safepharm Laboratories Ltd, UK Project no: 545/54. Report dated 23 November 1994. Cheminova Agro A/S report CHA Doc No: 9-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-2, TSCA Subpart B, Section 798.1100). The study was conducted between 7 July 1994 and 1 August 1994.

The test material (chlorpyrifos technical; Cheminova Agro A/S; Batch 341-HRR-153; certified purity 99.3%) was ground to a fine powder and applied dermally to male and female Sprague-Dawley rats (Harlan UK Ltd; approximately 10-14 weeks old at the start of the main study). The test sites were clipped areas on the back and flanks of each animal, and these sites were moistened with arachis oil BP prior to dosing. Surgical gauze was applied over the test area and semi-occluded with self-adhesive bandage for the 24-h exposure period. After the 24-h contact period, the bandage was carefully removed and the treated skin wiped with cotton wool moistened with arachis oil.

In a range-finding study, one animal/sex/dose was tested at doses of 100, 500, 1000, or 2000 mg/kg, and deaths and overt signs of toxicity were recorded 30 minutes, and 1, 2, and 4 h after dosing and then daily for five days. Based on the results of the range-finding study, the main study consisted of a group of animals (5/sex/dose) treated with the test material at a dose of 2000 mg/kg, in the same manner as the range-finding study. The test sites were examined for evidence of primary irritation and scored according to the method of Draize. Deaths and overt signs of toxicity were recorded 30 minutes, and 1, 2, and 4 h after dosing and then daily for 14 days. Individual bodyweights were recorded prior to dosing on day 0 and on days 7 and 14, or at death. At the end of the dosing period, all animals were killed and subjected to gross pathological examination, including opening of the abdominal and thoracic cavities. No tissues were retained.

Results

No deaths were reported in either the range-finding study or the main study. In the range-finding study, common signs of toxicity were ataxia, hunched posture, lethargy, decreased respiration rate and laboured respiration, and occasional additional findings included red/brown stains around the eyes or mouth and occasional body tremors.

In the main study, clinical signs were similar to those seen in the range-finding study, with occasional incidents of exophthalmos also reported. No signs were reported later than 5-6 days after dosing. No signs of skin irritation were noted during the study. No abnormalities were noted at necropsy.

Under the conditions of this study, the acute dermal LD50 for the test material in Sprague-Dawley rats was > 2000 mg/kg.

Anon (1995c) Acute dermal toxicity study of chlorpyrifos technical in rabbits. Fredrick Institute of Plant Protection and Toxicology, India. Report no. 2748, dated 16 August 1995. Project no. 05-168-95. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

Technical chlorpyrifos (Ficom Organics, India; stated purity 96.2%; batch no. C503085) was mixed with distilled water and applied to the clipped abdominal skin area of groups of Himalayan albino rabbits (Fredrick Institute, India; 1.2 - 1.6 kg at start of experiment; 3 animals/sex/dose) at doses of 0 (distilled water control), 1000, 1250, and 1500 mg/kg (dose volume not stated). After application of the test material, the application sites were covered with gauze pads and fixed to the bodies with tape, then the trunks were covered with wrapping for 24 h. At the end of the exposure period, the bandage/gauze pads were removed and the treatment sites cleaned with distilled water. All animals were observed for mortality and/or signs associated with toxicity soon after dosing and then every hour for the first day, then daily during the 21-day observation period. Body weights were measured prior to dosing and then on days 7, 14, and 21. All surviving animals were necropsied for gross pathological observations at the end of the observation period.

Results

Rabbits exposed to 1500 mg/kg of the test material displayed tremors and salivation after 3 h of administration. The length of time that these signs were observed was not stated, nor was the incidence for this finding given. Mortality was 0/6, 2/6, 3/6, and 4/6 at 0, 1000, 1250, and 1500 mg/kg, respectively. The mortality data for the separate sexes was not provided. The time of deaths was not stated. Gross pathological examination did not reveal any abnormalities.

Under the conditions of this study, the acute dermal LD50 in rabbits was 1233.57 mg/kg (95% confidence limits: 993.82-1531.15 mg/kg).

Jackson D & Ogilvie SW (1994a) Chlorpyrifos Tech: Acute dermal toxicity (LD50) test in rats.

Inveresk Research International, Scotland. Project No. 555546; Report No: 10439; 9 August 1994. [Lerace Pty Ltd; Submission 11204; A3162/31, Box 31]

This study was conducted in compliance with UK Department of Health GLP regulations and in accordance with OECD Test Guideline No: 402 and US EPA Guidelines, Subdivision F, 81-2. The acute dermal toxicity potential of Chlorpyrifos Tech (Luxembourg Industries, Israel; batch 007/94; 99% purity) was tested in rats (Sprague-Dawley; Harlan Olac Ltd, UK; 5/sex/group), with the test material applied evenly onto a gauze dressing that was subsequently applied to the intact, clipped skin on the back of each animal and covered with an occlusive dressing for 24 h. Each animal received a single dermal dose of chlorpyrifos at 2000 mg/kg, and the animals were observed for 14 days after treatment for mortality and/or signs of toxicity. The doses for this study were selected after a range-finding study was conducted in 2 animals/sex/dose at 500, 1000, 1500, and 2000 mg/kg.

There were no deaths during the study. The signs of toxicity included piloerection, ataxia, and tremors on day 3 in a single female treated at 2000 mg/kg, and red nasal discharge occurring within 4 hours of dosing in surviving males in the main study. There were no significant pathological findings reported. Under the conditions of this study, the dermal LD50 for the test material was >2000 mg/kg.

Nissimov S & Nyska A (1984a) Pyrinex Tech: Acute dermal toxicity in rabbits. Life Science Research Israel, Study conducted 19 April - 3 May, 1984. LSRI Report MAK/059/PYR, 12 May 1984. [Makteshim; Submission 11471; Amalgamated Chemicals; Submission 1396]

This study was conducted according to OECD and US EPA test guidelines, and was subject to Quality Assurance inspection. To assess the acute dermal toxicity potential of Pyrinex Tech (95.5% chlorpyrifos; Makteshim Chemical Works), the crystalline test material was first heated to a liquid form in a water bath for approximately one hour at 50^{o} C, and this material was then applied as a single dose (approximately 2000 mg/kg) to a clipped area of skin between the limb girdles of male and female albino rabbits (5/sex/dose; local strain; Loebenstein Laboratory Animals, Israel). The test site constituted approximately 10% of the total body surface area (~200 cm^2), and the test material was kept in place under a porous gauze dressing and an adhesive bandage for 24 h. Animals were inspected four times on the day of dosing, and twice daily thereafter, with the test terminated on Day 15.

No mortalities occurred during the study, and no clinical signs of toxicity were reported. Signs of skin irritation, namely scales and healed ulcers, were observed at necropsy. Under the conditions of this study, the acute dermal LD50 in rabbits was >2000 mg/kg.

Buch SA, Gardner JR & Birnie JV (1980) Pyrinex: Acute percutaneous toxicity study in rats. Life Science Research, UK. LSR Report: 80/MAK022/300, 14 July 1980. [Makteshim; Submission 11471]

This study was conducted in accordance with US EPA Guidelines 163.81-2. To determine the acute dermal toxicology potential of technical chlorpyrifos (Pyrinex; Makteshim; purity not stated), the test material was moistened slightly with a small quantity of physiological saline and applied to the skin of male and female rats (CD Sprague Dawley strain; Charles River, UK), and covered by a layer of unmedicated gauze and a waterproof plaster. In a preliminary range-finding component of the study, the test material was applied to the abraded skin (2 animals/sex/dose) of rats at doses of 500, 750, 1000,

3000, and 5000 mg/kg body weight. In the main study, the test material was applied to the intact skin of one group of 10 males and 10 females at a dose level of 5000 mg/kg, and to the abraded skin of a group of 10 males and 10 females, also at 5000 mg/kg. A control group (5 males and 5 females) received saline only, applied to abraded skin. Animals were observed three times during the first hour, and then at least twice daily until day 15. Bandages were removed after 24 h, with the test site wiped to remove excess test material.

In the preliminary study, mortality was confined to 1/2 males at 5000 mg/kg (found dead on day 3), and 1/2 females at 750 mg/kg (found dead on day 4). It was reported that clinical signs associated with treatment were observed at doses of 750 mg/kg and above, and consisted of reduced motor activity, ataxia, abdominal hunching, lethargy, debility, tremor, diarrhoea, orbital secretion, snout staining, and lack of grooming.

In the main study, the signs observed included unkempt appearance, tremor and decreased motor activity, and these were seen between days 2-12. No clinical signs were seen in 4/10 males from the non-abraded group, nor in 4/10 females from the abraded group. No control animals dies during the main study. In the animals administered with 5000 mg/kg chlorpyrifos to the intact skin, mortality was confined to 1/10 males. In the groups treated with 5000 mg/kg to abraded skin, 2/10 males and 1/10 females died.

Under the conditions of this study, the dermal LD50 was >5000 mg/kg, for both intact and abraded skin.

Henck JW & Kociba RJ (1980) Three samples of Dursban insecticide: Acute oral toxicity and acute percutaneous absorption potential. Dow Report A1A-130, undated. [Dow; Submission 11462; reference 16]

To test and compare the acute dermal toxicity potential of three different batches of technical chlorpyrifos (Dursban; Dow; batches 6289-610, 693-76, 693-80; purity 97.1-100%), the test material was administered to groups of male and female rabbits (New Zealand White albino; Langshaw Farms, USA; 2/sex/dose), with the undiluted test material applied to the clipped intact skin on the trunk of the animals, and covered with a plastic sleeve for 24 h. After this time, the test sites were washed with soap and water, and animals were observed for mortality and/or clinical signs for 14 days. The dose levels were as follows:

batch 6289-610: 630, 1300, 2520, 5000 mg/kg;
batch 693-76: 630, 1300, 1600, 2520 mg/kg;
batch 693-80: 630, 1300, 2520, 5000 mg/kg.

Clinical signs ranged from slight lethargy at the lower doses to severe diarrhoea and severe ataxia at the higher dose levels.

The percutaneous LD50 values (95% confidence intervals) were as follows:

Sample 6289-610: 1580 mg/kg (828-2606 mg/kg)

Sample 693-76: 1598 mg/kg (1243-1919 mg/kg)
Sample 693-80: 1801 mg/kg (1023-3152 mg/kg)

3.1.3 Acute inhalational studies

Blagden SM (1994) Chlorpyrifos technical: Acute inhalation toxicity study. Four-hour exposure (nose only) in the rat. Safepharm Laboratories Ltd, UK Project no: 545/55. Report dated 22 November 1994. Cheminova Agro A/S report CHA Doc No: 10-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD; Japanese MAFF notification 3850; Japanese Health and Welfare notification 313 and subsequent notification 870) and designed to satisfy OECD Guidelines (403), and US EPA Guidelines (FIFRA Section 81-3). The study was conducted between 31 August 1994 and 5 October 1994.

A group of male and female young adult Sprague-Dawley rats (5/sex; Charles River UK Ltd) was exposed (nose-only) to the test material (chlorpyrifos technical; Cheminova Agro A/S; Batch 341-HRR-153; certified purity 99.3%) as a vapour atmosphere for a period of 4 h. The test material was first melted, then compressed air was blown through the test material and ducted directly into exposure chambers. The chamber atmosphere was sampled for concentration analysis at approximately hourly intervals during the exposure period. All animals were observed for clinical signs at hourly intervals during exposure, immediately after removal from restraining tubes after exposure, one hour after termination of exposure, and then daily for 14 days. Individual bodyweights were recorded on the day of exposure, and days 7 and 14. At the end of the study, all animals were killed and subjected to internal and external macroscopic examination.

Results

The actual vapour atmosphere concentration was 36 mg/m^3, and the chamber flow rate was maintained at 10 litres/minute, providing 20 air changes/h. No animals died during the study. During exposure, wet fur was commonly observed, and there were incidents of increased or decreased respiratory rates. Following exposure, all animals displayed hunched posture and piloerection, and two animals has red/brown staining around the eyes and/or snout. No abnormal signs were observed from day 1 until the end of the study. No abnormalities were noted at necropsy.

Under the conditions of this study, the acute inhalation LD50 in Sprague-Dawley rats was > 36 mg/m^3 (the maximum attainable concentration).

Hardy CJ & Jackson GC (1984) Dursban technical: Acute inhalation toxicity in rats. The Dow Chemical Company, Report DWC 411/84774, or DET 368, dated Dec 10, 1984. GLP [Dow; Submission 238, part 2 vol 1. A3162/5 Box 42]

Rats (5/sex/group, albino HC/CFHB, 6-8 weeks old) were exposed by inhalation to vaporised chlorpyrifos (Dursban technical, lot EK 830516110) at 0 or 200 mg/m^3 for 4 h, and then observed for 2 weeks. There were no deaths. Signs of toxicity were limited to salivation, closing/partial closing of eyes, abnormal body posture, abnormal respiration and discharge from the snout. There were no

treatment-related pathological findings. The LC50 for chlorpyrifos was >200 mg/m^3.

Anderson BT, Walker SA & Punler MJ (1995) Chlorpyrifos Tech: Acute inhalational toxicity study in rats. Inveresk Research International, Scotland. Project. No: 654130; Report No: 10425; 14 February, 1995. [Lerace Pty Ltd; Submission 11204; A3162/31, Box 31]

This study was conducted in compliance with OECD (403) and US EPA guidelines for acute toxicity testing, and with internationally recognised standards of GLP. Technical chlorpyrifos (Luxembourg Industries, Israel; batch 007/94; 99% purity) was administered to rats (Sprague-Dawley; Harlan Olac Ltd, UK; 5/sex/group) by a 4 h nose-only inhalational exposure at measured concentrations of 150 or 230 mg/m^3. All animals were observed during exposure for signs of intoxication, and then observed for mortality and/or clinical signs for 14 days following exposure. At the end of the observation period, animals were subjected to gross pathological examination. Some intermittent apparatus problems were experienced during the study, with blockages occurring due to the use of the test material in its powder form. The nominal doses used (corresponding to measured doses of 150 or 230 mg/m^3) were 23600 and 13300 mg/m^3, respectively.

Clinical signs, which had disappeared by day 3 of the study, were confined to unkempt appearance in two females, and encrustations around the eye of a single male, at 150 mg/m^3. No mortality was reported during the study. Under the conditions of this study, the 4 h LC50 was >230 mg/m^3 chlorpyrifos.

Anon (1996a) Acute inhalation study with chlorpyrifos technical in Wistar rats. Fredrick Institute of Plant Protection and Toxicology, India. Report no. 2968, dated 30 January 1996. Project no. 05-297-1995. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

Wistar rats (5/sex/dose; Fredrick Institute, India; 6-8 weeks old) were exposed to technical chlorpyrifos (Ficom Organics, India; stated purity 96.2%; batch no. C503085) for 4 h (whole body) at nominal concentrations of 3850, 7220, 9620, and 14430 mg/m^3. The test material was initially dissolved in acetone to obtain a concentration of 200 mg/ml, and this solution was infused through a nebuliser to generate particles less than 5µ in size. The solution was infused at a rate of 0.2, 0.375, 0.5, and 0.75 ml/min, respectively, to achieve the nominal test concentrations. Air was drawn through the chamber at a rate of 10 litres/min. Control animals were similarly exposed, but to acetone only.

Sampling of the chamber atmosphere (n = 3) revealed mean actual concentrations of 180 $\pm$ 60, 470 $\pm$ 160, 1190 $\pm$ 140, and 1760 $\pm$ 110 mg/m^3, respectively, at the nominal concentrations listed above. No explanation was provided for the large differences between nominal and actual concentrations.

All animals were observed for 14 days for mortality and/or signs of toxicity. Body weights were recorded prior to exposure and at 7 and 14 days, and all animals were subjected to gross pathology examination at the end of the study.

Results

Mortality incidence was 0/10, 1/10, 3/10, 9/10, and 10/10 at 0, 180, 470, 1190, and 1760 mg/m^3, respectively, and these deaths occurred up to 48 h after exposure, but the sex of the dead animals was not provided. Clinical signs of toxicity were reported to include tremor and paralysis, which persisted for up to 48 h, but the incidence of these findings, and the doses at which they occurred, were not provided. Common lesions were reported in control and treated animals, but the details of these findings were not provided.

Under the conditions of this study, the LC50 of chlorpyrifos technical in rats was 560 mg/m^3 (95% confidence limits 360-950 mg/m^3).

Buch SA (1980) Pyrinex Tech: Acute inhalation toxicity in rats. Life Science Research, UK. LSR report 80/MAK/025/362, 27 August 1980. [Makteshim; Submission 11471; Amalgamated Chemicals; Submission 1396]

This study was conducted in accordance with Section 163.81-3 of the EPA Proposed Guidelines for Registering Pesticides in the US; Hazard Evaluation; Human and Domestic Animals. To test the acute inhalational toxicity of Pyrinex Technical (chlorpyrifos; 95.2% purity; Makteshim Chemical Works; Batch D1/5-3), the active ingredient was prepared as a 60% w/w solution in xylene, and administered by nose-only exposure to groups of young adult Wistar rats (Sprague-Dawley strain; Charles River UK) at continuous atmospheric actual concentrations of 1690, 2230, 2980, 3560, and 4070 mg/m^3, for a single 4 h period. Negative control and vehicle (xylene) control groups were similarly treated in this study. Groups consisted of 5 animals/sex/dose, except at doses of 4.07 mg/m^3, and with xylene alone, where 10 males and 5 females were used. No males were exposed to the lowest Pyrinex concentration of 1.69 mg/m^3. Animals were observed for 14 days following the test period, for mortality and signs of intoxication.

During the exposure period, clinical signs were generally confined to bradypnoea and hypnoea, but these effects were seen in animals administered with xylene alone, as well as those animals that received pyrinex/xylene. No clinical signs were observed in negative control animals during this period. In males, clinical signs observed during the period after treatment were generally confined to piloerection and lack of grooming, while other signs were seen in females exposed to the test material, including proneness, ataxia, tremor, and decreased motor activity. Mortality was confined to females, with 1/5, 2/5, 3/5, 2/5, and 4/5 animals dying at concentrations of 1690, 2230, 2980, 3560, and 4070 mg/m^3, respectively.

Under the conditions of this study, the acute LC50 (95% confidence intervals) in rats following a single 4-h nose only exposure to a 60% solution of chlorpyrifos/xylene was 2890 mg/m^3 (2010-4160 mg/m^3) in females, and >4070 mg/m^3 in males.

Landry, TD, Dittenber DA, Lomax LG & Momany-Pfruender JJ (1986) Chlorpyrifos: An acute vapor inhalation toxicity study with Fischer 344 rats. The Dow Chemical Company Study No.K-44793-74 dated 3 December, 1986. [Dow; Submission 11462, reference 3]

Young (7-10 weeks) Fischer 344 rats (CrL) were exposed to chlorpyrifos vapour (Dursban R, batch

not stated) for 6 h in either whole body or nose-only apparatus. The first group of 12 rats/sex were exposed whole-body at 0 and 6 ppb (0.085 mg/m^3) for 6 h, and then sacrificed within 2 h for plasma cholinesterase determination, while the remaining 6 animals/sex/dose were held for 2 weeks prior to cholinesterase determination and necropsy. Another group of 6 rats/sex was exposed whole-body at 0 and 3 ppb (0.05 mg/m^3) for 6 h and then sacrificed within 2 h for plasma cholinesterase determination and necropsy. A third group of 6 rats/sex was exposed nose-only at 0 and 14 ppb (0.2 mg/m^3) for 6 h with blood samples taken within 2 h later for plasma cholinesterase determination. These animals were observed for 2 weeks prior to sacrifice and necropsy. Body weights and clinical signs were recorded.

The measured time weighted average (TWA) exposures for the three groups were 6.1, 3.5 and 14.4 ppb, respectively. There were no unequivocal signs of toxicity attributed to exposure. One male (6 ppb) was lethargic on test day 8 and died on test day 9 and this was attributed to physical trauma. Relative body weights were unaffected by treatment, although all animals on test including controls lost weight between days 11-15. The gross pathology observations recorded no treatment-related findings. Plasma cholinesterase levels (see table below) were reduced in both sexes after whole body exposure at 6 ppb, but not at 3 ppb whole body or at 14 ppb nose-only.

Rat plasma cholinesterase activity after exposure to chlorpyrifos vapour

Exposure Apparatus	Sampling Time	Exposure Concentration	Plasma Cholinesterase (U/ml) Males	Plasma Cholinesterase (U/ml) Females
whole body	0-2 h	0	8.6	15.3
		6 ppb (0.085 mg/m^3)	7.5*	11.7*
whole body	2 weeks	0	7.4	20.1
		6 ppb (0.085 mg/m^3)	6.7	21.0
whole body	0-2 h	0	6.8	18.5
		3.5 ppb (0.05 mg/m^3)	6.5	19.0
nose only	0-2 h	0	7.0	23.8
		14 ppb (0.2 mg/m^3)	7.1	22.4

* statistically significant difference from controls

Kenny TJ, Coombs DW & Hardy CJ (1987) Chlorpyrifos technical acute inhalation toxicity in rats 4-hour exposure. Huntingdon Research Centre, UK. HRC Report MBS 20/87575, 6 October, 1987. [Makteshim; Submission 11471]

This study was conducted in accordance with GLP standards of the US EPA (Title 40 Code of Federal Regulations Part 160, 1983), Japan MAFF (Notification 3850, 1984), and the OECD (1982). A Quality Assurance Statement has been issued for this study. Young albino Wistar rats (Crl:COBS WIBR; Charles River, UK) were subjected to a single 4-h whole-body exposure to chlorpyrifos (96.8% purity; Batch 489205; source not stated) to investigate the acute inhalational toxicity potential of the test material. Groups of males and female animals (5/sex) were exposed to the test material at a concentration of 1020 mg/m^3, while negative control animals (5/sex) were exposed to air only in an identical exposure system. Animals were observed for 14 days after exposure for mortality and signs

of intoxication.

During the exposure period, clinical signs in treated animals included hunching, rapid breathing, sneezing, and closure of the eyes. During the observation period, clinical signs in treated animals included brown staining around the snout and jaws, lachrymation, salivation, abnormal body posture, and whole-body tremors. The tremors were seen in all treated females from days 3-7, but were not observed in males. No signs of intoxication were observed in control animals. There was no mortality during the study.

Under the conditions of this study, the 4-h whole body LC50 was >1020 mg/m^3.

Ludwig PD & Powers DR (1966) Blood cholinesterase analysis on animals exposed to varying concentration of Dursban dispersed as a thermal fog and liquid aerosol. The Dow Chemical Company. Report dated March 11, 1966 [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Pigs, sheep, dogs and rats (3 or 4 animals/group, no other details) were exposed to a thermally-generated fog (in diesel oil), or aerosol spray of Dursban (in xylene) (dogs and sheep only), when confined in a spray chamber for 4 h including the brief (time unspecified) period of fog/aerosol injection. Blood samples were taken at various times after exposure for cholinesterase estimations, but no technical details were provided. The calculated maximum chamber concentrations of Dursban were 28000, 113000 and 226000 mg/m^3, however no meaningful estimates of inhalational exposure or the dermal exposure contribution to cholinesterase depression can be made from this study.

Cholinesterase activity (pH Stat method) was reported for 2 days pre-treatment and 2-5 days post-treatment; the maximum recorded reduction in cholinesterase activity is reported for each exposed animal in the table below.

The largest reduction was 38% seen with the fog at 226000 mg/m^3 in dogs. This effect was reversible and by the end of 6 days post-exposure, cholinesterase activity had returned to normal in the dog. A maximum decrease in cholinesterase activity of 26% was seen in dogs exposed to 226000 mg/m^3 of the aerosol Dursban. Other species tested were less susceptive to the anticholinesterase activity of Dursban.

Dogs and monkeys

This report was presented as a summary document, and no details of the source of the results were given. A po dose of 10 µmoles/kg DOWCO 179 (chlorpyrifos; 97%) was given to dogs (3) and monkeys (3), followed by blood sampling at regular intervals for cholinesterase determination. The results (summarised below) show that plasma cholinesterase was much more sensitive to the inhibitory action of DOWCO 179 than erythrocyte cholinesterase (both species).

Cholinesterase activity (as % of control)

Specimen	Species	Time (hours)							
		0	**4**	**8**	**24**	**48**	**144**	**168**	**192**
Erythrocytes	dog	170	81*	88	78	89	86	100	-
Plasma	dog	258	93	17	30	56	65	93	95
Erythrocytes	monkey	360	60	66	80	82	73	90	90
Plasma	monkey	260	6	8	14	30	54	58	53

(*Cholinesterase activity measured as µl CO_2/30 min).

Phillips JE & Lomax LG (1989) 1% aqueous dilution of Dursban acute aerosol inhalation toxicity study in Fischer 344 rats. The Dow Chemical Company Report No. M-004803-004A, dated October 19, 1989. Guideline 81-3. GLP. QA. [Dow; Submission 11462, reference 13]

Rats (Fischer 344, 11 weeks old, 5/sex) were exposed (whole body) to an aqueous solution of Dursban TC (GHD-2424-39-17) containing 1% chlorpyrifos for 4 h. Clinical signs (daily), body weight changes (days 1, 2, 4, 8, 11 and 15) and gross pathology findings at terminal sacrifice (day 15) were reported.

The achieved exposure concentration was 3200 mg/m^3 (TWA) of the formulation, equating to 32 mg/m^3 of chlorpyrifos. The MMAD of the particles was generally less than 5 microns and hence the particles were respirable, but additional exposure would have occurred from skin absorption and ingestion. There were no deaths during the study. Bodyweights were transiently depressed, but there were no clinical signs or treatment-related findings at necropsy. The LC50 for the 1% formulation exceeds 3200 mg/m^3.

Soule RD & Wolf MA (1967) Results of air sampling and cholinesterase studies on rats and chicks exposed to Dursban during field applications in Brevard County, Florida. **The Dow Chemical Company. Report No: T35.12-44793-12/13, dated Sep 29, 1967 [Dow; submission 238, part 2 vol 1. A3162/5 Box 42, & Submission 11462, reference 8]**

In this poorly reported study, rats (young adults) and chicks (8-10 days) were exposed (maximum 30 mins) to either an aerial spray or thermally-generated fog of Dursban (chlorpyrifos). The aerial spray produced concentrations of Dursban from 3.9-8.1 µg/m³. While the fog contained concentrations of between 17.5-23.0 ug/m³. A maximum reduction in serum cholinesterase activity of 24% was observed in chicks and 20% in the rats at the sample time of 7-9 h after exposure. There were no signs of toxicity in either species as a result of their exposure to Dursban. This study was not of regulatory standard.

3.1.4 Eye irritation studies

Summary of eye irritation studies with chlorpyrifos technical

Species	Effects	Outcome	Reference
Rabbit	Slight conjunctival redness	Slight irritant	Taylor & Olsen (1963)
Rabbit	Conjunctival effects only at 1 h due to mechanical injury	Non-irritant	Jones (1985a)
Rabbit	Slight to moderate conjunctival redness,	Slight irritant	Jackson & Ogilvie (1994b)

	slight chemosis, slight discharge		
Rabbit	Slight conjunctival redness, (slightly less in irrigated eyes)	Slight irritant	Buch & Gardner (1980b)
Rabbit	Diffuse corneal opacities in some animals (reversible), conjunctival irritation and chemosis	Moderate irritant	Dreher (1994d)
Rabbit	Very slight iridial and conjunctival irritation at 4 h only.	Non-irritant	Anon (1995e)

Taylor ML & Olson KJ (1963) Toxicological properties of 0,0-diethyl-0-3,5,6-trichloro-2-pyridyl phosphorothioate. The Dow Chemical Company. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Few procedural details are provided in this study and the results are reported here in tabular form only.

Eye irritation studies with chlorpyrifos (purity and batch not stated)

Rabbit - eye 6 animals	undiluted	unwashed	slight conjunctival redness in 2/6 for <24h, in 1/6 for <48h and in 3/6 persisting >168h
Rabbit - eye 6 animals	undiluted	washed	as above

Jones JR (1985a) Dursban F : Eye irritation study in the rabbit. Dow Chemical Europe Report DET 424, dated Jan 25, 1985 (Hazleton Laboratories Report: 4201-50/393, dated November 1984) OECD 405 [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Three young adult female New Zealand white rabbits each had 100 mg of finely ground Dursban placed into the left conjunctival sac, with the untreated right eye serving as control. A transient conjunctival irritation was noted in all animals 1 h after treatment (mean Draize score of 5.0 for conjunctiva), and one animal showed slight iridial inflammation (mean Draize score of 0 for the iris). All animals had normal eyes when scored at 24 h, with mean Draize scores of zero. The transient irritation may have been due to a direct physical irritant effect of the powder.

Jackson D and Ogilvie SW (1994b) Chlorpyrifos Tech: Primary eye irritation test in rabbits. Inveresk Research International, Scotland. Project No: 555546; Report No: 10441; 9 August 1994. [Lerace Pty Ltd; Submission 11204; A3162/31, Box 31]

This study was conducted in compliance with OECD guideline 403, and US EPA guidelines, Subdivision F, 81-4, and in accordance with GLP requirements. The eye irritation potential of Chlorpyrifos Tech (Luxembourg Industries, Israel; batch 007/94; 99% purity) was tested in six young male New Zealand White rabbits (Harlan Olac Ltd, UK), with 100 mg of test material placed into the right eye of each rabbit, and the untreated left eyes of animals serving as controls. One rabbit was tested first and observed 1 h after instillation of test material for signs of severe irritation. In the absence of such signs, the remaining animals were treated the following day. The eyes were examined for signs of irritation at 1, 24, 48, and 72 h after treatment, and scored for irritation based on the US EPA recommended scoring system.

There were no signs of corneal or iridial irritation. Slight to moderate conjunctival redness (Mean Draize

Score (MDS); 1 h: 1; 24 h: 0.83), slight chemosis (MDS; 1 h: 0.83, 24 h: 0.16), and discharge (MDS; 1 hr: 1.6; 24 hr: 0.83) were noted up to 24 h post instillation, but no signs of irritation were reported at 48 h. Under the conditions of this study, the test material was a slight eye irritant to rabbits.

Dreher DM (1994d) Chlorpyrifos technical: Acute eye irritation test in the rabbit. Safepharm Laboratories Ltd, UK Project no: 545/57. Report dated 23 November 1994. Cheminova Agro A/S report CHA Doc No: 12-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-4, TSCA Subpart E, Section 798.4500). The study was conducted between 28 July 1994 and 4 August 1994.

The eye irritation potential of the test material (0.1 g; chlorpyrifos technical; Cheminova Agro A/S; Batch 341-HRR-153; certified purity 99.3%), was assessed in New Zealand White rabbits (3 animals/sex; David Percival Ltd, UK; 12-20 weeks old at the start of the study), by instilling the test substance into the conjunctival sac of the right eye, with the left eye remaining untreated and serving as control. One rabbit was initially treated. After consideration of the ocular responses in the first animal, the remaining five animals were treated. To minimise the initial pain on application, a drop of local anaesthetic (0.5% proxymetacaine hydrochloride) was instilled into both eyes of these animals 1-2 minutes before treatment. Assessment of ocular damage/irritation was made approximately 1, 24, 48, and 72 h following treatment, according to the numerical evaluation of Draize.

Results

Residual test material was observed around the eyes of all animals 1 h after treatment. Diffuse corneal opacities (score 1 for degree and area of opacity) were observed in two animals at the 24 h examination, but these effects had disappeared by 48 h, and no other corneal effects were observed. Iridial inflammation (score 1) was noted in four animals at 1 h, and this effect persisted for 24 h in one of these animals, and for 48 in another. No iridial effects were observed after 72 h. Conjunctival irritation was observed in all animals, with redness (score 2: diffuse, deeper crimson red at 1 to 24 h; score 1: vessels definitely injected above normal at 48 h), chemosis (score 2: obvious swelling with partial eversion of lids up to 48 h), and discharge (scores 2-3: moistening of the lids and hairs just adjacent to lids, or a considerable area around the eye; only at 1 h). No signs of conjunctival irritation was observed at 72 h.

Maximum individual irritation scores were seen at 1 h, and ranged from 12 to 19, with a mean score of 16.3. The mean scores at 24, 48, and 72 h were 9.3, 2.5, and 0, respectively. The overall scores according to EEC Council Directive 67/548/EEC were 2 (mean 0.11), 3 (0.17), 11 (0.61), and 8 (0.44), for corneal opacity, iridial inflammation, conjunctival redness, and conjunctival chemosis, respectively.

Under the conditions of this study, the test material was considered to be a moderate eye irritant in New Zealand White rabbits.

Anon (1995e) Irritation of chlorpyrifos technical to mucous membrane in rabbits. Fredrick Institute of Plant Protection and Toxicology, India. Report no. 2746, dated 16 August 1995. Project no. 05-166-95. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

Technical chlorpyrifos (100 mg; Ficom Organics, India; stated purity 96.2%; batch no. C503085) was instilled into the conjunctival sac of the left eyes of Himalayan albino rabbits (Fredrick Institute, India; 3/sex/group; 1.1-1.3 kg), and the right eyes of the animals served as controls. A group of control animals was similarly treated with distilled water only. All animals were observed for signs of ocular irritation at 1, 4, 24, 48, and 72 h, and then daily up to day 7. Ocular lesions were graded and scored according to the method of Draize.

Results

At the 4-h observation, very slight iridial irritation (score 1) and conjunctival irritation (score 1) were reported. It was not clear from the report whether the conjunctival irritation was redness, discharge or chemosis. No signs of irritation were reported at 24 or 72 h following instillation of the test material.

Under the conditions of this study, the test material was not an eye irritant in rabbits.

Buch SA & Gardner JR (1980b) Pyrinex Tech: Irritance to rabbit eye. Life Science Research, UK. LSR report 80/MAK/023/143, 30 April, 1980. [Makteshim; Submission 11471; Amalgamated Chemicals; Submission 1396]

This study was conducted in compliance with the regulations of the US EPA, 191.81-4, 1981. To test the eye irritation potential of technical chlorpyrifos (Makteshim Pyrinex technical, purity not stated), the test material (fine powder; 100 mg) was placed into the right eyes of nine young adult albino rabbits (6 females/3 males; Morton Commercial Rabbits, UK; New Zealand White strain). The behaviour of the animals was observed for several minutes after instillation of the test material to allow assessment of the initial pain response. Irrigation of the eyes of three animals was conducted using saline solution no sooner than 30 seconds after treatment, while the eyes of the remaining animals were not irrigated. Ocular examinations were conducted after 24, 48, and 72 h, and at 4 and 7 days, according to the method of Draize.

Most animals displayed slight initial pain responses following instillation of the test material. No signs of irritation were observed in the cornea nor in the iris of any animals in this study. Slight conjunctival redness was observed in all animals with unwashed eyes, and in 2/3 animals whose eyes were irrigated after 30 seconds. This irritation disappeared in most animals by days 3-4, but persisted for 7 days in a single animal (unirrigated). The mean irritation score in rabbits with unwashed eyes was 2.3 at 24 h, and 0.3 at 7 days, while in the rabbits with irrigated eyes, these scores were 1.3 and 0, respectively. Under the conditions of this study, the test material was a slight eye irritant in rabbits.

3.1.5 Dermal irritation studies

Summary of dermal irritation studies conducted with chlorpyrifos

Species	Effects	Outcome	Reference
Rabbit	Slight to mild hyperaemia, slight burn, slight exfoliation	Slight irritant	Taylor & Olsen (1963)
Rabbit	No effects	Non-irritant	Jones (1985b)
Rabbit	Slight erythema	Slight irritant	Jackson & Ogilvie (1994d)
Rabbit	Well-defined or very slight erythema (intact or abraded skin), very slight oedema (one animal)	Slight irritant	Buch & Gardner (1980a)
Species	**Effects**	**Outcome**	**Reference**
Rabbit	Very slight to well-defined erythema and very slight to slight oedema. No effects at 72 h	Slight irritant	Dreher (1994c)
Rabbit	No signs of erythema or oedema	Non-irritant	Anon (1995d)

Taylor ML & Olson KJ (1963) Toxicological properties of 0,0-diethyl-0-3,5,6-trichloro-2-pyridyl phosphorothioate. The Dow Chemical Company. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Few procedural details are provided in this study and the results are reported here in tabular form only.

Skin irritation studies with chlorpyrifos (purity and batch not stated)

Rabbit	10 daily applications undiluted & dry	intact bandaged belly skin	progression from mild hyperaemia to slight burn to slight exfoliation. Full healing in <21 d
Rabbit	9 daily applications undiluted & wet (water)	intact bandaged belly skin	progression from slight hyperaemia to slight burn (1/2) to slight exfoliation. Full healing in <21 d
Rabbit	3 daily applications undiluted & dry	abraded bandaged belly skin	progression from slight hyperaemia to slight burn (1/2) to slight exfoliation. Full healing in <21 d
Rabbit	3 daily applications undiluted & wet (water)	abraded bandaged belly skin	progression from slight hyperaemia to slight exfoliation (1/2). Full healing in <21 d

Jones JR (1985b) Dursban F: Primary skin irritation and corrosivity study in the rabbit. Dow Chemical Europe Report DET 421, dated Jan 25, 1985 (Hazleton Laboratories Report: 4176-50/392, dated November 1984) OECD 404 [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Finely ground Dursban F (500 mg, batch EK 830516110), moistened with distilled water was applied under an occlusive dressing to about 6 cm² of clipped skin on the back of three young adult female NZ white rabbits as a single 4 h application. The dressing was removed and the area washed prior to scoring for irritation using the Draize scale. A primary irritation index of 0 was obtained, indicating that Dursban F was a non-irritant to the skin of the rabbit under the conditions of this study.

Jackson D & Ogilvie SW (1994d) Chlorpyrifos Tech: Primary skin irritation test in rabbits. Inveresk Research International, Scotland. Project No: 555546; Report No: 10440; 9 August 1994. [Lerace Pty Ltd; Submission 11204; A3162/31, Box 31]

This study was conducted in compliance with OECD guideline 404, and US EPA guidelines, Subdivision F, 81-5, and in accordance with GLP requirements. The skin irritation potential of Chlorpyrifos Tech (Luxembourg Industries, Israel; batch 007/94; 99% purity) was tested in six young male New Zealand White rabbits (Harlan Olac Ltd, UK), with 500 mg of test material (moistened with water) applied under a gauze patch to the clipped intact skin on the trunk of each rabbit, and then covered with an occlusive dressing. The dressings were removed after 4 h, and the skin wiped to remove residual test material. The skin was examined for signs of irritation at 1, 24, 48, and 72 h after patch removal, and scored for irritation based on the US EPA recommended scoring system. Further assessments were conducted at 4, 5, and 6 days after patch removal to determine the reversibility of skin effects.

Slight erythema (score 1) was noted in 3/6 test sites at 1 h. This effect persisted until 72 h at one test site, and until day 5 in another animal. No skin irritation was seen at day 6. Under the conditions of this study, the test material was a slight skin irritant to rabbits.

Buch SA & Gardner JR (1980a) Pyrinex tech: Irritance to rabbit skin. Life Science Research, UK. LSR report 80/MAK/024/144, 30 April, 1980. [Makteshim; Submission 11471; Amalgamated Chemicals; Submission 1396]

This study was designed and conducted in accordance with the US EPA guidelines 163.81-5, 1978. The test material Pyrinex tech (chlorpyrifos; Makteshim; purity not stated) was tested for its skin irritation potential in six young adult male New Zealand White rabbits (Morton Commercial Rabbits, UK). Single doses of the test material (0.5 g fine powder) were impregnated onto unmedicated lint patches, and applied to shaved test sites (abraded and intact) on the dorsal skin of the rabbits. Two test patches were applied to both the intact and abraded sites of each animal. The test patches were held in place under impermeable dressings, which were removed after a 23 h exposure period. Treatment sites were then wiped with paper towel to remove any excess test material from the site, then the reactions at the test sites were assessed by the method of Draize at 24 and 72 h, and at 7 days after administration of the test material.

Well-defined or very slight erythema was seen in all animals at 24 h, both on intact and abraded sites. Very slight oedema was also seen in the intact and abraded anterior test sites of a single animal at 24 h. Very slight erythema was seen at the intact and abraded anterior test sites of a single animal at 72 h and at 7 days. No skin reactions were seen in other animals at these time intervals. The primary irritation index was calculated to be 0.67.

Under the conditions of this study, the test material was considered to be a slight skin irritant to rabbits.

Anon (1995d) Primary skin irritation of chlorpyrifos technical in rabbits. Fredrick Institute of Plant Protection and Toxicology, India. Report no. 2747, dated 15 August 1995. Project no. 05-167-95. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

Technical chlorpyrifos (500 mg; Ficom Organics, India; stated purity 96.2%; batch no. C503085) was applied to the clipped intact and abraded skin on the trunks of a group of Himalayan albino rabbits (Fredrick Institute, India; 1.2 - 1.5 kg at start of experiment; 3 animals/sex). After application of the test material, the trunks were wrapped with plastic sheets and held in place with tapes for 24 h. A control group was treated with distilled water. After the exposure period, the coverings were removed, but the report did not indicate whether the test sites were washed or cleaned at this time. The skin reactions were observed at 24 and 72 h after application for signs of irritation, and these signs were scored according to the method of Draize.

Results

No signs of erythema or oedema were reported in intact or abraded skin at the observation intervals.

Under the conditions of this study, the test material was not a skin irritant in rabbits.

Dreher DM (1994c) Chlorpyrifos technical: Acute dermal irritation test in the rabbit. Safepharm Laboratories Ltd, UK Project no: 545/56. Report dated 23 November 1994. Cheminova Agro A/S report CHA Doc No: 11-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-5, TSCA Subpart E, Section 798.4470). The study was conducted between 13 July 1994 and 16 July 1994.

The test material (0.5 g; chlorpyrifos technical; Cheminova Agro A/S; Batch 341-HRR-153; certified purity 99.3%), moistened with 0.5 ml distilled water, was applied to clipped skin areas on the back of New Zealand White rabbits (5 males: 1 female; David Percival Ltd, UK; approximately 12-20 weeks old at the start of the study). Surgical gauze was applied over the test area and secured in position with a strip of surgical adhesive tape, and then the trunk of each rabbit was wrapped in an elasticised corset. After the 4-h contact period, the corset and patches were carefully removed and the treated skin wiped with cotton wool soaked in distilled water. One hour after removal of the patches, and approximately 24, 48, and 72 h after patch removal, the test sites were examined for evidence of dermal irritation and scored according to the method of Draize.

Results

Very slight (score 1) to well-defined (score 2) erythema and very slight (score 1) to slight (score 2) oedema were observed in all animals between 1 and 24 h after patch removal. At 48 h, very slight erythema (3 animals) and very slight oedema (1 animal) only were observed. No signs of skin irritation were observed at 72 h. The sum of 24 and 72 h readings (S) was 10. The Primary Irritation Index

(S/12) was 0.8. This score translates to a mild irritant classification according to Draize.

Under the conditions of this study, the test material was considered to be a slight skin irritant in rabbits.

3.1.6 Dermal sensitisation studies

Carreon RE & Wall JM (1983) Dursban F : Guinea pig skin sensitization potential. The Dow Chemical Company. Report: HET-K-044793-056, dated August 29, 1983 [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Test A: Two groups of 10 male Hartley albino guinea pigs were treated with either Dursban (10% solution of Dursban F in Dowanol DPM/Tween 80 (9:1)) or a positive control (DER 331 epoxy resin in Dowanol DPM/Tween 80 (9:1)) in a non-standard skin sensitising assay. Each treatment consisted of the application of 0.1 ml of test substance to the clipped and occluded back-skin. There were ten consecutive daily applications and 0.2 ml of Freund's Adjuvant was injected intradermally adjacent to the treatment site at the time of the 6th treatment. Primary irritation was scored daily after patch removal. Fourteen days after the last treatment the challenge dose test material was applied uncovered to one freshly clipped flank and the solvent carrier to the other. Skin responses were scored at 24 and 48 h after application.

Test B: This entire procedure was replicated in another experiment.

Test C: Another two groups of 10 male Hartley albino guinea pigs were treated with either Dursban (10% solution of Dursban F in Dowanol DPM/Tween 80 (9:1)) or a positive control (DER 331 epoxy resin in Dowanol DPM/Tween 80 (9:1)) in a non-standard skin-sensitising assay. Each treatment consisted of the application of 0.1 ml of test substance to the clipped and occluded back-skin. There were 4 applications in 9 days, and 0.2 ml of Freund's Adjuvant was injected intradermally adjacent to the treatment site at the time of the 3rd treatment. Primary irritation was scored daily after patch removal. Fourteen days after the last treatment the challenge dose test material was applied uncovered to one freshly clipped flank and the solvent carrier to the other. Skin responses were scored at 24 and 48 h after application.

No experimental data were provided for these tests. The report states that for Tests A, B and C respectively, 4/10, 3/10 and 0/10 guinea pigs were weakly sensitised, while the positive control substance induced sensitisation in 9/10, 9/10 and 8/10 respectively. In the absence of scoring data this study cannot be adequately evaluated.

Henck JW & Lockwood DD (1978) Skin sensitization potential of Dursban F insecticide. The Dow Chemical Company. Report HET-AO-4122-(1), dated September 15, 1978. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Two groups of 10 male Hartley albino guinea pigs were treated with either Dursban (10% solution of Dursban F in Dowanol DPM glycol ether or a positive control (10% epoxy resin in Dowanol DPM /Tween 80 (9:1)) in a skin-sensitising assay reported as “repeated insult patch test”. No technical details of procedures or results are reported; however the outcome was reported as 0/10 and 10/10 for sensitisation by Dursban F and the positive control respectively. In the absence of any technical details

including scoring data, this study cannot be adequately evaluated.

Dreher DM (1994e) Chlorpyrifos technical: Magnusson & Kligman maximisation study in the guinea pig. Safepharm Laboratories Ltd, UK Project no: 545/58. Report dated 23 November 1994. Cheminova Agro A/S report CHA Doc No: 13-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-6, TSCA Subpart E, Section 798.4100). The study was conducted between 29 June 1994 and 4 August 1994.

The skin sensitisation potential of the test material (chlorpyrifos technical; Cheminova Agro A/S; Batch 341-HRR-153; certified purity 99.3%), was assessed in female albino Dunkin-Hartley guinea pigs (David Hall Ltd, UK; 8-12 weeks old at the start of the study). The test material was melted at approximately 70^{o} C prior to formulation, and freshly prepared as follows:

Intradermal induction: 25% w/v in arachis oil BP and 25% w/v in a mixture of Freund's Complete Adjuvant plus arachis oil BP (1:1)

Topical induction: undiluted as supplied.

Topical challenge: undiluted as supplied and 75% v/v in arachis oil BP

Determination of the concentration, homogeneity and stability of the test material preparations was not conducted. No concurrent positive control group was used in this study, but an historical positive control response of between 39 and 80% was reported in four studies using the same source of animals (with a variety of positive control substances) conducted at a similar time to this study, and so the test system appears to be suitable for determining the skin sensitisation potential of compounds in guinea pigs.

Range-finding studies: The concentration of test material to be used at each stage of the main study were determined by "sighting tests" as follows:

Intradermal induction: Four animals were intradermally injected with preparations of test material (1, 5, 10, or 25% in arachis oil BP), and the highest concentration that did not cause local necrosis, ulceration, or systemic toxicity was selected for this stage of the main study.

Topical induction: Two guinea pigs (intradermally injected with Freund's Complete Adjuvant twenty-one days earlier) were treated with undiluted test material and three preparations of the test material (75, 50, and 25% in arachis oil BP). The highest concentration causing only mild to moderate dermal irritation after a 48-h occlusive exposure was selected for this stage of the main study.

Topical challenge: The undiluted test material and three preparations of the test material (75, 50, and 25% in arachis oil BP) were applied to the flanks of two guinea pigs and occluded for 24 h. These

guinea pigs had been treated identically to control animals of the main study, up to day 14. The highest non-irritant concentration of the test material and one lower concentration were selected for this stage of the main study.

Main study: Thirty guinea pigs were used for the main study (twenty test and ten control). Body weights were measured at the beginning and end of the study.

Induction: A row of three injections were made to the clipped shoulder region of each animal. In test animals, the injections were:

i) Freund's Complete Adjuvant plus distilled water 1:1
ii) a 25% w/v dilution of test material in arachis oil BP
iii) a 25% w/v dilution of test material in a 1:1 preparation of Freund's Complete Adjuvant plus arachis oil BP

In control animals, the induction procedure was identical to that used in test animals, except that the injections were as follows:

i) Freund's Complete Adjuvant plus distilled water 1:1
ii) arachis oil BP
iii) Freund's Complete Adjuvant plus arachis oil BP 1:1

One week after injections, the same area on the shoulder region was clipped, and treated with a topical application of the undiluted test material. The test material (0.2-0.3 ml) was applied on filter paper, held in place by a strip of surgical adhesive tape, covered with an overlapping sheet of aluminium foil, then further secured with an elastic bandage for 48 h. The degree of erythema and oedema were quantified according to the method of Draize at 1 and 24 h after removal of the patches.

Challenge: On day 21 of the study, the undiluted test material (0.1-0.2 ml) was applied to the clipped right flank of each animal on a square of filter paper held in place by a strip of adhesive surgical tape, and occluded in a similar manner to the induction phase of the study for 24 h. The test material was also applied to a separate skin site on the clipped right flank at a 75% v/v concentration in arachis oil BP, and the vehicle alone similarly applied to the clipped left flank, and occluded. After removal of the dressing, the challenge sites were swabbed with cotton wool soaked in diethyl ether to remove residual material. Evaluation of the skin sites was made at 24 and 48 h after removal of the dressing.

Results

Very slight (score 1; 10 animals) to well-defined (score 2; 2 animals) erythema and very slight (score 1; 1 animal) oedema was observed 1 h after the topical induction in test animals, but no signs of irritation were observed at 24 h. No skin reactions were observed in control animals following topical induction.

No skin reactions were observed in at the test or vehicle control sites following topical challenge application. One animal was found dead at this stage of the study, but cause of death was not determined.

Under the conditions of this study, the test material was not a skin sensitiser in guinea pigs.

Anon (1996b) Allergy and skin sensitization potential of chlorpyrifos technical in guinea pigs. Fredrick Institute of Plant Protection and Toxicology, India. Report no. 2928, dated 15 March 1996. Project no. 05-291-95. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

The skin sensitisation potential of technical chlorpyrifos (100 mg; Ficom Organics, India; stated purity 96.2%; batch no. C503085) was tested in male Hartley guinea pigs (Fredrick Institute, India; 300-700 g), with ten animals in the test group, and five animals in each of the control and positive control groups. Chlorpyrifos technical was prepared as a 1% solution in 0.5% carboxymethyl cellulose.

Test and positive control groups received induction exposures to the shaved left flank skin area, followed by challenge exposure 14 days after the third induction dose. Control animals received a challenge exposure only to 0.5 ml of 0.1% chlorpyrifos technical.

Induction: The test material was applied to the skin on the flank of treated animals and retained under a patch for 6 h. This induction exposure was conducted on days 0, 7, and 14 (ie., three induction exposures in total). Positive control animals similarly received exposures to 0.3% 1-chloro-2, 4-dinitrobenzene).

Challenge: Test and control group animals were given a single challenge dose of 0.5 ml of 0.1% chlorpyrifos technical, and positive control animals were given 0.5 ml of 0.3% 1-chloro-2,4-dinitrobenzene, on their respective right flanks. No details were provided on the length of this challenge exposure period.

Following the challenge exposure, local skin reactions were recorded at 24 and 48 h. The total irritation score for each animal (sum of erythema and oedema scores) was calculated on a scale of 0 (no sensitisation) to seven (severe sensitisation), and the mean severity of sensitisation score was calculated. The incidence of skin sensitisation reactions was calculated as the number of animals with scores of 1 or greater, divided by the total number of animals. The sensitisation ranking was calculated as the mean severity divided by the maximum of the irritation scale (ie. seven), and the test compound was graded as a sensitiser on the basis of this ranking.

Results

Test animals displayed barely perceptible (score 1; 4/10 animals) erythema or slight oedema (score 1; 2/10 animals) at 24 h post-challenge, but no signs of irritation were observed at 48 h. Using the study's scoring system, the sensitisation ranking was 9%, and on this basis the test material was ranked as a weak sensitiser by the study authors. A single control animal displayed barely perceptible erythema at 24 h, but no other signs of skin irritation were seen in this group. Positive control animals displayed barely perceptible to deep red erythema (score 1-3) and slight to marked oedema (score 1-3) at 24 h

post-challenge, and barely susceptible erythema and slight to marked oedema at 48 h, with an incidence of 100% for these findings, and a sensitisation ranking of 40-51% (moderate skin sensitisation).

Under the conditions of this study, the test material was not considered to be a skin sensitiser in guinea pigs.

Jones JR (1985c) Dursban F: Delayed contact hypersensitivity study in the guinea pig (Buehler Test). Dow Chemical Europe. Report DET 475, dated March 20, 1985. (Hazleton Laboratories Europe, report 4311-50/422 dated January 1985). OECD 406. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

After a dose-ranging study indicated a suitable dosing regimen, young albino Dunkin-Hartley guinea pigs (6/sex) were treated with Dursban F in a standard Beuhler skin sensitisation assay. The test substance (0.3 ml, 100% w/v in polyethylene glycol on a lint pad) was applied for 6 h under an occlusive bandage to the freshly clipped left flank of the animals on days 1, 8 and 15. The challenge doses of Dursban F and solvent only were applied for 6h on day 29 to the right and left flanks respectively. The negative controls were untreated until day 29, at which time they received identical treatment to the test-substance animals. The challenge sites from both groups were evaluated at 24 and 48 h after patch removal.

There were no adverse skin reactions noted on exposure to the test substance or vehicle alone in either test or control groups.

Jackson D & Ogilvie SW (1994c) Chlorpyrifos Tech: Magnusson-Kligman maximisation test in guinea pigs. Inveresk Research International, Scotland. Project No: 555546; Report No: 10442; 9 August 1994. [Lerace Pty Ltd; Submission 11204; A3162/31, Box 31]

This study was conducted in accordance with OECD Guideline 406 and US EPA Guidelines, Subdivision F, 81-6. The skin sensitisation potential of Chlorpyrifos Tech (Luxembourg Industries, Israel; batch 007/94; 99% purity) was tested in young female Dunkin-Hartley guinea pigs (David Hall Ltd, UK), using the Magnusson-Kligman Maximisation Test. The test consists of an induction procedure, with intradermal injections of the test material, followed after one week by topical application, and a challenge procedure conducted three weeks after the induction procedure. A preliminary dose-ranging study was conducted using eight guinea pigs, with injections made to the shaved scapular region and topical applications made to the shaved flanks of test animals. Four guinea pigs were subjected to injections of test material (75%, 50%, 25%, 10%, 5%, 2%, and 1% w/v in maize oil) and four guinea pigs exposed to topical applications of test material (75%, 50%, 25%, 10%, 5%, 2%, and 1% w/v in maize oil). The test sites were assessed for irritation at 24, 48, and 72 h after injection, and at 24 and 48 h after patch removal (48-h exposure). Following the range-finding study, a concentration of 25% test material in maize oil was selected for the injection induction phase of the main study, and 75% test material in maize oil selected for the topical induction phase of the study. A concentration of 75% in maize oil was also selected for the challenge phase of the main study.

In the main study, hair was shaved from the scapular region of 20 test animals and 10 control animals. Test animals received six intradermal injections, with two injections of each of the following: 0.1 ml Freund's Complete Adjuvant (FCA); 0.1 ml test material (25% in maize oil); and 0.1 ml of a 50:50 emulsion of test material:FCA (final concentration of test material 25%). The control animals were

similarly treated using the vehicle instead of test material. The test sites were examined at 1 h and 24 h after injection. Six days after the injection phase, the injection sites were again shaved and wetted with a 10% aqueous solution of sodium lauryl sulfate to enhance the possibility of sensitisation. After 24 h, a patch containing test material (75% in maize oil; dose volume not stated) was applied to the test site on each animal and covered with an occlusive dressing for 48 h. The test sites were examined at 1 h and 24 h after patch removal. Two weeks after the start of topical induction, test and control animals were challenged with a 75% solution of test material in maize oil, and with the vehicle alone. These applications were made to the shaved left flank of animals, and held in place for 24 h, using a similar method to that used for the topical induction applications. The degree of response was determined approximately 24 and 48 h after removal of the challenge patch.

No concurrent positive control was used in this study, but the sensitivity of the guinea pigs used in this study was assessed periodically by the testing facility using 2-mercaptobenzothiazole, a known sensitiser.

During the induction phase of the study, slight or discrete erythema was observed in all animals (test and control) at 1 and 24 h after injection, and at 1 h after topical application. A number of animals, test and control, also displayed such erythema at 24 h after topical application. During the application procedure, two test group animals were ataxic, thin, and subdued, and were removed from the study. Prior to the challenge application, a single test animal was removed from the study due to lesions on the dorsal test site. A single test animal showed a positive response to challenge, with slight to discrete erythema observed. No vehicle control animals reacted to the challenge application. Under the conditions of this study, the test material was not a skin sensitiser to guinea pigs.

Berman CL (1987) Evaluation of chlorpyrifos (Pyrinex) for dermal sensitization of Guinea pig. Arthur D Little Inc, USA, Report ADL 59487-01, 21 October 1987. [Makteshim; Submission 11471]

This study was conducted in compliance with the GLP regulations of the US EPA (40 CFR Part 160). The skin sensitisation potential of technical chlorpyrifos (Makteshim; Batch 489205; 96.8% purity) was tested in albino guinea pigs (Dunkin-Hartley; Hazleton Research Products, USA). The test material was dissolved in 100% dimethylsulfoxide (DMSO), and diluted in DMSO to achieve the desired test concentrations. Dinitrochlorobenzene (DNCB) was prepared at a concentration of 0.1% (w/v) for use as a positive control, and solubilised on the day of use by the addition of Tween 80. Test material was applied to the shaved flank regions of animals, with induction applications made to the left flank, and challenge applications to the right flank. Test or control materials (0.2-0.5 ml) were applied to a patch of filter paper or cotton and placed on the test site, under an occlusive bandage, for 6 h. After the test periods, the dressings were removed, and the test sites washed with 70% ethanol.

A dose range-finding study was conducted to determine the highest non-irritating concentration of the test material, with animals exposed to test material at concentrations ranging from 0.25 to 5.0% chlorpyrifos in DMSO for six hours. Based on the results of this range finding study, a chlorpyrifos concentration of 1.5% was selected for the main study.
In the main study, three groups of animals were used. One group of 20 animals (Group II) was sensitised and challenged with chlorpyrifos; a group of 10 animals was challenged with chlorpyrifos without any prior sensitisation period (Group I); and a positive control group (Group III; 10 animals) was sensitised

and challenged with 0.1% DNCB. Animals in Groups II and III each received three induction doses of the appropriate material (one application/week for three weeks). The first induction application to Group II was with 1.5% chlorpyrifos. As this resulted in a slight skin reaction, subsequent applications to animals in Groups I and II were conducted with 1.0% chlorpyrifos. Two weeks after the final induction dose, all animals were challenged, with test or control materials applied for a six hour period. Twenty-four hours after challenge application, the area was treated with a depilatory, and cleaned with 70% ethanol. Dermal reactions were scored approximately one hour after the sites were cleaned. Forty-eight hours after the challenge dose, test sites were again scored for dermal reactions.

In the animals treated with a single chlorpyrifos dose (Group I), irritation ranging from scattered mild redness to moderate and diffuse redness was seen at 24 h (5/10 animals) and 48 h (4/10 animals; mild redness only). In animals induced and then challenged with chlorpyrifos (Group II), only 1/20 animals displayed scattered mild redness after the challenge dose at 24 h, and no skin reaction was seen at 48 h. The positive control group displayed mild redness at the 24 h inspection (9/10 animals) and at 48 h (4/10 animals).

Under the conditions of this study, the test material was not a skin sensitiser in guinea pigs.

3.1.7 Potentiation Studies

Anon (1964) Potentiation studies with Dursban in combination with Ruelene and Malathion in rats. The Dow Chemical Company. Report dated April 22, 1964. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

No substantial procedures or technical details were provided for this study. LD50s were calculated for Dursban, Ruelene and Malathion from separate experiments in which single oral doses of the compounds in corn oil were administered at 5 different doses to groups of 5 male rats. The experimentally derived LD50s (95% CI) were 245 mg/kg (219-273 mg/kg) for Dursban, 1020 mg/kg (880-1880 mg/kg) for Ruelene and 1370 mg/kg (1280-1470 mg/kg) for malathion. Additional studies using similar methods determined the LD50 resulting from co-joint administration of the compounds as well as studies where Dursban was administered four hours prior to the dose of Ruelene or Malathion. The Dursban/Ruelene combination showed no potentiation under either procedure, but Dursban/Malathion showed a 3-fold potentiation after both procedures with LD50s of about 135 mg/kg (co-joint) and 158 mg/kg (4 h apart) vs 420 mg/kg "expected". This study was not of a regulatory standard.

Norris JM (1970) Potentiation study on Dowco 179 and Vapona insecticide. The Dow Chemical Company Report # T35.12-44793-24, dated January 13, 1970. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

No substantial procedures or technical details were provided for this study. LD50s were calculated for Dowco 179 (97% chlorpyrifos), Vapona insecticide (93% chlorpyrifos), and a 1:1 mixture of the two based on 4 or 5 oral doses in corn oil administered to fasted male albino Sherman rats (5/dose). The experimentally derived mg/kg LD50s (95% CI) were 118 (77-181), 59 (38-91) for Dowco 179 and Vapona insecticide respectively. The 1:1 mixture of the two products yielded an LD50 of 135 mg/kg (97-188) cf the "expected" value of 79 mg/kg, and hence no potentiation was inferred. This study was not of regulatory standard.

3.1.8 Antidote treatment

Davies RE & Kynoch SR (1970) Acute subcutaneous toxicity of Dursban to rats and the effect of treatment with 1. Atropine sulphate 2. Atropine sulphate and PAM . Report variously labelled : A1A-275, Dow Chemical Europe 3641/70/453, dated 22 September, 1970. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42, Submission 11462, reference 15]

Main Study: Following a preliminary range-finding study, fasted CFY rats (70-104g, 5/sex/group) were injected subcutaneously with Dursban (Dowco 179, lot CD-935, 97%) in a vehicle of Tween 20:dimethyl sulphoxide:water in ratio 2:3:5 and injection volume ca. 0.1 ml. Doses ranged between 0 and 200 mg/kg (6 dose levels) and animals were observed for two weeks post-dose. Signs included lethargy, tremors, excessive lacrimation and salivation and bradypnoea, and deaths occurred from 19h-4 d, but survivors appeared normal at two weeks. All animals at 200 mg/kg died, and the derived LD50 (95% CI) was 147 (120-179) mg/kg.

Atropine sulphate treatment: Groups of rats (5/sex/dose) received subcutaneous injections of Dursban (7 dose levels, 0-320 mg/kg) in a similar procedure to the main study with each animal receiving a subcutaneous injection of atropine sulphate (1.74% in water, 1.0 ml/kg) four hours after Dursban dosing or when signs of toxicity were observed, and subsequently at 24 h intervals for seven days. Deaths occurred within 22 h to 5 d. The derived LD50 was 205 (165-254) mg/kg.

Atropine sulphate + PAM: Groups of rats (5/sex/dose) received subcutaneous injections of Dursban (8 dose levels, 0-800 mg/kg) in a similar procedure to the main study with each animal receiving a subcutaneous injection of atropine sulphate (1.74%) and PAM (2-hydroyiminomethyl-1-methyl pyridinium methiodide) (5%) in water at 1.0 ml/kg 4 h after Dursban dosing or when signs of toxicity were observed, and subsequently at 24 h intervals for seven days. Deaths occurred within 22 h to 6 d. The derived LD50 was 540 (365-799) mg/kg.

Humiston CG (1969) A study of possible antidotes for Dursban exposures. The Dow Chemical Company, File No. T35.12-1244793-23, undated. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

This study reports experiments with rats and dogs which investigate the effectiveness of PAM and atropine as antidotes to Dursban toxicity. This study was not evaluated as it was not of regulatory standard, there being insufficient technical detail provided. This study was not of regulatory standard.

Swart RW (1968) Tests to determine the antidotal activity of atropine and protopam chloride for Dursban toxicity. The Dow Chemical Company Study No:TA-423, dated October 1, 1968 [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

This study reports experiments with cattle which investigate the effectiveness of pralidoxime chloride and atropine as antidotes to Dursban toxicity. This study was not evaluated as it was not of regulatory standard, there being insufficient technical detail provided. This study was not of regulatory standard.

3.2 Acute Toxicity of Chlorpyrifos End Use Products

3.2.1 Dursban 20 MEC

Summary of acute toxicity of Dursban MEC formulation (20% aqueous solution of microencapsulated chlorpyrifos).

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rat	albino	M+F	Oral	undiluted	LD50: >25850 mg/kg	Hart & O'Malley (1985a)
Rabbit	NZ white	M+F	Dermal	undiluted	LD50: >10000 mg/kg	Hart & O'Malley (1985b)
Rabbit	NZ white	F	Eye Irritation	undiluted	Slight eye irritant	O'Malley & Ebbens (1985)
Rabbit	NZ white	F	Skin irritation	undiluted	Slight skin irritant	Hart & O'Malley (1985c)

Hart GE & O'Malley KD (1985a) Acute Oral Toxicity Study with ACP 5-85, MC00002 (Formulated) in Albino Rats. Riker Laboratories, Inc., St. Paul, Minnesota USA Study No. 0985AR0152, conducted 9 April to 9 May, 1985 and dated 13 May 1985. [Dow; Submission 1073, reference A3162/15, B18]

(Quality-assured study claimed to comply with GLP [EPA FIFRA] but no GLP statement signed or test guidelines identified)

Acute toxicity data was provided on Dursban 20 MEC manufacturing concentrate, a 20% aqueous solution of microencapsulated chlorpyrifos. This was given the code name ACP 5-85 (MC00002).

The test material was administered by gastric intubation to fasted male and female albino rats (5/sex/dose; Charles River Breeding Laboratories, USA, age unstated, initial bodyweight 193-236 g) at doses of 1.03, 10.34, and 25.85 g/kg of the formulation, corresponding to doses of 206, 2068, and 5170 mg/kg body weight of chlorpyrifos, with dose volumes of 1, 10, and 25 ml/kg, respectively, at the doses above. The test material was administered undiluted. After administration of the test material, the animals were observed for 14 days, at which time surviving animals were sacrificed, and gross necropsies were conducted, in which the heart, lungs, liver, kidneys and gastrointestinal tract were examined.

A single female died on day three of the study, at a dose of 10.34 g/kg test material. This animal displayed dyspnoea and salivation on day two of the study. No other mortality or significant clinical signs associated with chlorpyrifos administration were reported during the study, with the exception of salivation and hyperacusis in a single high dose male, which were seen only at 6 hours post dosing. At necropsy, the decedent displayed haemorrhagic lungs, but no abnormalities were observed among the animals which survived until termination.

Under the conditions of this study, the acute oral LD50 for the test material was >25.85 g/kg body weight (approximately >5170 mg chlorpyrifos/kg body weight).

Hart GE & O'Malley KD (1985b) Acute Dermal Toxicity Screen with ACP 5-85, MC00002 (Formulation) in Albino Rabbits. Riker Laboratories, Inc. Study No. 0985AB0151, conducted from 22 April to 6 May 1985 and dated 8 May 1985. [Dow; Submission 1073, reference A3162/15, B18]

(Quality-assured study claimed to be conducted in accordance with the US EPA FIFRA Good Laboratory Practice Regulations, but no compliance statement signed and no test guidelines specified).

The acute dermal toxicity potential of Dursban 20 MEC (ACP 5-58 (MC00002), an aqueous formulation containing 20% microencapsulated chlorpyrifos) was determined using male and female New Zealand albino rabbits (Hazleton-Dutchland; 5/sex/dose, initial bodyweight 1.93 - 2.48 kg). A single dose of the undiluted test material (10 000 mg/kg) was applied to the clipped intact skin of each animal, and retained in place for 24 h under an occlusive plastic dressing, after which time the residual test material was removed with water. To prevent oral ingestion of the test compound, rabbits were fitted with flexible collars. The animals were weighed on days 0, 7 and 14 and observed for mortality and behaviour for fourteen days after administration, at which time the survivors were sacrificed and necropsied grossly including examination of the heart, lungs, liver, kidneys and gastrointestinal tract.

No mortality occurred during the study, and no clinical signs of intoxication, skin irritation or pathological abnormalities were observed. Under the conditions of this study, the dermal LD50 for the test material, containing 20% chlorpyrifos, was >10 000 mg/kg.

O'Malley KD & Ebbens KL (1985) Acute Ocular Irritation Test with ACP 5-85, MC00002 (Formulation) in Albino Rabbits. Riker Laboratories, Inc., St. Paul, Minnesota USA Study No. 0985EB0150 conducted from 2 April to 9 April 1985 and dated 7 May 1985. [Dow; Submission 1073, reference A3162/15, B18]

(Quality-assured study claimed to be conducted in accordance with the US EPA FIFRA Good Laboratory Practice Regulations, but no compliance statement signed and no test guidelines specified).

A 0.1 ml volume of the test substance (Dursban 20 MEC, coded ACP 5-85 (MC0002), an aqueous formulation containing 20% microencapsulated chlorpyrifos) was instilled into the conjunctival sac of the right eye of six female New Zealand albino rabbits (Hazleton Dutchland, age and initial bodyweight unstated), with the left eye acting as the untreated control. The treated eyes were not washed, and were examined at 1 hour, and 1, 2, 3 and 7 days.

At 1 h post treatment, very slight conjunctival redness, and chemosis and/or discharge were observed in four animals, but these effects had disappeared at the examination at 1 day. The mean Draize score for the group at the one hour examination was 2.7, and this was the total mean score for the study, with no other ocular irritation seen during the study. The test substance did not cause any damage to the cornea or iris. Under the conditions of this study, the test substance was considered to be a slight eye irritant in rabbits.

Hart GE and O'Malley KD (1985c) Primary Skin Irritation Test with ACP 5-85, MC00002 (Formulation) in Albino Rabbits. Riker Laboratories, Inc., St. Paul, Minnesota USA Study No.

0985EB0149, conducted from 2 April to 5 April 1985 and dated 7 May 1985. [Dow; Submission 1073, reference A3162/15, B18]

(Quality-assured study claimed to be conducted in accordance with the US EPA FIFRA Good Laboratory Practice Regulations, but no compliance statement signed and no test guidelines specified).

A 0.5 ml volume of the test substance (Dursban 20 MEC, coded ACP 5-85 (MC0002), an aqueous formulation containing 20% microencapsulated chlorpyrifos) was applied to two sites on the clipped backs and flanks of six female New Zealand albino rabbits (Hazleton Dutchland, age and initial bodyweight unstated). On the day of application, one site was abraded by making four epidermal incisions, while the other was left intact. The test substance was applied to the sites, immediately covered with a gauze patch, and the trunk of each animal was then wrapped in an impervious plastic sheet for 24 h. The dressings were then removed and any residual test article removed by washing with water. The test sites were examined for signs of skin irritation at one hour and 48 hours after removal of the test article.

Barely perceptible erythema was observed on the abraded skin of two animals, and on the intact skin of one animal after 1 h but not at 48 h. No other signs of irritation were observed during the study. The mean scores for erythema and oedema of abraded sites was 0.3, and for intact skin was 0.2, with an overall Primary Irritation Index of 0.1/8.0. Under the conditions of this study, the test substance was considered to be slightly irritating to the skin of rabbits.

3.2.2 Dursban 50W and Lorsban 50W & WP and XRM-4700

Carreon RE &Yano BL (1985) Dursban 50W: Acute oral toxicity in rats and rabbits. Dow Chemical USA Report HET M-004700-002, dated May 6 1985. EPA guidelines 81-1. QA. [Dow; Submission 11462, reference 40]

Rats

The diluted test material (25% aqueous dilution of Dursban 50W) was administered by gastric intubation to male and female Fischer 344 rats (6/sex/dose; Charles River Breeding Laboratories, USA) at doses of 320, 630 or 1300 mg/kg of the formulation; an additional group of males received 500 mg/kg and an additional group of females received 160 mg/kg. Rats were weighed weekly, observed for two weeks after dosing and then necropsied.

Lethargy and body tremors were noted at all doses, while excessive lacrimation, salivation, diarrhea and rapid shallow respiration were noted at some doses. No animals died at 160 mg/kg, 1female died at 320 mg/kg, 2 males at 500 mg/kg and all animals at 630 and 1300 mg/kg died. Deaths occurred on days 1-5. Bodyweights of survivors were normal, and there were no findings at necropsy. The LD50 for male rats was 494 (429-583) mg/kg, and for females was 382 (289-553) mg/kg.

Rabbits

The diluted test material (25% aqueous dilution of Dursban 50W) was administered by gastric intubation to male and female NZW rabbits (5/sex/dose; Hazleton, Dutchland Inc., USA) at doses of 500, 1000

or 2000 mg/kg of the formulation. Rabbits were weighed weekly, observed for two weeks after dosing and then necropsied.

Decreased appetite was noted at all dose levels. Lethargy, body tremors, salivation, diarrhea and rapid shallow respiration were noted at 2000 mg/kg. Animals which died spontaneously exhibited facial/perineal soiling with faeces and fluid, decreased faecal content and secondary non-specific gastrointestinal irritation. No animals died at 500 mg/kg, 1 male and 1 female at 1000 mg/kg and 5/5 males and 4/5 females at 2000 mg/kg, with deaths occurring within 1-2 days. There were no findings at necropsy. The LD50 for male rabbits was 1175 (854-1814) mg/kg, and for females was 1414 (925-4231) mg/kg.

Nitschke KD & Yano BL (1983) Dursban 50W insecticide: An acute aerosol inhalation study. Dow Chemical USA report No.: A1A-255, signed December 1983 [Dow; Submission 11462, reference 41]

Rats (Fischer 344, 10-weeks old, 5/sex/group) were whole body exposed for 4 h to filtered air or 1500 mg/m^3 of the test substance, identified as Dursban 50W insecticide, Lot #DM83011705, composed of Dursban F 53.2% and inerts 46.8%. Animals were weighed and clinical signs recorded regularly during a 2 week post-exposure period, prior to necropsy and gross pathology examination.

The measured TWA concentration in the exposure chamber was 1500 mg/m^3, and the MMAD of the test material was 5.5 microns (raw data not provided). All animals survived till scheduled sacrifice. The test substance was observed for several days post-exposure as a brownish material on the face and forepaws of some (females only) rats. No raw data was presented for survival, bodyweights, clinical signs or the gross pathology findings. The authors state that: hyperexcitability was recorded in one female rat on days 5-7 post-exposure; bodyweights were decreased on day 1, but bodyweight gains recovered to control values thereafter; there were no treatment-related findings at necropsy and gross pathology examination. Under the conditions of this study the LC50 for Dursban 50W insecticide exceeded 1500 mg/m^3.

Jeffrey MM (1986b) Lorsban 50 WP Insecticide: Primary dermal irritation study in New Zealand White rabbits. Dow Chemical USA Report HET M004700-003C dated January 16 1986. Guideline: EPA 81-5; GLP (dated 1991) [Dow; Submission 11462, reference 36]

Lorsban 50WP insecticide (Dow Chemical Canada, lot reference GHD-1109-84; containing 49.4% technical chlorpyrifos (Dursban F); 0.5 g of water wettable grey powder) was applied to the clipped back skin of six (1M, 5F) NZ white rabbits (Hazleton Dutchland, USA), under a moistened gauze patch (2.5 cm^2) for 4 h. The patch was then removed and the application site was wiped clean. The application sites were scored for erythema, oedema and necrosis at 30 minutes, 24, 48 and 72 h after patch removal. This study was conducted in accordance with the intent of GLP.

Scores were uniformly zero at 30 minutes, and all other scoring times. the Primary Irritation Index was 0.0. Three rabbits (males) displayed slight erythema at 24, 48 and 72 h. Under the conditions of this study, Lorsban 50WP insecticide was not a skin irritant in rabbits.

Streeter CM & Carreon RE (1986) Lorsban 50 W: Dermal sensitization potential in guinea pigs. Dow Chemical USA Report No.: HET M-004700-003, report dated January 8, 1986; GLP undated; QA; Guideline: EPA 81-6. [Dow; Submission 11462, reference 37]

Lorsban 50W insecticide (Dow Chemical Canada, lot reference GHD-1109-84; containing 49.4% technical chlorpyrifos (Dursban F); grey powder) was tested in a skin sensitisation assay in ten male Hartley albino guinea pigs (Charles River Breeding Laboratories, USA). In the induction phase, the animals were shorn and depilated (day 0) and then received four dermal applications (days 2, 5, 7, 9) of 0.1 ml of the diluted test formulation (10% in Dowanol DPM (dipropylene glycol monomethyl ether/Tween 80; 9:1)) on gauze patches under occlusive dressing. The patches remained in place for 48 h prior to removal and replacement with a fresh patch. At the time of the 3rd application (day 7), Freund's Adjuvant was intradermally injected at multiple points adjacent to the patch. An additional group of 10 guinea pigs was similarly treated with a positive control DER331 (10% epoxy resin) substance. Each time the patches were removed, observations for erythema and/or oedema were made.

In the challenge phase, two weeks after the fourth application, the test material or the positive control was applied to one shorn flank, and the solvent to the other shorn flank of the appropriate animals and left uncovered. The application sites were graded for sensitization response at 24 and 48 h after the challenge application.

Only summary results were provided. When challenged with the positive control material, slight to marked erythema was noted in 10/10 animals at 24 and 48 h. When challenged with the test formulation, no erythema was observed at any test sites at either 24 or 48 h. Under the conditions of this study, Lorsban 50W insecticide was not considered to be a skin sensitiser in guinea pigs.

3.2.3 Dursban TC Insecticide

Summary of acute toxicity of Dursban TC formulation.

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rat	Fischer 344	M F	Oral	aqueous	LD50: 226 mg/kg LD50: 50-500 mg/kg	Carreon et al (1985)
Rabbit	NZ white	M+F	Oral	undiluted	LD50: >500 mg/kg	Jeffrey et al (1986b)
Rabbit	NZ white	M F	Dermal	undiluted	LD50: 1265 mg/kg LD50: 930 mg/kg	Jeffrey et al (1986a)
Rat	Fischer 344	M F	Inhalation	undiluted	LC50: 3900 mg/m^3 2600-3600 mg/m^3	Streeter et al (1985)
Rabbit	NZ white		Eye Irritation	undiluted	Severe eye irritant	Carreon (1985b)
Rabbit	NZ white	M+F	Skin irritation	undiluted	Slight skin irritant	Carreon (1985a)
Guinea pig	albino	M	Skin sensitisation	undiluted	Moderate skin sensitiser	Carreon (1986b)

Carreon RE, Battjes JE & Eisenbrandt DL (1985) Dursban TC Insecticide : Acute oral toxicity study in Fischer 344 rats. The Dow Chemical Company Laboratory Report Code: HET-M-004795-001A, 16 August 1985. [Dow; Submission 1080]

The study was conducted according to the New and Revised Health Effects Test Guidelines of the US EPA, 81-1, 1984, and a quality assurance statement was issued for this study. The test material (Dursban TC; containing 44.6% chlorpyrifos) was administered to eight groups of Fischer 344 rats (Charles River Breeding Laboratories, US; 7-week old; 6/sex/dose) via oral gavage (undiluted or as a 10% aqueous solution) at doses of 50, 500, 1000, or 2000 mg test material/kg body weight. Food was provided to all rats following dosing, and animals were observed for 14 days after treatment. All animals were submitted for a complete necropsy examination.

No animals died at 50 mg/kg, and no clinical signs of intoxication were observed at this dose level. At 500 mg/kg, 5/6 males died between days 2-4, and 6/6 females died by day 2 of the study. At 1000 and 2000 mg/kg, all animals died before day 2 of the study. At doses of 500 mg/kg and above, clinical signs consistent with organophosphate intoxication were observed in animals, and included lethargy, loss of motor coordination, body tremors, excessive lacrimation/salivation, diarrhea, laboured breathing, and unconsciousness. The time of onset of these signs decreased with increased dose levels.

The LD50 was calculated to be 226 mg/kg for male rats (95% confidence interval 100-413 mg/kg), and between 50 and 500 mg/kg for females.

Jeffrey MM, Battjes JE & Yano BL (1986b) Dursban TC Termiticide : Acute oral toxicity study in New Zealand white rabbits. The Dow Chemical Company, Laboratory Report Code: HET-M-004795-001E, 17 March, 1986. [Dow; Submission 1080]

This study was conducted in accordance with the intent of the Good Laboratory Practices for Non-Clinical Studies; the US EPA; FIFRA November 1983; EPA Guidelines 1982. The test material (Dursban TC; Dow Chemical, USA; containing 42.8% chlorpyrifos) was administered undiluted by oral gavage to groups of New Zealand white rabbits (source not stated; 5/sex/dose) at doses of 200 or 500 mg test material/kg body weight. Animals were observed for 14 days following treatment. All animals that survived the study period were sacrificed and submitted to pathological examination.

Mortality was confined to a single female from the 500 mg/kg dose group, with the animal found dead on day 4 of the study, apparently from pneumonia which may have occurred due to administration of the test material into the lung. Decreased appetite was reported for all males at 200 mg/kg, and for all animals at 500 mg/kg, but no other clinical signs of intoxication were reported. Gross pathological examination did not reveal any effects that were attributed to chlorpyrifos administration.

Under the conditions of this study, the oral LD50 in rabbits was >500 mg/kg.

Jeffrey M M, Battjes JE & Lomax LG (1986a) Dursban TC Termiticide : Acute dermal toxicity study in New Zealand white rabbits. The Dow Chemical Company, Laboratory Report Code: HET-M-004795-001E; 31 January 1986. [Dow; Submission 1080]

The study was conducted in accordance with the intent of the GLP for Non-Clinical Study (Federal Register, Dec 22, 1978, Part II, vol 43, no 247, 59986-60025), the US EPA: FIFRA GLP Procedures (Federal Register, Nov 29, 1983, Part IV, Vol 48, No 230, 53946-53969); Pesticide Assessment Guidelines: Subdivision F-Hazard Evaluation, Human and Domestic Animals, EPA, October 1982. The

undiluted test material (Dursban TC; Dow Chemical, USA; containing 44.6% chlorpyrifos) was applied to the clipped dorsal and ventral trunk regions of New Zealand white rabbits (Hazleton Dutchland, USA; 5/sex/dose) at doses of 200, 800, or 2000 mg test material/kg body weight, and retained in place under a gauze dressing and an occlusive covering for 24 hours. After the treatment period, the dressings were removed and any residual test material removed by washing with mild soap and water. Animals were observed closely for 14 days after treatment. All animals were subjected to necropsy examination.

At 200 mg/kg, no animals died, and no clinical signs of intoxication were reported. At 800 mg/kg, 1/5 males died (day 5) and 1/5 females died (day 4), while at 2000 mg/kg, 4/5 males and 5/5 females died (between days 2-4). Clinical signs of intoxication at 800 and 2000 mg/kg included loss of appetite, loss of motor coordination, reluctance to move, lethargy, shallow respiration. Slight to moderate erythema, oedema, and necrosis were reported in animals at all dose level, and in addition marked erythema was observed in animals at 800 mg/kg.

Under the conditions of this study the dermal LD50 was calculated to be 1265 mg/kg for males (95% confidence interval 625-7809 mg/kg), and 930 mg/kg for females (547-1913 mg/kg).

Streeter CM, Battjes JE & Johnson KA (1985) Dursban TC Insecticide : An acute aerosol inhalation study of formulation XRM-4795 with rats. The Dow Chemical Company, Laboratory Report Code: HET M-004795-002, 14 May 1985. [Dow; Submission 1080]

The study phases of this report were inspected by Quality Assurance staff in accordance with FDA and EPA GLP regulations. The acute inhalational toxicity of Dursban TC (Dow; containing 44.6% technical chlorpyrifos) was investigated in Fischer 344 rats (Charles River Breeding Laboratories; approximately 10 weeks old at initiation of treatment; 6 animals/sex/dose unless otherwise stated), with animals exposed to a single four-hour, whole-body exposure of the test material under dynamic airflow conditions at doses of 0, 2600 (females only), 3600, and 4700 mg/m^3. The mean mass median aerodynamic diameter of these aerosols was 1.97 µ, while the mean geometric standard deviation of the particle size distribution was 2.04. Animals were routinely observed at least once a day for two weeks after treatment for clinical reactions. Gross pathological examinations were conducted on all rats surviving at least one day post exposure.

No control animals died during the study, and no females died at 2600 mg/m^3. At 3600 mg/m^3, 1/6 males and 6/6 females died within 3 days of exposure, while all animals exposed to 4700 mg/m^3 of the test material died within 2 days of exposure. During the exposure period, rats displayed clinical signs of intoxication, including lacrimation, salivation, shallow breathing, and incoordination. Clinical signs in surviving animals post exposure included chromodacryorrhea, inguinal urine stain, reddish perinasal encrustation, tremors, and hypersensitivity.

Under the conditions of this study, the 4-h LC50 for the test material was determined to be 3900 mg/m^3 in male rats, and between 2600 and 3900 mg/m^3 in female rats.

Carreon RE (1985b) Dursban TC: Primary eye irritation study in New Zealand white rabbits. The Dow Chemical Company Laboratory Report Code: HET M-004795-001(D), 8 April 1985. [Dow; Submission 1080]

This study was conducted in accordance with Pesticide Assessment Guidelines of EPA; Subdivision F-Hazard Evaluation; Human and Domestic Animals; 81-4, October 1982. Dursban TC (Dow; containing 44.6% technical chlorpyrifos; 0.1 ml) was instilled as a single application into the right eyes of six NZ white rabbits (Hazleton Dutchland, USA), and these treated eyes were left unwashed. The left eye acted as an untreated control. Examination of the eyes occurred at 1, 24, 48 and 72 hours, and 7, 14 and 21 days, post-instillation.

Following application, rabbits appeared to experience moderate discomfort. Examination of the treated eyes at 1 h revealed moderate conjunctival redness and swelling (irritation score of 1 or 2), discharge (score 1), reddening of the iris (score 1) and slight opacity of the cornea. At 24 h, the conjunctival effects were most noticeable in the majority of animals (irritation scores of 2 or 3 for chemosis, redness, and discharge), with diffuse, crimson redness, and individual vessels not easily discernible; obvious swelling with partial eversion of lids or swelling with the lids about half closed; and discharge, with moistening of the lids and hairs, and considerable area around the eyes. By day 7 post treatment, easily discernible translucent areas of the cornea were seen in two animals (irritation score 2), with another two animals displaying scattered or diffuse areas of opacity (irritation score 1). Iridial redness was seen in all animals for at least 3-7 days. All signs of irritation were resolved in all but one rabbit at 21 days post-exposure. Under the conditions of this study, Dursban TC was a severe eye irritant in the rabbit.

Carreon RE (1985a) Dursban TC: Primary dermal irritation study in New Zealand white rabbits. The Dow Chemical Company, Laboratory Report Code: HET M-004795-001(C), 8 April 1985. [Dow; Submission 1080]

This study was conducted in compliance with FDA and EPA GLP regulations, and Pesticide Assessment Guidelines of the EPA; Subdivision F-Hazard Evaluation: Human and Domestic Animals; 81-5, October 1984. A single dose of 0.5 ml of undiluted Dursban TC (Dow; containing 44.6% technical chlorpyrifos) was applied under a gauze patch to the healthy intact dorsal clipped skin of 2 male and 4 female NZ white rabbits (Hazleton Dutchland USA) for a contact period of 4 h. The treatment site was covered with an occlusive dressing following application of the test substance, and the test site was washed with water at the end of the exposure period.

The 4-h exposure resulted in very slight (1/6 animals) to well-defined (3/6 animals) hyperaemia, but no oedema was observed in any animals. Hyperaemia was still evident at 72 h post-application, with an irritation score of 2 in one animal, and a score of 1 in two animals. The Primary Irritation Score was calculated to be 0.63/8.0. Under the conditions of this study, Dursban TC was a slight skin irritant in the rabbit.

Carreon RE (1986b) Dursban TC Insecticide Formulation(s): Dermal sensitization potential in the guinea pig. The Dow Chemical Company, Laboratory Report Code: HET M-004795-001(F), 17 February, 1986. [Dow; Submission 1080]

This study was conducted in accordance with the intent of the Good Laboratory Practices for Non-Clinical Studies (Federal Register, December 1978); the Environment Protection Agency; FIFRA GLP Procedures (Federal Register, November 1983), EPA Guidelines (October 1982).

To determine the skin sensitisation potential of the formulation Dursban TC (Dow; containing 44.6% technical chlorpyrifos), ten male Hartley albino guinea pigs (Charles River Breeding Laboratories, USA) received four dermal applications of 0.1 ml of the test formulation as a 10% Dowanol DPM solution (dipropylene glycol monomethyl ether/Tween 80; 9:1) made to the clipped back regions over a period of 10 days. Additional groups of 10 guinea pigs each were similarly treated with a vehicle control (the Dursban TC formulation without the active ingredient Dursban XP), and a positive control (10% epoxy resin) substance. Each application was made under a gauze patch then covered with an occlusive dressing for 48 h. After the first application was removed, a second application (0.1 ml) was made. At the time of the third application, a total of 0.2 ml of Freund's Adjuvant was injected intradermally at multiple points adjacent to the application site. Forty-eight hours after this application, the patch was again removed, and a fresh application of 0.1 ml of test material was made. Each time the patches were removed, observations for erythema and/or oedema were made.

A 2-week break from treatment followed the induction period. During the induction period the treatment site was regularly examined for skin reactions. Challenge doses (0.1 ml) of the respective test and control substances were applied to previously treated sites on one side, and with Dowanol DPM/Tween 80 (9:1) on the other, and the skin reactions noted at 24 and 48 h.

No signs of skin irritation were seen in any group during the induction phase of the study. When challenged with the positive control material, slight to marked erythema was noted in 19/20 animals. When challenged with the test formulation (10% Dursban TC), slight erythema was observed in 5/10 animals at 24 h, and 5/10 animals at 48 h (six different animals in total), while none of the 10 guinea pigs challenged with the blank formulation (minus Dursban XP) showed signs of erythema. Under the conditions of this study, the test material was considered to be a weak skin sensitiser in guinea pigs.

Carreon RE (1986a) Dursban TC Insecticide (Formulation B): Dermal sensitization potential in the guinea pig. The Dow Chemical Company, Laboratory Report Code: HET M-004817-001, 9 April, 1986. [Dow; Submission 1080]

This study was conducted in accordance with the intent of the Good Laboratory Practices for Non-Clinical Studies (Federal Register, December 1978); the Environment Protection Agency; FIFRA GLP Procedures (Federal Register, November 1983), EPA Guidelines (October 1982).
To determine the skin sensitisation potential of the formulation Dursban TC (Formulation B) (Dow; containing 43.0% technical chlorpyrifos), ten male Hartley albino guinea pigs (Charles River Breeding Laboratories, USA) received four dermal applications of 0.1 ml of the undiluted test formulation made to the clipped back regions over a period of 10 days. An additional group of 10 guinea pigs were similarly treated with a positive control [DER 331; 10% epoxy resin in DOWANOL DPM (dipropylene glycol monomethyl ether/Tween 80; 9:1) (dipropylene glycol monomethyl ether/Tween 80; 9:1)] substance. Each application was made under a gauze patch then covered with an occlusive dressing for 48 h. After the first application was removed, a second application (0.1 ml) was made. At the time of the third application, a total of 0.2 ml of Freund's Adjuvant was injected intradermally at multiple points adjacent to the application site. Forty-eight hours after this application, the patch was again removed, and a fresh application of 0.1 ml of test material was made. Each time the patches were removed, observations for erythema and/or oedema were made.

A 2 week break from treatment followed the induction period. During the induction period the treatment

site was regularly examined for skin reactions. Challenge doses (0.1 ml) of the respective test and control substances were applied to previously treated sites on one flank, and with Dowanol DPM/Tween 80 (9:1) on the other, and the skin reactions noted at 24 and 48 h.

One animal died during the study, but its death was not attributed to chlorpyrifos administration. No signs of skin irritation were seen in any group during the induction phase of the study. When challenged with the positive control material, moderate to marked erythema was noted in 9/10 animals. When challenged with the test formulation (undiluted Dursban TC Formulation B), all animals displayed a skin sensitisation response, with slight erythema observed in 4/9 animals at 24 h, and 3/9 animals at 48 h, and moderate erythema observed in 5/9 animals and 6/9 animals, at 24 and 48 h, respectively. Under the conditions of this study, the test material was considered to be a moderate skin sensitiser in guinea pigs.

3.2.4 Dursban ULV

Summary of acute toxicity of Dursban ULV formulation

Species	Strain	Sex	Study Type	Vehicle	Outcome*	Reference
Rat	SD	M+F	Oral	undiluted	LD50: 541 µl/kg	Jones (1977d)
Rat	SD	M+F	Dermal	undiluted	LD50: >5000 µl/kg	Jones (1977c)
Rat	SD	M	Inhalation	undiluted	LC50: >1690 mg/m^3	Crouch & May (1977)
Rabbit	NZ white	M	Eye Irritation	undiluted	Slight eye irritant	Jones (1977b)
Rabbit	NZ white	M	Skin irritation	undiluted	Slight skin irritant	Jones (1977a)

* Calculated as volume or weight of product

Jones JR (1977d) Acute oral LD50 in the rat: Dursban ULV. Hazleton Laboratories Europe, report: 875-50/24, November 1977. [Dow; Submission 11462]

The acute oral LD50 for Dursban ULV (Dow; chlorpyrifos concentration not stated) was determined in Sprague-Dawley rats (Bantin and Kingman, UK). The undiluted test material was first administered by oral gavage to four groups of fasted rats (2/sex/dose; dose volume 0.2 to 1.6 ml) at doses of 200, 400, 800, and 1600 µl/kg, in a range-finding study. Mortality was 2/4 and 4/4 at 800 and 160 µl/kg, respectively. Based on these results, the doses for the main study were 400, 670, 800, 950, 1350, and 1600 µl/kg, with 5 animals/sex/dose. Animals were observed for clinical signs for 14 days after treatment.

Clinical signs including prostration, convulsions, exophthalmos and chromodacryorrhea were generally confined to animals treated at doses of 950 µl/kg and above. Mortality was 1/10, 3/10, 7/10, 9/10, 9/10, and 10/10, at 400, 670, 800, 950, 1350, and 1600 µl/kg, respectively. Under the conditions of this study, the LD50 (95% confidence limits) was 700 µl/kg (583-840 µl/kg).

Jones JR (1977c) Acute dermal LD50 in the rat: Dursban ULV. Hazleton Laboratories Europe Report no: 932-50/33, November 1977. [Dow; Submission 11462, reference 21]

To determine the dermal LD50 of the chlorpyrifos formulation Dursban ULV (Dow; concentration not

stated), the undiluted test material (5 ml/kg) was applied to the shaved intact skin on the backs and flanks of Sprague-Dawley rats (Charles River, UK; 5/sex). The test sites were then covered with waterproof dressings for 24 h, after which time the dressings were removed, and residual test material removed by washing with warm water. The animals were observed for signs of toxicity at 15, 30, and 60 minutes, and at 4 h. Overall mortality was assessed at 48 h.

No deaths occurred during the study, but all animals displayed a marked brown secretion around the nose, hunched posture and chromodacryorrhea at 24 h, and to a lesser extent at 48 h.

Under the conditions of this study, the dermal LD50 of the test material was >5 ml/kg.

Crouch CN & May JW (1977) Acute toxicity by inhalation of Dursban ULV in rats. Hazleton Laboratories Europe, Report No: 874-50/23, September 1977. [Dow; Submission 11462, reference 22]

Dursban ULV (Dow; concentration not stated), was administered undiluted, as an aerosol, by nose-only inhalation to rats for 4 h. The animals (Sprague-Dawley; Bantin and Kingman, UK; 8/sex/dose) were exposed to measured concentrations of 0 (control; 5/sex), 790, 940, 1280, and 1690 mg/m^3 of the test material. The highest dose was a reflection of the maximum concentration achievable in this study.

No deaths associated with treatment occurred during the exposure period. On the day following exposure, 2/16 animals in the 1690 mg/m^3 group died, and 1/16 animals in the 1280 mg/m^3 group died. Clinical signs, including ruffled fur, chromodacryorrhea, nasal secretion, and lethargy, were reported at all exposure concentrations, but these signs disappeared after 2-3 days. At the highest dose level, animals also showed signs of tremor, twitching, and uncoordinated movement. Under the conditions of this study, the LC50 for the test material was >1690 mg/m^3.

Jones JR (1977a) Primary skin irritation studies in the rabbit Dursban ULV (EF312). Hazleton Laboratories Europe Study No.: 910-50/28, dated October 1977 [Dow; Submission 11462, reference 23]

This study was carried out to determine the primary irritation index of Dursban ULV (EF312). No details of this formulation were provided. The test substance (colourless liquid, 0.5 g) was applied to two sites on the clipped backs of six male New Zealand White rabbits (The Buxted Rabbit Co. Sussex). On the day of application, one site was abraded by making three epidermal incisions, while the other was left intact. After the test substance was applied to the sites they were immediately covered with a gauze patch, followed by a cotton wool pad, and the trunk of each animal was then wrapped in a bandage for 24 h. One h and 48 h after removal of the patches the test sites were examined for signs of skin irritation.

Four rabbits showed slight erythema (score 1) at both sites at 24 h but not at 72 h, and no sites showed oedema at either scoring time. No other signs of irritation were observed during the study. The mean scores for erythema and oedema of abraded and intact sites were 8/6 = 1.3, and hence the primary irritation index was 1.3/4 = 0.3 and the material was regarded as a slight irritant to rabbit skin.

Jones JR (1977b) Eye irritation study in the rabbit Dursban ULV (EF312). Hazleton Laboratories Europe Study No: 918-50/29, dated November 1977 [Dow; Submission 11462,

reference 24]

This study was carried out to determine the ocular irritancy of Dursban ULV (EF312). No details of this formulation were provided. The test substance (colourless liquid, 0.1 ml) was instilled into the left conjunctival sac of six male New Zealand White rabbits (The Buxted Rabbit Co. Sussex), with the untreated right eyes serving as control . The eyes were scored using the Draize scale at 1, 2, 3, 4 and 7 days after treatment.

Slight to mild conjunctival redness (6/6) and slight discharge (3/6) were seen on day one, and no other signs of irritation were seen at other scoring times. The total score was 22/110 for day 1 and zero at all other times. This formulation was a slight eye irritant in rabbits.

3.2.5 Lorsban 50 EC

Summary of acute toxicity of Lorsban 50EC formulation

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rat	SD	M F	Oral	water	LD50: 310 mg/kg LD50: 230 mg/kg	Anon (1985a)
Rabbit	NZ white	M+F	Dermal	undiluted	LD50: >2000 mg/kg	Anon (1985b)
Rat	Fischer 344	M F	Inhalation	undiluted	LC50: 2500 mg/m^3 LC50: 2000 mg/m^3	Streeter et al (1986)
Rabbit	NZ white	F	Eye Irritation	undiluted	Moderate eye irritant	Anon (1985c)
Rabbit	NZ white	F	Skin irritation	undiluted	Slight skin irritant	Anon (1985d)

Anon (1985a) Acute oral toxicity in the Albino Rat. Toxicity Research Laboratory Ltd, TRL Study # 033-011, Completed 2 December, 1985. [Dow; Submission 1053]

This study was conducted in compliance with GLP for Non-Clinical Laboratory Studies regulations. The test material Lorsban 50 EC Insecticide (Dow; chlorpyrifos concentration not stated) was dissolved in distilled water and administered to young adult male and female rats (Crl:CD (SD)BR; Charles River Breeding Laboratories; 5/sex/dose) at doses of 156.3, 312.5, or 625 mg/kg (males), and at 156.3, 221, 312.5, 442, or 625 mg/kg (females). The dosage volume was 10 ml/kg. Animals were observed for mortality and clinical signs of intoxication during the day of administration and twice daily thereafter for 14 days.

No animals died at 156.3 mg/kg, while a single female died at 221 mg/kg. At 312.5 mg/kg, a single male died, but all females died at this dose. At doses of 442 mg/kg and 625 mg/kg in females, and at 625 mg/kg in males, all animals died following administration of the test material. A range of clinical signs were reported, including hypoactivity, salivation, lacrimation, diarrhea, prostration, tremors, ataxia, and convulsions, with the incidence of a number of these signs increasing with dose. Clinical signs were not seen in surviving rats after five days. The LD50 value was determined to be 310 mg/kg for males (95% confidence interval 247-389 mg/kg), and 230 mg/kg for females (174-304 mg/kg). All deaths occurred within four days of administration.

Anon (1985b) Acute dermal toxicity in the Albino Rat. Toxicity Research Laboratory Ltd, TRL

Study # 033-012, completed 16 December, 1985. [Dow; Submission 1053].

This study was conducted in compliance with GLP for Non-Clinical Laboratory Studies regulations. The test material (Lorsban 50EC; Dow: chlorpyrifos concentration not stated) was applied to the shaven intact skin on the trunk of rabbits (New Zealand white; Oak Hill Rabbit Ranch, USA; 16-17 weeks old; five animals/sex/dose) to give a dose of 2000 mg/kg body weight. The test material was covered with a gauze dressing and tape for 24 h, after which time the site was washed with water to remove any residual test material. The animals were observed for mortality and clinical signs of toxicity frequently during the four hours after dosing, and twice daily thereafter for 14 days.

No animals died during this study. No clinical signs of intoxication were observed during the study. Slight erythema and oedema were seen in all animals, while exfoliation and fissuring were also seen in most of the animals. Under the conditions of this study, the dermal LD50 for this test material was >2000 mg/kg.

Streeter CM, Battjes JE & Lomax LG (1986) Lorsban 50 EC insecticide : Acute aerosol inhalation study in rats. The Dow Chemical Company, Laboratory Report Code: HET DR-0155-1753-002, 9 October, 1986. [Dow; Submission 1053]

This study was conducted consistent with FDA and EPA GLP Regulations. To assess the acute inhalational toxicity of Lorsban 50 EC (Dow; containing 50.1% chlorpyrifos; lot HM 831108/1), the test material was administered for four hours by whole-body exposure to Fischer 344 rats (minimum 8 weeks old at time of exposure; Charles River Breeding Laboratory; six animals/sex/dose) at time-weighted average concentrations of 1500 (females only), 2200, and 3100 mg/m^3. Animals were observed for mortality and clinical signs of intoxication for up to 14 days after exposure. The mean mass median aerodynamic diameter of the aerosols was 2.33µ, and the mean geometric standard deviation of the particle size distribution was 2.09.

During the exposure period, only a few of the animals were able to be observed in the chamber. At 1500 mg/m^3, animals displayed salivation, lacrimation and laboured breathing during exposure. On day 1 following exposure, all animals displayed salivation and lacrimation, but these effects were not seen at later observations during the study. At 2200 mg/m^3, animals in the exposure chamber displayed eye squint, salivation, lacrimation, and laboured breathing, with lacrimation persisting in all animals for two days, and in some animals for up to five days. Tremors were also seen in all animals for two days, and in some animals for up to six days. At 3100 mg/m^3, eye squint, lacrimation, laboured breathing, salivation, and lethargy were seen in all animals on the first two days of the study. Signs such as staining of the muzzle, periocular region and perineum, and hyper-reactivity, were also seen during the observation period at all dose levels. No females died at 1500 mg/m^3; 1/6 males and 4/6 females died at 2200 mg/m^3 from days 5-14, and all animals exposed at 3100 mg/m^3 died between days 2-5.

The LC50 was determined to be 2000 mg/m^3 for females (95% confidence intervals 1500-3100 mg/m^3), and 2500 mg/m^3 (<3100 mg/m^3) for males.

Anon (1985c) Primary eye irritation in the albino rabbit. Toxicity Research Laboratory, TRL Study # 033-013, completed 19 November, 1985. [Dow; Submission 1053]

This study was conducted in accordance with GLP requirements. To evaluate the eye irritation potential

of Lorsban 50 EC, a formulation containing chlorpyrifos (Dow; concentration of active ingredient not stated) was instilled into the everted lower eyelids (0.1 ml aliquots) of six female New Zealand white rabbits (16-17 weeks old; Oak Hill Rabbit Ranch, USA). The treated eyes were examined and scored for irritation at 24, 48, 72, and 96 h after compound administration, and also at 7, 10, and 14 days. The untreated left eyes of animals served as controls.

Slight to moderate conjunctival redness and chemosis was seen in all animals, with redness persisting until 96 h in 4/6 animals, and for 7 days in 2/6 animals. At 24 h, conjunctival redness ranged from diffuse beefy redness (score 3; 3/6 animals) to diffuse crimson (score2; 3/6 animals). A purulent discharge was observed around the conjunctiva in all test animals. Corneal opacities were noted in all animals, with scattered to diffuse areas of opacity (score 1) seen in 5/6 animals, and easily discernible translucent areas (score 2) seen in 1/6 animals. While some opacities persisted for 96 h, no corneal opacity was seen at the 7 day examination.

Under the conditions of this study, the test material was a moderate eye irritant in rabbits.

Anon (1985d) Primary dermal irritation in the albino rabbit. Toxicity Research Laboratory, TRL Study # 033-014, Completed 11 November, 1985 [Dow; Submission 1053].

This study was conducted in accordance with the standards set forth in the OECD Guideline for testing of Chemicals (12 May 1981), and in accordance with GLP procedures. The primary dermal irritation potential of Lorsban 50 EC (Dow; containing chlorpyrifos; concentration of active ingredient not stated) was tested in six female New Zealand white rabbits (16-17 weeks old; Oak Hill Rabbit Ranch, USA). The dorsal area of the trunk of each animal was clipped of fur, and the test material (0.5 ml) was applied to a small area of skin (approximately 6 cm^2) and covered with a gauze patch. The test site was then covered in a semi-occlusive dressing for four hours, after which time the test site was rinsed with water to remove any residual test material. The test site was observed for erythema and oedema in accordance with the Draize technique at 30 min after patch removal, and then at 24, 48, and 72 h, and 4, 5, and 6 days after application.

Very slight (barely perceptible) erythema (score 1) was seen in all animals for up to 5 days, while very slight (barely perceptible) oedema (score 1) was also seen in one animal at 4.5 h, and another animal at up to 24 h post administration. Under the conditions of this study, the test material was a slight skin irritant in rabbits.

Anon (1985e) Dermal sensitization in guinea pigs. Toxicity Research Laboratory Ltd. TRL Study # 033-015, completed 28 February, 1986. [Dow; Submission 1053]

This study was conducted according to GLP procedures, and the OECD Guidelines for Testing of Chemicals. A formulation containing chlorpyrifos (Lorsban 50 EC; Dow; concentration of active ingredient not stated) was tested for dermal sensitisation potential in young, adult male guinea pigs (Murphy Breeding Laboratories, USA) using the method of Buehler. Prior to the initiation of the study, a pilot test was conducted to determine a non-irritating concentration of the test material, using a single dose of the formulation. This study reported that the test material was not irritating to the skin of guinea pigs.

During the induction phase of the main study, the skin of the backs and flanks of ten animals was clipped of hair, and the undiluted test material (0.5 ml) was applied three times/week (with at least one day intervening) for a total of ten applications. To avoid cumulative irritation, the test applications were applied to one of three test sites, either on the back or either flank of the animals. After application, the test material was covered with a pad, and with an impervious sheeting for a six hour exposure period, after which the animals were returned to their cages. The test sites were scored for erythema and oedema 24 and 48 h after each application. Two weeks after the final induction application, the animals were challenged once with a single dose of the undiluted test material. The challenge dose was applied to the same test sit as induction doses 1, 4, 7, and 10. A positive control group, also consisting of ten male guinea pigs, was treated similarly, but with a 0.1% (w/v) dilution of 1-chloro-2,4-dinitrobenzene in water.

Following induction doses with the test material, very slight (barely perceptible) erythema was seen in treated animals, but generally only on one or two occasions during the study. Well-defined erythema was seen in a single animal only, after the fourth induction dose, and very slight oedema was also seen in this animal following this induction dose. Oedema was not reported in other treated animals. No reaction was observed following challenge doses of the test material. Following the challenge with the positive control material, very slight erythema was seen in three animals, and well defined erythema in a single animal. Very slight oedema was seen in a single animal only, following the positive control challenge dose.

Under the conditions of this study, the test material was not a skin sensitiser to guinea pigs. However, due to the equivocal nature of the response to the positive control material, this study does not adequately address the skin sensitisation potential of Lorsban 50 EC.

3.2.6 Dursban 2E

Carreon RE & Wall JM (1985) Dursban 2E formulation : Dermal sensitization potential in the guinea pig. The Dow Chemical Company, Laboratory report Code: HET M-004501-004, 21 February, 1985. [Dow; Submission, 1053]

This study was conducted in compliance with US FDA and EPA GLP regulations. To test the skin sensitisation potential of the chlorpyrifos formulation Dursban 2E (Dow; containing 25% chlorpyrifos technical), the test material was applied to the shaved skin on the back of ten male Hartley albino guinea pigs (Charles River Breeding Laboratories, USA), with four applications (each 0.1 ml of a 1% solution in Dowanol DPM/Tween * (9:1)) made over the 7-day induction period. Each application was made on a gauze patch, and then secured in place under an occlusive dressing for 48 h before the next application was made. At the time of the third application, a total of 0.2 ml of Freund's Adjuvant was injected intradermally at multiple points adjacent to the application site. After the final application of test material, the patches were removed, and animals were allowed to recover for a 14-day period. A group of 10 male guinea pigs similarly received applications of a positive control material, DER 331 epoxy resin, as a 10% solution in the same vehicle used on the test animals. After the rest period, both flanks of each animal were clipped and challenged with the test material on one side and the solvent on the other. The test material was applied as a 0.5% dilution, as the 1% dilution resulted in skin irritation in a number of animals during the induction phase of the study. Challenge applications were not covered, and skin responses at these sites were observed at 24 and 48 after application.

During the induction phase of the study, slight skin irritation (hyperaemia) was observed in 4/10 animals that was treated with the 1% solution of the test material. No signs of skin irritation or sensitisation were observed following the challenge dose with the 0.5% solution of test material. The positive control material resulted in moderate to marked erythema reactions in all animals upon challenge. Under the conditions of this study, the test material was not a skin sensitiser in guinea pigs.

3.2.7 Lorsban LV/XRM-4656

Carreon RE, Johnson KA & Wall JM (1982) Experimental Lorsban Formulation (XRM-4656); acute toxicological properties and industrial handling hazards. Study No: HET M-4556-(1), Dated 8 September, 1982 [Dow; Submission 11462, reference 1]

A Lorsban formulation (23% chlorpyrifos) described variously as chlorpyrifos solution and GHD-0544-38 was tested for acute oral and dermal toxicity, eye irritation and skin irritation.

*Acute oral toxicity: R*ats (Fischer 344, 6/sex/group) were administered 320, 630, 1300 or 2500 mg/kg of the formulation as a 10% corn-oil solution by single dose gavage. Clinical signs were noted for the two week observation period, and surviving animals were sacrificed and subjected to gross necropsy. Clinical signs included body tremors, watery eyes, excessive salivation, lethargy and laboured respiration. There were no abnormalities noted at gross necropsy. Deaths occurred on days 1-4 and were recorded as 0, 0, 5/12 (4M, 1F) and 12/12 for 320, 630, 1300 or 2500 mg/kg doses respectively. The oral LD50 was 1173 mg/kg for males and 1530 mg/kg for females.

Acute eye irritation: Aliquots of the undiluted test material (0.1 ml) were instilled into the lower conjunctival sac of the right eye of six NZW rabbits (6M) without washing, and similarly into the sac of 3 rabbits (1M, 2F) with washout after 30 seconds. The left eye of each animal served as a control. Animals were returned to their cages immediately following installation of the test material, and assessment of ocular effects were made for all rabbits at 1, 2, 3, 4, 7, 10 and 15 days after dosing, and scored according to the method of Draize.

Slight conjunctival redness (Draize score 1) was seen in all animals at 1, 24, and 48 h, but this effect had disappeared by 72 h in all animals. Additionally, slight conjunctival chemosis and discharge were seen in 2/3 animals at 1 h and at 24 h, but these effects were not seen at the 48 h examination. A single animal displayed slight iridial irritation at the 1 h examination only.

Under the conditions of this study, the test material was considered to be a slight eye irritant in rabbits.

Streeter CM, Battjes JE & Yano BL (1986) Lorsban LV Insecticide: An acute aerosol inhalation study of formulation XRM-4656 in rats. The Dow Chemical Company Report No.: HET-M004656-003 dated April 16 1986. QA [Dow; Submission 11462, reference 31]

This study was performed to assess the acute inhalational toxicity of a chlorpyrifos formulation known as Lorsban LV: formulation XRM-4656. Few details were provided, but the formulation was described as an amber liquid, density 1.040 g/ml Ref: GHD-1137-1, purity 95.9%, and containing 22.95%

Dursban F. The test material was administered for four hours by whole body exposure to Fischer 344 rats (minimum 8 weeks old at time of exposure; Charles River Breeding Laboratory; six animals/sex/dose) at time-weighted average concentrations of 4600 mg/m^3. Additional similar experiments with additional rats achieved 4600 and 3700 mg/m^3 for males and females respectively. Animals were observed for mortality and clinical signs of intoxication for up to 14 days after exposure, and then necropsied.

The mean mass median aerodynamic diameter of the aerosols was 2.27 µ, and the mean geometric standard deviation of the particle size distribution was 1.85. During the exposure period, only a few of the animals were able to be observed in the chamber. On days 1 and 2 following exposure at 4600 mg/m^3, most animals displayed salivation, lacrimation, lethargy, reddish stains (muzzle) and urine perineal stains, with laboured breathing and reddish stains (periocular) appearing only on day 2. Signs at later observation times additionally included hyper-reactivity and unkempt fur, but were less frequent, especially in males, such that by day 14 only urine perineal stains (2M, 3F) and alopecia (muzzle and periocular, 3 F) were present. In a second experiment, males exposed at 4600 mg/m^3 displayed a similar pattern of signs to those listed above, such that by day 14 2/6 displayed urine perineal stains only. Another experiment in which females were exposed at 3700 mg/m^3 also recorded a similar pattern of clinical signs to that listed above but included trembling seen in all rats from days 1-3. By day 14 the only signs recorded were urine perineal stains (5/6) and alopecia (muzzle and periocular, 5/6). Bodyweights dropped in all rats following exposure but tended to recover to pre-exposure levels by day 14. Deaths occurred in 1/6 males (died day 14) and 3/6 females (died days 4, 7 & 8) in the first experiment (M & F, 4600 mg/m^3) and the surviving females in this group recorded enlarged adrenals at terminal necropsy. The LC50 (4h) for Lorsban LV experimental formulation XRM-4656 containing ca. 23% chlorpyrifos was determined to be 4600 mg/m^3 for females and >4600 mg/m^3 for males.

3.2.8 Pyrinex ME Insecticide

Summary of acute toxicity of Pyrinex ME formulation

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rat	SD	M+F	Oral	undiluted	LD50: >2000 mg/kg	Dreher (1993a)
Rat	SD	M+F	Dermal	undiluted	LD50: >2000 mg/kg	Dreher (1993b)
Rabbit	NZ white	not stated	Eye Irritation	undiluted	Slight eye irritant	Privman (1994)
Rabbit	NZ white	F	Skin irritation	undiluted	Slight skin irritant	Dreher (1993c)

Dreher DM (1993a) Pyrinex ME: Acute oral toxicity (limit test) in the rat. Safepharm Laboratories Ltd, UK. Project no: 306/187, 21 May 1993. [Makteshim; Submission 11471, 10994]

This study was conducted in accordance with the OECD Guidelines for Testing of Chemicals (1987) no: 401. The test material, Pyrinex ME (chlorpyrifos concentration not stated), was administered via oral gavage to male and female Sprague-Dawley rats (Charles River, UK) to establish the acute oral toxicity potential of the compound. As a range finding study, a single male and female rat were administered the test material at a dose of 2000 mg/kg, and the animals were then observed for mortality or signs of toxicity at 30 min, and at 1, 2, and 4 h after dosing, then daily for 5 days. As there were no deaths at this dose level, the 2000 mg/kg dose was chosen for the main study. Five animals/sex were administered

the test material at a dose of 2000 mg/kg, with a dose volume of 1.85 ml/kg. Observations for death and/or clinical signs of toxicity were made at 30 minutes, and 1, 2, and 4 h after dosing, then daily for 14 days. At the end of the main study, animals were killed and subjected to gross pathological examination.

No deaths or clinical signs of toxicity were reported during the main study, and no abnormalities were noted at necropsy. Under the conditions of this study, the oral LD50 for the test material, Pyrinex ME, was considered to be >2000 mg/kg in rats.

Dreher DM (1993b) Pyrinex ME: Acute dermal toxicity (limit test) in the rat. Safepharm Laboratories Ltd., UK. Project no: 306/188, 21 May, 1988. [Makteshim; Submission 11471, 10994]

This study was conducted in accordance with the OECD Guidelines for Testing of Chemicals (1981) no: 402. Pyrinex ME, a microencapsulated chlorpyrifos formulation (concentration not stated) was applied undiluted to the intact shorn skin on the back and flanks of ten Sprague-Dawley rats (5/sex; Charles River, UK) at a dose of 2000 mg/kg, and a dose volume of 1.85 ml/kg. The test material was then covered with gauze and a semi-occlusive dressing and bandage, and retained in place for 24 h. After the exposure period, the treated skin was cleaned with distilled water to remove any residual test material. Animals were observed for mortality and signs of toxicity at 30 minutes, 1, 2, and 4 h after dosing, and daily thereafter for 14 days. At the end of the study the animals were killed and subjected to gross pathological examination.

No deaths or signs of toxicity were observed during the study. No signs of skin irritation were reported, and no abnormalities were noted at necropsy. Under the conditions of this study the dermal LD50 for the chlorpyrifos formulation Pyrinex ME was >2000 mg/kg in rats.

Dreher DM (1993c) Pyrinex ME: Acute dermal irritation test in the rat. Safepharm Laboratories Ltd., UK. Project no: 306/189, 11 May, 1988. [Makteshim; Submission 11471, 10994]

This study was conducted in accordance with the OECD Guidelines for Testing of Chemicals (1981) no: 404. To assess the skin irritation potential of the microencapsulated chlorpyrifos formulation, Pyrinex ME (Makteshim; concentration not stated), the test material was applied undiluted (0.5 ml) to the clipped dorsal/flank areas of three female New Zealand White rabbits (David Percival Ltd., UK). The test material was introduced under a gauze patch, and secured in place under a semi-occlusive dressing for four hours, after which time the test sites were swabbed with distilled water to remove any residual test material. Approximately one hour after removal of the patches, and at 24, 48, and 72 h later, the test sites were examined for evidence of primary irritation and scored according to the method of Draize.

Mild erythema (irritation score 1) was seen in two rabbits at 1 h, but only persisted in one animal until 24 h. No signs of skin irritation were seen at 48 h. The Primary Irritation Index was 0.2. Under the conditions of this study, the test material was considered to be a slight skin irritant to rabbits.

Privman I (1994) Pyrinex ME: Primary eye irritation in rabbits. Life Science Research Israel

Ltd. LSRI report MAK/238/PYR, 3 October 1994. [Makteshim; Submission 11471, 10994]

This study was conducted in accordance with OECD GLP requirements, and according to OECD Guidelines 405. To assess the eye irritation potential of the chlorpyrifos formulation Pyrinex ME (concentration not stated), aliquots of the undiluted test material (0.1 ml) were instilled into the lower conjunctival sac of the left eye of three healthy young adult albino rabbits (sex not stated; A. Loebenstein Laboratory Animals, Israel). The right eye of each animal served as a control. Animals were returned to their cages immediately following installation of the test material, and assessment of ocular effects were made for all rabbits at 1, 24, 48, and 72 h after dosing, and scored according to the method of Draize.

Slight conjunctival redness (Draize score 1) was seen in all animals at 1, 24, and 48 h, but this effect had disappeared by 72 h in all animals. Additionally, slight conjunctival chemosis and discharge were seen in 2/3 animals at 1 h and at 24 h, but these effects were not seen at the 48 h examination. A single animal displayed slight iridial irritation at the 1 h examination only.

Under the conditions of this study, the test material was considered to be a slight eye irritant in rabbits.

3.2.9 Pyrinex 48 EC Formulation/4EC Formulation

Summary of acute toxicity of Pyrinex 48EC formulation

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rat	SD	M F M+F	Oral	maize oil	LD50: 541 mg/kg LD50: 497 mg/kg LD50: 508 mg/kg	Hamani (1986)
Rabbit	NZ white	M+F	Dermal	undiluted	LD50: >2000 mg/kg	McSheehy (1986c)
Rat 4EC formulation	SD	M F M+F	Inhalation	undiluted	LC50: 3400 mg/m^3 LC50: 3100 mg/m^3 LC50: 3300 mg/m^3	Hoffman (1988)
Rabbit	NZ white	F	Eye Irritation	undiluted	Moderate eye irritant	McSheehy (1986b)
Rabbit	NZ white	F	Skin irritation	undiluted	Slight skin irritant	McSheehy (1986a)
Guinea pig	local strain	M+F	Skin sensitisation	various	moderate skin sensitiser	Crown & Marom (1989)

Hamani S (1986) Pyrinex 48 EC: Acute oral toxicity study in the rat. Life Science Research Israel. LSRI report MAK/038/PYR, 1 June 1986. [Makteshim; Submission 11471]

This study was conducted in accordance with OECD Guidelines for Testing of Chemicals, Section 401, and EPA FIFRA Guidelines, Subdivision F, Series 81-1, and was subject to QA inspection. The test material Pyrinex 48 EC (Makteshim; batch 560008; chlorpyrifos concentration not stated) was prepared in maize oil, and administered to young adult male and female rats (remote Sprague-Dawley strain; Charles River, UK) by oral gavage, with a dose volume of 5 ml/kg. To determine the dose levels for the study, a preliminary study was conducted using 2 animals/sex/dose, with doses ranging from 75-1200 mg/kg bodyweight. In this preliminary study, deaths occurred at doses of 300-1200 mg/kg. On the basis of the preliminary results, the doses used in the main study were 200, 270, 360, 490, and 893 mg/kg bodyweight, with a dose volume of 5 ml/kg. Animals were inspected for mortality and clinical signs four times on the day of dosing, and twice-daily thereafter until day 14. Animals were killed at study termination, and gross pathological examinations performed.

In the main study, no deaths occurred at 200, 270, or 360 mg/kg. At 490 mg/kg, 2/5 males and 3/5 females died, while at 893 mg/kg, all animals died. Deaths were noted between days 3 and 6, with all animals in the high dose group dead by day 4 of the study. Clinical signs of intoxication included decreased motor activity, ataxia, hunching, salivation, and urogenital staining. Under the conditions of this study, the oral LD50 for the test material (95% confidence intervals) was 497 mg/kg (386-638 mg/kg) in females, 541 mg/kg (411-711 mg/kg) in males, and 508 mg/kg (438-590 mg/kg) in males and females combined.

McSheehy TW (1986c) Pyrinex 48 EC: Acute dermal toxicity study in rabbits. Life Science Research Roma Toxicology Centre SPA. LSR-RTC report 143-008/T/037/86, 30 December 1986. [Makteshim; Submission 11471]

This study was conducted in accordance with GLP regulations of the OECD and US FDA. The acute dermal toxicity of the test material, Pyrinex 48 EC (containing chlorpyrifos; Makteshim; batch 560008; stated purity 45.2% w/w), was investigated using New Zealand White rabbits (Charles River Italia). The

results of an initial range-finding study, using 2 male and 2 female animals, was used to determine the dose selection for the main study, with the method of pre-treatment, treatment, and observations similar in both range-finding and main studies.

Approximately 24 h before test material application, an area on the back of test animals was clipped free of hair. On the day of treatment, the test material was applied to a lint patch and applied to the test site, then held in place with an occlusive dressing for 24 h, after which time the dressing was removed, and the test site washed with warm water to remove residual test material. Two animals/sex were used in the range-finding study, and 5 animals/sex in the main study, with a dose of 2000 mg/kg body weight applied to all animals. Animals were observed for mortality and signs of intoxication for the first hour after application, then at frequent intervals on the remainder of the day of application, and twice daily on subsequent days for a total of 14 days after application. Animals were killed on day 14, and subjected to a gross pathological examination.

Mortality was confined to a single female in the main study, which died during the observation period (day 13). No clinical signs were reported in animals during the range-finding study, nor in animals in the main study immediately after application of the test material. A number of animals displayed eye and/or nasal discharge during the observation period of the main study, but no other clinical signs were reported. Under the conditions of this study, the dermal LD50 for the test material was considered to be >2000 mg/kg bodyweight.

Hoffman GM (1988) An acute inhalation toxicity study of chlorpyrifos 4EC in the rat. Bio/Dynamics Inc., USA. Project No: 87-8018, 3 June 1988. [Makteshim; Submission 11471]

Three groups of Sprague-Dawley rats (aged 7-10 weeks at exposure; 5/sex/group; Charles River Breeding Laboratories, USA) were exposed to atmospheres containing the test material (Chlorpyrifos 4EC; containing 48.77% chlorpyrifos; Pennwalt) for a 4-h period in individual exposure chambers, with all animals held for at least a 14-day observation period post-exposure. The measured exposure concentration of the test material was 3000, 3500, and 4800 mg/m^3, corresponding to nominal concentrations of 8300, 14000, and 27000 mg/m^3, for the test groups in this study. All animals were observed immediately prior to exposure, then at approximately fifteen minute intervals during the first hour of exposure, and hourly for the remainder of the exposure. Detailed observations were recorded 30 minutes after exposure, then hourly for two hours, and daily for the remainder of the study. All decedent animals and survivors were subjected to a gross pathological examination. This study was conducted in accordance with US EPA Guidelines, Section 81-3, and in accordance with US EPA GLP requirements 40 CFR Part 160 (FIFRA) and 40 CFR Part 792 (TSCA).

At 4800 mg/m^3, all animals died within 3 days after exposure, while 7/10 animals died at 3500 mg/m^3 (3/5 males and 4/5 females) within 3 days after exposure, and 3/10 animals died after one day of exposure to 3000 mg/m^3 (one male and two females). During the exposure period, the signs of toxicity included lacrimation, laboured breathing, gasping, and decreased activity, while post-exposure signs included respiratory effects (laboured breathing, gasping), lacrimation, salivation, tremors, hunched appearance, uncoordinated gait, and prostration. Total recovery from these effects was not seen during the recovery period in surviving animals.

Under the conditions of this study, the LC50 (95% confidence limits) was 3100 mg/m^3 (2700-

3700 mg/m^3) for females; 3400 mg/m^3 (3000-3900 mg/m^3) for males; and 3300 mg/m^3 (3000-3600 mg/m^3) for males and females combined.

McSheehy TW (1986a) Pyrinex 48 EC: Primary skin irritation study in rabbits. Life Science Research Italy, Roma Toxicology Centre SPA. LSR-RTC report 143-009/T/026/86, 24 December 1986. [Makteshim; Submission 11471]

This study was conducted in accordance with GLP regulations of the OECD and US FDA. The skin irritation potential of the undiluted test material (Pyrinex 48EC; Makteshim; Batch 560008; 45.2% chlorpyrifos) was tested in six female rabbits (New Zealand White; Charles River Italia). Areas of skin on the back of test animals were clipped free of hair approximately 24 h prior to administration of test material. On the day of treatment, 0.5 ml of test material was placed on an unmedicated gauze patch on the test site on the left side of the animals, and an untreated path similarly placed on the right side of the animals to act as control. The patches were covered and held in place with a waterproof plaster for 4 h, after which time the dressings were removed, and the test sites washed with warm water to remove residues of the test material. Assessment of the skin irritation at the test sites was made at approximately 30 minutes, 1, 24, 48, and 72 h after patch removal, and at 24 h intervals until the skin lesions were resolved. Skin lesions were scored according to the method of Draize.

Erythema was seen in all animals, ranging in severity from slight to moderate-severe (irritation scores 1-3). Slight to well-defined erythema (irritation score 1-2) persisted for up to 72 h in 4/6 animals. No erythema was seen at 96 h. Exfoliation persisted for up to 15 days in several animals. Slight oedema was observed in a single animal at the 24 h examination only. Under the conditions of this study, the test material was a slight skin irritant in rabbits.

McSheehy TW (1986b) Pyrinex 48 EC: Eye irritation study in rabbits. Life Science Research Italy, Roma Toxicology Centre SPA. LSR-RTC report 143-010/T/025/86, 24 December 1986. [Makteshim; Submission 11471]

This study was conducted in accordance with the principles of GLP of the OECD and US FDA. The eye irritation potential of the test material Pyrinex 48EC (Makteshim; 45.2% chlorpyrifos; batch 560008) was tested in 12 female New Zealand White rabbits (Charles River Breeding Laboratories, Italy). An aliquot of the undiluted test material (0.1 ml) was instilled onto the corneal surface of the right eye of all animals, and the left eye of each animal acted as the untreated control. Animals were exposed to the test material for periods of 24 h (six animals), four seconds (three animals), or 30 seconds (three animals). At the end of the required contact period, the treated eyes were flushed with a stream of lukewarm water for approximately five minutes to remove the test material. Eyes were examined at 1, 24, 48, 72, and 168 h for signs associated with treatment. Irritation was scored according to the method of Draize. The method of pre-treatment, treatment, flushing and observations conducted were essentially similar for all groups.

Scattered or diffuse areas of opacity (irritation score 1), covering one quarter of the cornea or less, were observed in 4/6 animals treated for 24 h. Another animal in this group displayed more severe corneal effects (irritation score 2), with easily discernible translucent areas on more than one quarter of the surface area, and scattered opacities were seen in this animal up to 72 h. No corneal effects were seen

after 7 days. In the animals with eyes flushed after 4-30 seconds, some scattered or diffuse areas of opacity were reported, but these effects were also seen in the untreated eyes of these animals at a similar incidence.

No iridial effects were observed in this study. Conjunctival effects were seen in animals exposed to the test material for 24 h, and ranged from blood vessels being definitely injected (irritation score 1) at one hour in all animals, to diffuse, crimson redness (irritation score 2) in one animal at 24 and 48 h. No effects were seen in these animals at the 72-h inspection. In animals exposed for 4 seconds, 2/3 animals displayed slight conjunctival irritation at one hour only. Slight to obvious swelling of the eyelids were seen in animals from all treatment groups at 1-24 h, but these effects had disappeared at 48 h. Very slight discharge was observed at 1 h in treated eyes that had been flushed after 4-30 seconds, while in the animals exposed to the test material for 24 h, considerable discharge was seen at 1 h, but persisted for up to 48 h in a single animal only.

Under the study conditions the test material was a moderate eye irritant to rabbits.

Crown S & Marom M (1989) Pyrinex 48 EC: delayed sensitisation study in Guinea pigs. Life Science Research Israel. LSRI report MAK/164/PYR48EC, 16 April, 1989. [Makteshim; Submission 11471]

This study was conducted in accordance with OECD and US EPA FIFRA guidelines, and in compliance with GLP standards (OECD; US EPA). The skin sensitisation potential of Pyrinex 48 EC (Makteshim; chlorpyrifos concentration not stated) was assessed in albino guinea pigs (A. Loebenstein Laboratory Animals, Israel; local strain) using a modified version of the Magnusson and Kligman maximisation test. The study consisted of a preliminary skin irritation screen and the main study. The test material was formulated in the following manner:

Study	Method	Vehicle	Concentration (%)	Dose/site
Prelim.	Intradermal	FCA emulsion or maize oil	0.5, 1, 2, 5 w/v	0.1 ml
Prelim.	Topical	Maize oil	25, 50, 70, 100 w/v	0.5 ml
Main study: primary induction	Intradermal	FCA emulsion or sesame oil	Pyrinex 48EC 2% w/v	0.1 ml
Main study: secondary induction	Topical		Pyrinex 48EC 100%	0.5 ml
Main study: challenge	Topical		Pyrinex 48EC 100%	0.5 ml

In the preliminary study, intradermal injections of the test material were made to the shaved skin over the scapulae of two animals, with one animal receiving the test material suspended in maize oil, and the other animal receiving the test material suspended in Freund's complete adjuvant (FCA). Skin reactions were recorded at 24 and 48 h after administration. In the preliminary study, topical applications were also made to the shaved flanks of three animals, with the test material suspended in maize oil, then covered in occlusive dressings for 24 h. Skin reactions were assessed 2 and 24 h after removal of the dressings.

In the main study, 10 animals/sex/group were used, with the test group receiving both induction and challenge doses, while a control group received the challenge procedure only. The induction procedure

consisted of primary induction by intradermal injection on day 1, and secondary induction by topical application on day 8. These procedures were performed on the shaved area of the suprascapular region.

In the primary induction, three pairs of injections were made using FCA in emulsified form, test material in sesame oil, and test material in FCA. After the secondary induction procedure with undiluted test material, reaction to treatment was recorded 24 h after removal of the dressing.

The challenge procedure was performed on day 21 of the study, with an undiluted application of the test material to the shaved left flank of animals, followed with assessment of the sites at 24 and 48 h after removal of the dressings.

In the preliminary study, no response was observed following topical application of the test material. Intradermal injection of the test material at 1%, 2%, and 5% resulted in a slight erythematous response after 24 and 48 h, but no reaction was observed with 0.5% test material.

In the main study, intradermal induction with the test material in sesame oil resulted in patchy to severe erythema and oedema in 9/20 sites in males, and 2/20 sites in females. Treatment with FCA in emulsified form resulted in erythema (patchy to severe) and oedema in 19/20 sites in males and 18/20 sites in females. Similar responses were seen in 13/20 sites in males and 14/20 sites in females after treatment with the test material in FCA.

After topical induction treatment, using undiluted test material, severe erythema was seen in a single male only. In the challenge phase of the study, 6/20 control animals showed patchy to moderate erythema after 24 h, and a similar response was seen in 10/20 animals after 48 h. In animals induced with test material and then challenged, 16/20 animals has slight to severe erythema at 24 h, with the response being generally more severe than that seen in control animals. At 48 h, a similar response was seen in 18/20 animals.

Under the conditions of this study, the test material was a moderate skin sensitiser in guinea pigs.

3.2.10 WP 50

Vaughn C, Keeler PA & Jersey GC (1976) Acute toxicological properties in industrial handling hazards of M-4170, a wettable powder formulation containing 50% chlorpyrifos. The Dow Chemical Company Report dated: August 24 1976, reference: TACUTE AM F4170. No GLP certificate. QA. [Dow; Submission 11462, reference 2]

Chlorpyrifos 50% wettable powder formulation (described as an off-white powder) bearing reference No. GHI-5487-43 was tested to establish acute oral and dermal toxicity and eye and skin irritation properties. No raw data was presented in this summary report.

Acute oral toxicity: Fasted rats (S-D, 5/sex/dose)were administered 63, 126, 252 or 500 mg/kg of the test material by single dose gavage (vehicle unspecified). Clinical signs and bodyweights were recorded during a 2-week observation period prior to sacrifice and gross necropsy.

Clinical signs included body tremors, hyperactivity, nasal and eye secretions and lethargy. There were no abnormalities noted at gross necropsy. Deaths occurred on days 1-4 and were recorded as 0, 1 (F), 8/10 (3M, 4F) and 10/10 for 63, 126, 252 or 500 mg/kg doses respectively. The oral LD50 was 235 (120-445 95% CI) mg/kg for males and 180 (105-310 95% CI) mg/kg for females.

Acute dermal toxicity: Samples of the test material at 1000, 2000 and 3980 mg/kg were applied under an occlusive cuff to the clipped skin of NZW rabbits (2/sex/dose), and then 3-10 ml of water was injected under the cuff to aid skin contact. After 24-h exposure, the cuffs were removed and the area washed clean and dried. The animals were observed for two weeks.

Topical responses were slight to moderate redness and swelling (duration not stated) and clinical signs included transient diarrhea (all dose groups), temporary paralysis or incoordination (1F at 3980 mg/kg) and lethargy (1 M at 3980 mg/kg). One animal at 3980 mg/kg died on day 2 with signs of respiratory distress and nasal secretions. The surviving animals were normal in appearance and behaviour by the end of two weeks. The dermal LD50 of this formulation exceeds 3980 mg/kg.

Acute eye irritation: Samples of the undiluted test material (0.1 g) were instilled into the lower conjunctival sac of the right eye of six NZW rabbits (sex unspecified) with washing after 30 seconds, and similarly into the sac of the left eye of the same rabbits without washout. Assessment of ocular effects were made for all rabbits at 1, 2, 3, 7 and 14 days after dosing. No results are reported other than the summary conclusion that the formulation induced slight to moderate conjunctival irritation which was still present 14 d post-treatment. In the absence of data to the contrary, this formulation was considered a moderate eye irritant as effects were still present at 14 d.

Skin irritation: The formulation (0.1g, vehicle unspecified) was applied under an occlusive dressing to one abraded and one unabraded shaven site on the backs of six NZW rabbits. The skin reaction was scored when the patches were removed after 24 h and again 48 h later. No results are reported other than the summary conclusion that the formulation possessed a primary irritation score of 0.7/8.0, based on slight redness at the sites at 24 h, but none at 72 h. This formulation was considered to be a slight skin irritant.

3.2.11 1 lb/gallon EUP

Carreon RE & New MA (1981) Chlorpyrifos 1 LB/GAL: Acute toxicological properties. The Dow Chemical Company Report No. DR-0205-4413-(1), dated May 26, 1981. [Dow; Submission 11462, reference 6]

A formulation of chlorpyrifos was tested for acute oral and dermal toxicity and eye and skin irritation in rats. The formulation (13%) was identified as M-4543.

Acute oral toxicity: Fasted rats (CDF, 6/sex/dose) were administered 130, 250, 500, 1000, 2000, or 4000 mg/kg of the undiluted test material by single dose gavage. Clinical signs and bodyweights were recorded during a 2-week observation period prior to sacrifice and gross necropsy.

Clinical signs included diarrhea, body tremors, hyperactivity, nasal and eye secretions, ataxia, rapid shallow breathing and lethargy. There were no abnormalities noted at gross necropsy. Deaths occurred

on days 2-5 and were recorded as 0/12, 0/12, 1/12 (M), 3/12 (3F), 11/12 (5M, 6F) and 12/12 for the 130, 250, 500, 1000, 2000, or 4000 mg/kg doses respectively. The oral LD50 was 1414 (927-2598 95% CI) mg/kg for males and 1000 (733-1364 95% CI) mg/kg for females.

Acute dermal toxicity: Samples of the undiluted test material at doses of 2500 or 5000 mg/kg were applied under an occlusive cuff to the clipped skin of NZW rabbits (2/sex/dose). After 24-h exposure, the cuffs were removed and the area washed clean and dried. Clinical signs and bodyweights were recorded during a 2-week observation period prior to sacrifice and gross necropsy.

Topical responses were slight (4/8), moderate (3/8) or marked (1/8) redness, slight (4/8) or moderate (4/4) swelling and moderate (7/8) or marked (1/8) necrosis. Clinical signs included transient lethargy (all animals) and rapid shallow breathing (1 rabbit at 5000 mg/kg). One animal at 5000 mg/kg died on day 5. The surviving animals were normal in appearance and behaviour by the end of two weeks. The dermal LD50 of this formulation exceeds 5000 mg/kg.

Acute eye irritation: Samples of the undiluted test material (0.1 ml) were instilled into the lower conjunctival sac of the right eye of six NZW rabbits (sex unspecified) without washing, and similarly into the sac of the right eye of another three rabbits with washout after 30 seconds. The left eye was untreated and served as a control for all rabbits. Assessment of ocular effects were made for all rabbits at 1, 2, 3, 4, 7, 10, 14 and 21 days after dosing. The raw data were presented.

The rabbits experienced moderate to severe discomfort upon instillation of the test dose. The unwashed treated eyes in 2 animals still displayed corneal irritation at 21 days after treatment. The washed eyes were clear of irritation by day 10 after treatment. The Draize scores are presented in the table below. This formulation was a severe eye irritant.

Eye irritation scores* for rabbits treated with chlorpyrifos 1 lb/gallon EUP

	Group	day 1	day 2	day 3	day 4	day 7	day 10	day 14	day 21
unwashed	average	39	28	28	23	13	10	5	3
	range	30-59	4-59	2-64	0-40	0-37	0-37	0-19	0-10
washed	average	16	10	3	8	1	0	0	0
	range	6-25	0-26	0-4	2-22	0-2	0	0	0

* maximum score = 110

Skin irritation: The undiluted formulation (0.5 ml) was applied under an occlusive dressing to one abraded and one unabraded shaven site on the backs of six male NZW rabbits. The skin reaction was scored when the patches were removed after 24 h and again 48 h later, and the raw data was presented. The formulation possessed a primary irritation score of 3.3/8.0, based on moderate redness at the sites (6/6 rabbits) at 24 h and 72 h, superficial necrosis at 72 h (2/6) and slight (3/3) to moderate (3/3) swelling at 72 h. All rabbits exhibited moderate exfoliation over the application sites at 14 days, but no irritation at 21 days post treatment. This formulation was considered to be a moderate skin irritant.

3.2.12 25% WP Formulation (TF124X)

Hall CA & Hotson IK (1967) Report of a trial on the acute oral toxicity of Dursban for sheep. Dow Report A1A-392, 12 May 1967 [Dow; Submission 11462; reference 14]

To test the acute oral toxicity potential of a 25% WP formulation of chlorpyrifos (TF124X; Dow) in sheep, the test material was administered as an oral drench to 19 merino-X sheep, with 5 animals/group treated at dose levels of 50, 100, and 150 mg/kg, and a group of four animals treated at 200 mg/kg. The dose volume ranged from 40-160 ml/animal. The animals used in this study had previously been used in lice trials.

At the high dose level, a single animal died 21 h after treatment. No animals died at other dose levels, and clinical signs were restricted to coughing and heaving respiration seen in two animals at 200 mg/kg.

3.2.13 Lorsban 250 W

Summary of acute toxicity of Lorsban 250W formulation

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rabbit	NZ white	M+F	Eye Irritation	undiluted	Slight eye irritant	Jeffrey (1986a)
Rabbit	NZ white	M+F	Skin irritation	undiluted	Slight skin irritant	Jeffrey (1986c)
Guinea pig	Hartley	M	Skin sensitisation	25% in Dowanol DPM	Not a skin sensitiser	Jeffrey (1986d)
Guinea pig	Dunkin-Hartley	M	Skin sensitisation	50% in distilled water	Not a skin sensitiser	Jones and Collier (1987)

Jeffery MM (1986a) Lorsban 250 W Insecticide: Primary eye irritation study in New Zealand white rabbits. The Dow Chemical Company Report No.: HET DR-012403456-001C dated September 25, 1986 . GLP. QA. [Dow; Submission 11462, reference 32]

This study was conducted in accordance with the intent of GLP; the GLP certificate was signed in 1991. Lorsban 250W insecticide (Dow, lot No. 720270; containing 25.7% technical chlorpyrifos; 0.1 g of white powder) was instilled as a single application into the right eyes of six (3M, 3F) NZ white rabbits (Hazleton Dutchland, USA), and these treated eyes were left unwashed. The left eye acted as an untreated control. Examination of the eyes occurred at 1, 24, 48 and 72 h post-instillation.

Following application, rabbits appeared to experience slight discomfort. At 1 h after instillation all treated eyes displayed slight to moderate conjunctival redness (irritation score 2) and swelling (irritation score 3), discharge (irritation score 2-3) and reddening of the iris (irritation score 1). At 24 h slight conjunctival redness (irritation score 1-2; 6/6animals) and chemosis (3/6) were seen, at 48 h slight conjunctival redness (irritation score 1; 6/6 animals) and chemosis (1/6), and by 72 h all treated eyes were scored as free of effects. Under the conditions of this study, Lorsban 250W insecticide was a slight eye irritant in rabbits.

Jeffrey MM (1986c) Lorsban 250 W Insecticide: Primary dermal irritation study in New Zealand white rabbits. The Dow Chemical Company Report No.: HET DR-0124-3465-1001B dated September 30 1986 QA. [Dow; Submission 11462, reference 33]

This study was conducted in accordance with the intent of GLP. Lorsban 250W insecticide (Dow, lot No. 720270; containing 25.7% technical chlorpyrifos; 0.5 g of white powder) was applied to the clipped back skin of six (4M, 2F) NZ white rabbits (Hazleton Dutchland, USA), under a moistened gauze patch (2.5 cm^2) for 4 h . The patch was then removed and the application site was wiped clean. The application sites were scored for erythema, oedema and necrosis at 30 minutes, 24, 48 and 72 h after patch removal.

Scores were uniformly zero at 30 minutes, and three rabbits (males) displayed slight erythema at 24, 48 and 72 h (irritation score 1).

Under the conditions of this study Lorsban 250W insecticide was a slight skin irritant in rabbits.

Jeffery MM (1986d) Lorsban 250 W Insecticide: Dermal sensitization potential in the Hartley albino guinea pig. The Dow Chemical Company Report No.: HET-DR-0124-3465-001E dated October 28 1986. QA. [Dow; Submission 11462, reference 34]

Lorsban 250W insecticide (Dow, lot No. 720270; containing 25.7% technical chlorpyrifos; 0.5 g of white powder) was tested in a skin sensitisation assay in ten male Hartley albino guinea pigs (Charles River Breeding Laboratories, USA). In the induction phase, the animals received three dermal applications (6h,1/week, 3 weeks) of 0.1 ml of the diluted test formulation (25% in Dowanol DPM (dipropylene glycol monomethyl ether/Tween 80; 9:1)) in chambers placed over clipped regions of the left back. An additional group of 10 guinea pigs was similarly treated with a positive control DER331 (10% epoxy resin) substance. Each time the chambers were removed, observations for erythema and/or oedema were made. In the challenge phase, two weeks after the third application, the test material or the positive control was applied in a similar manner to the induction phase (6 h in chambers) to the right side of the back. The application sites were graded for sensitization response at 24 and 48 h after the challenge application.

No signs of skin irritation were seen in any group during the induction phase of the study. When challenged with the positive control material, slight to moderate erythema was noted in 6/10 (24 h) and 9/10 (48 h) animals. When challenged with the test formulation, slight erythema was observed in 2/10 animals at 24 h, and 2/10 animals at 48 h (3 different animals in total). Under the conditions of this study, Lorsban 250W insecticide was not considered to be a skin sensitiser in guinea pigs.

Jones JR & Collier TA (1987) Lorsban 250W: Modified Buehler contact sensitization study in the guinea pig. Dow Safepharm Laboratory Project No: 44/171. QA. OECD Guideline 406 [Dow; Submission 11462, reference 35]

After a dose ranging study, Lorsban 250W insecticide (Dow, lot No. 720270; containing 25.7% technical chlorpyrifos; grey powder) was tested in a skin sensitisation assay in 12 male albino Dunkin-Hartley albino guinea pigs (Interfauna, UK). In the induction phase, the animals received nine dermal applications (6 h, days 0, 2, 4, 7, 9, 11, 14, 16, 18) of 0.5 ml of the diluted test formulation (50% w/v in distilled water) on paper filters under occlusive dressing over clipped regions of the shoulder. An additional group of 12 guinea pigs was similarly treated with a solvent-only control. Observations for erythema and/or oedema were made ca. 24 h after each induction and immediately prior to the next

application. The challenge phase commenced on day 29 with the clipping of the flanks of the animals and the application in a similar manner to the induction phase, of 0.2 ml of the diluted test material to the right flank and solvent-only to the left flank. After 6 h the occlusive bandages were removed and the application sites were graded for sensitization response at 24 and 48 h after the challenge application.

No signs of skin irritation were seen in any group (0/12 controls, 0/12 test) during the challenge phase of the study. Appendices were provided detailing appropriate responses obtained in tests using a positive and negative control and using this protocol and strain. Under the conditions of this study, Lorsban 250W insecticide was not considered to be a skin sensitiser in guinea pigs.

3.2.14 Empire 20

Parcell BI & Healing G (1991) Skin sensitization in the Guinea-Pig of Empire 20. Huntingdon Research Centre, Huntingdon, Cambridgeshire England Study No. 91291D/DWC 611/SS, conducted from 3 April to 4 May 1991 and dated June 18 1991. [Dow; Submission 11462, reference 39]

(Quality-assured study, compliant with GLP [UK 1986, US FDA 21 CFR 1987, US EPA FIFRA and TSCA 40 CFR 1983/1989, Japan MHW 1982/1988, MAFF 1984 and MITI 1984, and OECD] and performed according to OECD Test Guideline 406 and EEC Directive 84/449)

After a dose ranging study, Empire 20 (off white suspension, 21.3% chlorpyrifos, batch X1891115-08, Dow Elanco, England) was tested in a skin sensitisation assay in 10 female albino Dunkin-Hartley albino guinea pigs (D. Hall, Newchurch, UK, 6-7 wk old, initial bodyweight 400-489 g). In the induction phase, the animals received three dermal applications (6h application, 1/week, 3 weeks) of 0.5 ml of the undiluted test formulation on gauze patches under occlusive dressing over clipped regions of the left shoulder. A control group of 10 guinea pigs was similarly treated but with the omission of the test compound. Observations for erythema and/or oedema were made ca. 24 h after each induction. The challenge phase commenced two weeks after the last induction application. In the challenge phase, the animals received one dermal application (6h) of 0.5 ml of the undiluted test formulation on gauze patches under occlusive dressing over clipped regions of the right shoulder. A control group of 10 guinea pigs was similarly treated but with the omission of the test compound. Observations for erythema and/or oedema were made 24, 48 and 72 h after removal of the patches. Daily observations of clinical signs were recorded as well as bodyweights pre- and post test.

There were no treatment-related clinical signs. Bodyweights were unaffected by treatment. There were no dermal reactions seen in any test or control animals during the induction or challenge phases. No concurrent positive controls were tested, but appendices were provided by the testing facility which detailed appropriate responses obtained in tests using a positive (formalin) and negative control and using this protocol and strain. Under the conditions of this test, the Empire 20 formulation was not a skin sensitiser in guinea pigs.

3.2.15 IWD-4325 Dursban PC

Summary of acute toxicity of Dursban PC formulation

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rat	SD	M	Oral	aqueous	LD50: 541 mg/kg	Jones (1996I)
Rat	SD	M+F	Dermal	undiluted	LD50: >4000 mg/kg	Jones (1996f)
Rabbit	NZ white	M+F	Eye Irritation	undiluted	Moderate eye irritant	Jones (1996g)
Rabbit	NZ white	F	Skin irritation	undiluted	Moderate skin irritant	Jones (1996j)
Guinea pig	Dunkin-Hartley	F	Skin sensitisation	aqueous	Not a skin sensitiser	Jones (1996h)

Jones JR (1996i) IWD-4325 (Dursban PC): Acute oral toxicity test in the rat. Safepharm Laboratories Limited, UK. SPL Project no: 790/011, 22 February 1996. Dow no: GHF-R-327. [Dow; Submission 11462, reference 49]

This study was conducted in compliance with GLP principles (UK Health; OECD) and in accordance with US requirements of CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58, and OECD Guidelines no: 401. A QA report has been issued for this study. The study was performed between 1 August and 29 August 1995. Test material (Dursban PC; IWD 4325; Dow; Batch HT-76-100), was administered by oral gavage (as an aqueous solution as required) to young male and female Sprague-Dawley rats (Charles River UK; 5-8 weeks old). To establish the appropriate dose levels for the main study, a range-finding study was performed using 1 animal/sex/dose, with test doses of 100, 500, and 2000 mg/kg, and a dose volume of 10 ml. The animals were observed for death and/or signs of overt toxicity at 30 minutes, and 1, 2, and 4 h after dosing, and daily for 5 days. Based on the results of this preliminary study, the main study was conducted using 5 males/group, with a dose volume of 10 ml, and dose levels of 80, 200, and 500 mg/kg. Observations were conducted as for the range-finding study, but the observation period was extended to 14 days after dosing. In addition, a single group of 5 females was similarly administered the test material at a dose of 80 mg/kg. All animals were subjected to gross pathological examination.

In the range-finding study, animals at 2000 mg/kg and the male at 500 mg/kg were found dead on the day of dosing or the next day. Common clinical signs included ataxia, decreased respiratory rate, hunching, lethargy, and occasional body tremors with fasciculations. Other signs were noted less often, and these included splayed or tiptoe gait and increased salivation.

No deaths were reported at 80 mg/kg, while 4/5 animals died at 200 mg/kg, and 5/5 animals died at 500 mg/kg. Deaths occurred between 4 h and 2 days after dosing. In the main study, the clinical signs were similar to those reported in the range-finding study, and these effects disappeared after 1-4 days. Two females at 80 mg/kg did not display any clinical signs of toxicity during the study. Under the conditions of this study, the acute oral LD50 (95% confidence limits) was calculated to be 152 mg/kg (105-219 mg/kg).

Jones JR (1996f) IWD-4325 (Dursban PC) - Acute dermal toxicity (limit test) in the rat. Safepharm Laboratories Limited, UK. SPL Project No: 790/012, 22 February 1996. Dow report No: GHF-R-317. [Dow; Submission 11462, reference 51]

This study was conducted in compliance with GLP principles (UK Health; OECD) and in accordance with US requirements of CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58, and OECD Guidelines no: 402. A QA report has been issued for this study. The study was performed between 7 August and 2 August 1995.

Undiluted test material (Dursban PC; IWD 4325; Dow; Batch HT-76-100) was applied to the intact clipped skin on the back and flanks of five male and five female rats (Charles River UK; 10-14 weeks old) at a dose level of 4000 mg/kg, with a dose volume of 3.75 ml/kg. Surgical gauze was placed over the treatment area, and the test site was then semi-occluded with a piece of self adhesive bandage. Residual test material was removed from the test site after 24 h. Animals were observed for mortality and/or signs of toxicity at 30 minutes, and 1, 2, and 4 h after dosing, then daily for 14 days. After removal of the dressing, the test sites were examined for evidence of primary irritation, and scored according to the method of Draize.

No deaths occurred during the study, and clinical signs included hunched posture, lethargy, decreased respiratory rate, and ataxia. No signs of skin irritation were observed during the study. Under the conditions of this study, the dermal LD50 for the test material was >4000 mg/kg. The test material was not irritating to the skin of rats.

Jones JR (1996g) IWD-4325 (Dursban PC) - Acute eye irritation test in the rabbit. Safepharm Laboratories Limited, UK. SPL Project No: 790/014, 22 February 1996. Dow Report No: GHF-R-318. [Dow; Submission 11462, reference 52]

This study was conducted in compliance with GLP principles (UK Health; OECD) and in accordance with US requirements of CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58, and OECD Guidelines no: 405. A QA report has been issued for this study. The study was performed between 10 July and 17 July 1995. An aliquot of the undiluted test substance (0.1 ml; Dursban PC; IWD 4325; Dow; Batch HT-76-100), was instilled into the conjunctival sac of the right eyes of three New Zealand White rabbits (1M, 2F; David Percival, UK; 12-16 weeks old). The untreated left eyes of the animals were used as controls. Assessment of ocular irritation was made approximately 1, 24, 48, and 72 h after treatment, and irritation was scored according to the method of Draize. An additional assessment was made on Day 7 of the study.

Scattered or diffuse areas of corneal opacity were observed in the treated eye of one animal at 24 and 48 h. This effect had resolved by 72 h. Slight iridial effects were seen in all animals at 1 h, and this persisted in a single animal until 48 h. Conjunctival redness was seen in all animals and ranged from slight to moderate (diffuse, deeper crimson), and persisted for up to 72 h, while slight to moderate conjunctival chemosis and discharge were also seen in all animals. The group-mean irritation scores ranged from 18.3 at 1 h to 1.3 at 72 h. No signs of irritation were seen at the Day 7 inspection. Under the conditions of this study, the test material was a moderate eye irritant to rabbits.

Jones JR (1996j) IWD-4325 (Dursban PC) :- Acute dermal irritation test in the rabbit. Safepharm Laboratories Project No.: 790/013, certificates dated February 22 1996, study performed 29 June - 6 July, 1995. QA. GLP. OECD guideline 404 [Dow; Submission 11462, reference 50]

Three female NZW rabbits (David Percival Ltd, UK, 12-16 weeks) were exposed for 4 h to a gauze patch containing 0.5 ml of the test material. The patch was applied to the shorn dorsal flank area under a semi-occlusive dressing. The test material was identified as a clear yellow to light brown liquid, Dursban PC, Batch #HT-76-100. After the 4-h exposure period, the patch was removed and the application area gently swabbed free of the test material. The application areas were scored for irritance (Draize) at 1, 24, 48 and 72 h later.

Slight to moderate erythema (score 1-2) was present at all three application sites at each of the 1, 24, 48 and 72 h scoring times. Very slight to slight oedema (score 1-2) was present at all three application sites at each of the 1, 24, 48 and 72 h scoring times. At seven days all signs of irritation had resolved other than some minor crust formation (2/3) and desquamation (1/3). The Primary Irritation Index was 22/6 = 3.7. Dursban PC was considered to be a moderate skin irritant to rabbit skin.

Jones JR (1996h) IWD-4325 (Dursban PC): Buehler delayed contact hypersensitivity study in the guinea pig. Safepharm Laboratories Limited, UK. SPL project No: 790/015, 22 February, 1996. Dow Report No: GHF-R-316. [Dow; Submission 11462, reference 53]

This study was conducted in compliance with GLP principles (UK Health; OECD) and in accordance with US requirements of CFR Part 160, 40 CFR Part 792 and 21 CFR Part 58, and OECD Guidelines no: 406. A QA report has been issued for this study. The study was performed between 5 July and 13 August 1995.

To assess the skin sensitisation potential, test material (Dursban PC; IWD 4325; Dow; Batch HT-76-100) was prepared as a 50% v/v solution in distilled water (for induction procedures) and as 25% and 10% v/v solutions in distilled water for challenge procedures. The test animals were female albino Dunkin-Hartley guinea pigs (David Hall Ltd., UK) aged approximately 8-12 weeks at the start of the main study. To determine the concentration of test material for the main study, preliminary tests were conducted. In the preliminary induction test, two animals were treated with 0.5 ml of the 50% solution of test material, with applications made to the clipped flanks under occlusive dressings for a 6 h exposure period. The degree of erythema and oedema was assessed at 24 and 48 h after dressing removal. For the preliminary challenge procedure, two animals that had received induction applications on days 0, 7, and 14 were treated with 0.5 ml of the two concentrations of test material (25% and 10%) in a topical challenge. The results of these tests were used to determine the concentrations used in the main study.

In the main study, 0.5 ml aliquots of a 50% v/v solution of test material were applied to the clipped intact skin on the left flanks of twenty animals, and held in place with an occlusive dressing for six hours. This procedure was repeated on days 7 and 14 of the study. Approximately 24 h after each induction dose, the degree of erythema and oedema was assessed. A control group (10 animals) received the vehicle only, using the same procedure as test animals. At day 28, challenge doses of 0.5 ml of a 25% solution of test material were applied to the shorn right flanks of all animals, and held in place under occlusive dressings for 6 h. The treatment sites were washed and assessed for signs of skin irritation at 24 and 48 h after removal of the dressings. In a similar procedure, a 10% solution of the test material was used as a challenge on the left flanks of all animals.

Following topical application with the test material, very slight to well-defined erythema with or without very slight oedema was observed. No skin reactions were observed following induction treatment in control animals. No skin reactions were observed following challenge in any animals during the study. No concurrent positive controls were used, but the validity of this testing protocol was routinely determined by the testing facility, and results of such recent tests have been provided by the testing laboratory in this report. Under the conditions of this study, the test material was not a skin sensitiser to guinea pigs.

3.2.16 Dursban Dust

Carreon RR & New MA (1981) Dursban Dust: Acute toxicological properties. Dow Chemical USA Study no: HET-4516-(1) dated July 28 1981. [Dow; Submission 11462, reference 38]

Dursban dust (identified as M-4516 and GHD-0408-18, yellow solid, from Dow Chemicals Agricultural Products Dept, Michigan. Composition: 1.0% chlorpyrifos from Dursban R and 99.0% yellow corn flour and inerts) was tested to evaluate the acute oral and dermal LD50's, and the eye and skin irritation potential.

Acute oral toxicity: Four groups (6/sex/dose) of fasted rats (CDF, CRL, 66-101 g) were administered 630, 1300, 2500 or 5000 mg/kg of the test material by single oral gavage. Clinical signs and body weights were recorded regularly and gross pathology examination performed at 2 weeks post-dose. There were no deaths, weight gains were normal, isolated muscle spasms were recorded in 2/6 females and body tremors in 6/6 males at 5000 mg/kg, and there were no treatment-related findings at necropsy (no pathology data provided). The acute oral LD50 for Dursban dust exceeds 5000 mg/kg.

Acute dermal toxicity: The test substance was applied at 5000 mg/kg with 5ml of water under an occlusive wrap to the clipped skin of four NZW rabbits (Langshaw Farms, Michigan, 2M & 2F). After 24 h the wrap was removed and the skin was washed and scored for irritation. The animals were observed regularly throughout the next 2 weeks, body weights were recorded weekly and gross pathology examination was conducted at necropsy.

All animals appeared lethargic on the day of treatment. Slight erythema (3/4) and slight oedema (1/4) were visible when the treatment wraps were removed. Observations at 1 and 2 weeks post treatment were unremarkable, and bodyweights were not significantly affected by treatment. Gross pathological examination at terminal necropsy did not record any treatment-related findings (raw data not provided). The acute dermal LD50 of Dursban dust (1%) exceeds 5000 mg/kg in rabbits.

Acute eye irritation: Aliquots of the undiluted test material (0.1 mg) were instilled into the lower conjunctival sac of the right eye of six NZW rabbits (Langshaw Farms, Michigan, mixed sex) without washing, and similarly into the sac of 3 rabbits with washout after 30 seconds. The left eye of each animal served as a control. Animals were returned to their cages immediately following installation of the test material, and assessment of ocular effects were made for all rabbits at 1, 2, 3, 4 and 7 days after dosing, and scored according to the method of Draize.

Slight conjunctival redness (Draize score 1) was seen in 3/6 and 0/3 of the treated unwashed and washed eyes respectively, but this effect had disappeared by 72 h in all animals. Under the conditions

of this study, the test material was considered to be a slight eye irritant in rabbits.

Acute skin irritation: The test substance (0.5 g) was applied under a gauze patch and occlusive wrap to one abraded and one unabraded site on the clipped back skin of six NZW rabbits (Langshaw Farms, Michigan, 4M, 2F) for 24 h. The sites were score for irritation after patch removal and 48 h later. Slight erythema was observed at 24 h in 3/6 unabraded and 6/6 abraded sites. No irritation was visible at the 72 h scoring interval. The primary irritation score was 0.37 out of a possible 8. Under the conditions of this study, the test material was considered to be a slight skin irritant in rabbits.

3.2.17 Dursban Micro-Lo

Summary of acute toxicity of Dursban Micro-Lo formulation

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rat	SD	M F	Oral	aqueous	LD50: > 250 mg/kg LD50: 297 mg/kg	Jones (1996a)
Rat	SD	M+F	Dermal	undiluted	LD50: >4000 mg/kg	Jones (1996b)
Rabbit	NZ white	F	Eye Irritation	undiluted	Severe eye irritant	Jones (1996d)
Rabbit	NZ white	M	Skin irritation	undiluted	Moderate skin irritant	Jones (1996c)
Guinea pig	Dunkin-Hartley	F	Skin sensitisation	aqueous	Not a skin sensitiser	Jones (1996p)

Jones JR (1996a) IWD-4192 (Dursban Micro-Lo Insecticide): Acute oral toxicity test in the rat. Safepharm Laboratories Project No.: 790/006, certificates dated February 22 1996. QA. GLP. OECD guideline 401 [Dow; Submission 11462, reference 44]

In a range finding study, individual (1/sex/dose) young (5-8 weeks) rats (Sprague-Dawley CD Charles River (UK), 5/sex) were administered an aqueous solution of 100, 500 and 2000 mg/kg of Dursban Micro-lo insecticide (a pale straw-coloured liquid, Batch #HN950427-06) by single oral gavage. Based on the results of this study (both rats at 2000 mg/kg died within 24 h), the main study was performed in which 5 females/dose were administered 250, 500 and 1000 mg/kg, and 5 males were administered 250 mg/kg of the same test substance by single oral gavage. The animals in the main study were observed for deaths or overt signs of toxicity up to 4 h after dosing and daily thereafter, body weights were recorded weekly and gross pathology was performed on all animals dying during the study and at terminal sacrifice at 2 weeks post-dose

Deaths were reported as 2 males at 250 mg/kg (2 h, 1 day), 1 female at 250 mg/kg (1 day), 5 females at 500 mg/kg (1 on day 1, 4 on day 2), and 5 females at 1000 mg/kg (4 on day 1, 1 on day 2). Clinical signs commenced within 0.5 h of dosing and included in most rats hunched posture, lethargy, decreased respiratory rate, laboured breathing and ataxia, and occasional incidence of increased salivation and exophthalmos. Surviving animals recovered within 4-7 days after dosing. bodyweight gains were not significantly affected by treatment and there were no treatment-related gross pathology findings at day 14 sacrifice. Animals dying and necropsied during the study were reported to have haemorrhagic lungs, dark liver and dark kidneys. Males were considered not to be markedly more sensitive than females to the oral toxicity of the test material. The acute oral LD50 of Dursban Micro-Lo Insecticide was calculated to be 297 (193-459) mg/kg in female rats.

Jones JR (1996b) IWD-4192 (Dursban Micro-Lo Insecticide): - Acute dermal toxicity (limit test) in the rat. Safepharm Laboratories Project No.: 790/007 certificates dated February 22 1996. QA. GLP. OECD guideline 402 [Dow; Submission 11462, reference 43]

Young (10-14 weeks) rats (Sprague-Dawley CD Charles River (UK), 5/sex) were dermally exposed to a dose of 4000 mg/kg of Dursban Micro-lo insecticide (a pale straw-coloured liquid, Batch #HN950427-06) for 24 h. The dose was administered on a gauze patch to ca. 10% of the shorn back and flank skin under a semi-occlusive dressing. After the 24 h exposure period, the patch was removed and the application area gently swabbed free of the test material. The animals were observed regularly during the 24 h exposure period and daily thereafter, and signs of toxicity and dermal irritance (Draize scale) noted. Bodyweights were noted weekly, and necropsy findings recorded on day 14.

There were no deaths during the study, and clinical signs of hunched posture, decreased respiratory rate and lethargy were recorded in most rats on day1, mainly females on day2, and not thereafter. There were no signs of dermal irritancy on any application sites on any day. Bodyweight gains were unaffected by treatment, and there were no abnormalities detected at necropsy. The dermal LD50 of Dursban Micro-Lo insecticide exceeds 4000 mg/kg in rats.

Jones JR (1996d) IWD-4192 (Dursban Micro-Lo Insecticide): Acute eye irritation test in the rabbit. Safepharm Laboratories Project No.: 790/009, certificates dated February 22 1996. QA. GLP. OECD guideline 405 [Dow; Submission 11462, reference 47]

The eye irritation potential of the test material was assessed in rabbits. The test material was identified as a pale straw-coloured liquid, Dursban Micro-lo insecticide, Batch #HN950427-06. An aliquot (0.1 ml) was instilled into the conjunctival sac of the right eye of one female New Zealand White rabbit (David Percival, UK; 12-16 weeks old), without washout; a local anaesthetic had been instilled into the eye 1-2 min before treatment. The untreated left eye of the animal was used as control. Assessment of ocular irritation was made approximately 1, 24, 48, and 72 h after treatment, and scored according to the method of Draize, and Group Mean Scores for irritation at each time point calculated according to a modified Kay & Calandra method.

Moderate to severe conjunctival redness (score 2-3), chemosis (score 2-3) and discharge (score 3) was evident at all scoring times. There was also slight iridial irritation (score 1) at all times. Additionally there was corneal opacity covering most of the area (score 4 for area), ranging from scattered (score 1 , 24 & 48 h) to easily discernible (score 2) at 72 h. Ectropion was observed at 72 h. The total Draize scores were 19, 39, 39 & 69 at 1, 24, 48 & 72 h respectively. The animal was sacrificed at 72 h due to obvious pain discomfort, and no further animals were tested. There was thus no day-7 eye irritancy score; however the effects were worsening and not resolving at 72 h, so Dursban Micro-lo insecticide was regarded as a severe irritant to the rabbit eye.

Jones JR (1996c) IWD-4192 (Dursban Micro-Lo insecticide): Acute dermal irritation test in the rabbit. Safepharm Laboratories Project No: 790/008. QA certificates dated February 22 1996. GLP. OECD guideline 404. [Dow; Submission 11462, reference 42]

Three male NZW rabbits (David Percival Ltd, UK, 12-16 weeks) were exposed for 4 h to a gauze

patch containing 0.5 ml of the test material. The patch was applied to the shorn dorsal flank area under a semi-occlusive dressing. The test material was identified as a pale straw-coloured liquid, Dursban Micro-lo insecticide, Batch #HN950427-06. After the 4 h exposure period, the patch was removed and the application area gently swabbed free of the test material. The application areas were scored for irritance (Draize) at 1, 24, 48 and 72 h later. Additional observations were made on days 7 and 14 to assess reversibility.

Erythema was clearly present at 1 h (3/3 well defined, score 2), peaked at 72 h (3/3 moderate to severe, score 3), and slowly decreased thereafter (2/3 slight at day 7, score 1) until day 14 (3/3 score zero). Oedema was clearly present at 1 h (3/3 slight, score 2), increased at 24 and 48 h (2/3 moderate - score 3, 1/3 slight - score 2), declined at 72 h (3/3 slight - score 2) and was absent by day 14. The sum of the 24 and 72 h readings was 29 and the Primary Irritation Index was 4.8. Dursban Micro-Lo insecticide was considered to be a moderate skin irritant in rabbits.

Jones JR (1996p) IWD-4192 (Dursban Micro-Lo Insecticide): Buehler delayed contact hypersensitivity study in the guinea pig. Safepharm Laboratories Project No.: 790/010, certificates dated February 22 1996, study performed July-August 1995. QA. GLP. OECD guideline 406 [Dow; Submission 11462, reference 48]

This study was designed to assess the skin sensitization potential of Dursban Micro-lo Insecticide in guinea pigs. The test animals were female albino Dunkin-Hartley guinea pigs (David Hall Ltd., UK) aged approximately 8-12 weeks at the start of the main study. The test material was identified as a pale straw-coloured liquid, Dursban Micro-lo insecticide, Batch #HN950427-06. The test material was prepared as a 25% v/v solution in distilled water (for induction procedures) and as 25% and 10% v/v solutions in distilled water for challenge procedures. To determine the concentration of test material for the main study, preliminary tests were conducted. In the preliminary induction test, 4 animals were treated with 0.5 ml of either a 50% or 25% solution of test material, with applications made to the clipped flanks under occlusive dressings for a 6 h exposure period. The degree of erythema and oedema was assessed at 24 and 48 h after dressing removal. For the preliminary challenge procedure, two animals that had received induction applications of solvent only on days 0, 7, and 14 were treated with 0.5 ml of each of 4 concentrations of test material (25%, 10%, 5% and 2%) in a topical challenge. The results of these tests were used to determine the concentrations used in the main study.

In the main study, 0.5 ml aliquots of a 25% v/v solution of test material on a cotton patch were applied to the clipped intact skin on the left flanks of twenty animals, and held in place with an occlusive dressing for 6 h. This procedure was repeated on days 7 and 14 of the study. Approximately 24 h after each induction dose, the degree of erythema and oedema was assessed. A control group (10 animals) received the vehicle only, using the same procedure as test animals. At day 28, challenge doses of 0.5 ml of a 25% solution of test material on a cotton patch were applied to the shorn right flanks of all animals, and held in place under occlusive dressings for 6 h. In a similar procedure, a 10% solution of the test material was used as a challenge on a separate site on the shorn right flanks of all animals. The treatment sites were washed and assessed for signs of skin irritation at 24 and 48 h after removal of the dressings using the method of Draize.

One treated animal died on day 13, and this was thought to be unrelated to treatment. The induction

phase of the main study recorded slight to moderate erythema (score 1 or 2) on 4/20 sites on day1, 11/20 on day 8 and 18/19 on day 15. Similarly oedema was recorded for 0, 1 and 6 sites on days 1, 8 and 15 respectively. No control sites recorded any irritation. During the challenge phase, scoring of the control animals recorded no irritation reactions at the 10% sites, and only slight erythema (score 1) at 2/10 of the 25% sites, and that at 24 h only. Similarly the treated animals recorded no irritation at the 10 % sites at either 24 or 48 h. The 25% challenge induced slight (score 1) erythema only at 4/19 sites at 24 h, and no erythema or oedema at any sites at 48 h. No concurrent positive controls were used, but the validity of this testing protocol was routinely determined by the testing facility, and appendices were provided detailing appropriate responses obtained in tests using a positive and negative control and using this protocol and strain. Under the conditions of this study, Dursban Micro-lo Insecticide was considered not to be a skin sensitiser to guinea pigs

3.2.18 Dursban Micro-Lo 2%

Jones JR (1996e) IWD-4192 (Dursban Micro-Lo) - 2% active ingredient:- Acute dermal irritation test in the rabbit. Safepharm Laboratories Project No.: 790/032, certificates dated February 22 1996. QA. GLP. OECD guideline 404 [Dow; Submission 11462, reference 45]

Three NZW rabbits (2M & 1F, David Percival Ltd, UK, 12-16 weeks) were exposed for 4 h to a gauze patch containing 0.5 ml of a 2% aqueous dilution of the test material. The patch was applied to the shorn dorsal flank area under a semi-occlusive dressing. The test material was identified as a pale straw-coloured liquid, Dursban Micro-lo insecticide, Batch #HN950427-06. After the 4-h exposure period, the patch was removed and the application area gently swabbed free of the test material. The application areas were scored for irritance (Draize) at 1, 24, 48 and 72 h later.

Slight erythema (score 1) was present at 1 h at one application site, but there were no other signs of irritance at any other scoring time. The Primary Irritation Index was 0.0. A 2% emulsion of Dursban Micro-Lo insecticide was considered to be non-irritant to rabbit skin.

Jones JR (1996q) IWD-4192 (Dursban Micro-Lo) - 2% active ingredient: Acute eye irritation test in the rabbit. Safepharm Laboratories Project No.: 790/033, certificates dated February 22 1996. QA. GLP. OECD guideline 405 [Dow; Submission 11462, reference 46]

The eye irritation potential of a 2% aqueous dilution of the test material was assessed in rabbits. The test material was identified as a pale straw-coloured liquid, Dursban Micro-lo insecticide, Batch #HN950427-06. An aliquot of the 2% dilution (0.1 ml) was instilled into the conjunctival sac of the right eyes of three New Zealand White rabbits (1M, 2F; David Percival, UK; 12-16 weeks old), without washout. The untreated left eyes of the animals were used as controls. Assessment of ocular irritation was made approximately 1, 24, 48, and 72 h after treatment, and irritation was scored according to the method of Draize, and Group Mean Scores for irritation at each time point calculated according to a modified Kay & Calandra method.

At 1 h there was slight conjunctival redness (score 1, 3/3 eyes), slight chemosis (score 1, 3/3 eyes) and slight to moderate discharge (score 1, 1/3 eyes; score 2, 2/3 eyes). Additionally at 1 h there was slight iridial irritation (score 1, 3/3 eyes) but no corneal involvement. By 24 h there was only slight conjunctival redness (score 1, 2/3 eyes) and chemosis (score 1, 1/3 eyes); by 48 h only conjunctival redness was

apparent (score 1, 2/3 eyes), and by 72 h all signs of irritation had ceased. The Group Mean Scores at 1, 24, 48 and 72 h respectively were: 12.3, 2.0, 1.3 and 0.0 . A 2% emulsion of Dursban Micro-Lo insecticide was considered to be a slight irritant to rabbit eyes.

3.2.19 Predator 300

Jones JR (1996l) Predator 300 Insecticide: Acute Oral Toxicity Test in the Rat. SafePharm Laboratories, UK. SPL study No.790/039, report code GHF-R-334, dated August 6, 1996. GLP, OECD 401 [Dow; Submission 11422, reference 7]

In a range finding study, individual (1/sex/dose) young (5-8 weeks) rats (Sprague-Dawley CD Charles River (UK), 5/sex) were administered undiluted test material (550 mg/kg) or an aqueous solution of 1000 and 500 mg/kg of Predator 300 Insecticide (a pale straw-coloured liquid, Batch #HT-76-198) by single oral gavage. Based on the results of this study (both rats at 1000 mg/kg died within 24 h; the males at 550 and 500 mg/kg died within one or two days of dosing), the main study was performed in which 5 males/dose were administered 300, 500 and 833 mg/kg, and 5 females 300 mg/kg of the same test substance by single oral gavage. The animals in the main study were observed for deaths or overt signs of toxicity up to 4 h after dosing and daily thereafter, body weights were recorded weekly and gross pathology was performed on all animals dying during the study and at terminal sacrifice at 2 weeks post-dose

Deaths were reported as 0/5 males and 0/5 females at 300 mg/kg, 3 males at 500 mg/kg (2/5 on day 1, 1/5 on day 2) and 5/5 males at 833 mg/kg (5/5 within 4 h). Clinical signs commenced within 0.5 h of dosing and included hunched posture, lethargy, decreased respiratory rate, laboured breathing and ataxia, splayed or tiptoe gait and (in males only) an occasional incidence of increased salivation and exophthalmos, signs of fasciculations, noisy respiration, chromodacryorrhea and increased lacrimation. Surviving animals recovered within 3-6 days after dosing. Bodyweight gains were not significantly affected by treatment and there were no treatment-related gross pathology findings at day-14 sacrifice. Animals dying and necropsied during the study were reported to have haemorrhagic lungs, dark liver and dark kidneys. The acute oral LD50 of Predator 300 Insecticide was calculated to be 475 (370-610) mg/kg in male rats.

Jones JR (1996k) Predator 300 Insecticide: Acute Dermal Toxicity (Limit Test) in the Rat. SafePharm Laboratories, UK. SPL study No.790/040, report code GHF-R-335, dated August 6, 1996. GLP, OECD 402 [Dow; Submission 11422, reference 8]

Young (10-14 weeks) rats (Sprague-Dawley CD Charles River (UK), 5/sex) were dermally exposed to a dose of 4000 mg/kg of Predator 300 Insecticide (a pale straw-coloured liquid, Batch #HT-76-198) for 24 h. The dose was administered on a gauze patch to ca. 10% of the shorn back and flank skin under a semi-occlusive dressing. After the 24-h exposure period, the patch was removed and the application area gently swabbed free of the test material. The animals were observed regularly during the 24-h exposure period and daily thereafter, and signs of toxicity and dermal irritance (Draize scale) noted. Bodyweights were noted weekly, and necropsy findings recorded on day 14.

There were no deaths during the study. There were no clinical signs or skin irritation seen during this

study. Bodyweight gains were unaffected by treatment, and there were no abnormalities detected at necropsy. The dermal LD50 of Predator 300 Insecticide was > 4000 mg/kg in rats.

Jones JR (1996m) Predator 300 Insecticide: Acute dermal irritation test in the rabbit. SafePharm Laboratories, UK. SPL study No.790/041, report code GHF-R-336, dated August 6, 1996. GLP, OECD 404 [Dow; Submission 11422, reference 11]

Three NZW rabbits (1F, 2 M) (David Percival Ltd, UK, 12-16 weeks) were exposed for 4 h to a gauze patch containing 0.5 ml of the test material. The patch was applied to the shorn dorsal flank area under a semi-occlusive dressing. The test material was identified as Predator 300 Insecticide (a pale straw-coloured liquid, Batch #HT-76-198). After the 4-h exposure period, the patch was removed and the application area gently swabbed free of the test material. The application areas were scored for irritance (Draize) at 1, 24, 48 and 72 h later. Additional observations were made on days 7 and 14 to assess reversibility.

Erythema was clearly present at 1 h (3/3 well defined, score 2) and declined thereafter. The score at 24 h was 2/3 sites with well defined erythema and 1/3 with slight (1/3), at 48 and 72 h there were 1/3 sites with well defined and 1/3 with slight erythema. These sites exhibited crust formation or desquamation at 7 and 14 d. Oedema was clearly present at 1 h (3/3 very slight - score 1), increased at 24 (1/3 slight - score 2, 1/3 very slight), declined at 48 and 72 h (1/3 very slight) and was absent by day 7. The sum of the 24 and 72 h readings was 12 and the Primary Irritation Index was 2.0. Under the conditions of this study Predator 300 Insecticide was considered to be a slight skin irritant in rabbits.

Jones JR (1996n) Predator 300 Insecticide: Acute eye irritation test in the rabbit. SafePharm Laboratories, UK. SPL study No.790/042, report code GHF-R-337, dated August 6, 1996. GLP, OECD 405 [Dow; Submission 11422, reference 10]

The eye irritation potential of the undiluted test material was assessed in rabbits. The test material was identified as Predator 300 Insecticide, comprising 300 g/litre chlorpyrifos (a pale straw-coloured liquid, Batch #HT-76-198). An aliquot of the test substance (0.1 ml) was instilled into the conjunctival sac of the right eyes of three male New Zealand White rabbits (David Percival, UK; 12-16 weeks old), without washout. The untreated left eyes of the animals were used as controls. Assessment of ocular irritation was made approximately 1, 24, 48, and 72 h after treatment, irritation was scored according to the method of Draize, and Group Mean Scores for irritation at each time point calculated according to a modified Kay & Calandra method.

Conjunctivae exhibited mild redness at 1 and 24 h (score 2, 3/3 eyes), and this decreased to slight redness (score 1) in all treated eyes at 48 and 72 h, but was not present at 7 d. Chemosis was mild at 1 h (score 2, 3/3 eyes), but decreased to slight (score 1, 3/3 eyes) at 24 and 48 h, decreased further at 72 h (score 1, 2/3 eyes) and was absent at 7 d. Discharge was marked at 1 h (score 3, 3/3 eyes), but declined to slight at 24 h (score 1, 3/3 eyes), to very slight at 72 h (score 1, 1/3 eyes), and was absent at 7 d. Additionally at 1 h there was slight iridial irritation (score 1, 3/3 eyes) but no corneal involvement apart from slight dulling seen in one eye at 1h. The Group Mean Scores at 1, 24, 48 and 72 h respectively were: 19.0, 8.0, 6.0 and 4.0 . Under the conditions of this study, Predator 300 Insecticide was considered to be a slight eye irritant in rabbits.

Jones JR (1996o) Predator 300 Insecticide: Buehler delayed contact hypersensitivity study in the guinea pig. SafePharm Laboratories, UK. SPL study No.790/043, report code GHF-R-338, dated August 6, 1996. GLP. OECD 406 [Dow; Submission 11422, reference 12]

This study was designed to assess the skin sensitization potential of Predator 300 Insecticide in guinea pigs. The test animals were female albino Dunkin-Hartley guinea pigs (David Hall Ltd., UK) aged approximately 8-12 weeks at the start of the main study. The test material was identified as Predator 300 Insecticide, comprising 300 g/litre chlorpyrifos (a pale straw-coloured liquid, Batch #HT-76-198) The test material was applied undiluted for induction procedures, and undiluted or as a 75% v/v solution in distilled water for challenge procedures. To determine the concentration of test material for the main study, preliminary tests were conducted. In the preliminary induction test, 2 animals were treated with 0.5 ml of 100%, 75%, 50% and 25% solution of test material, with applications made to the clipped flanks under occlusive dressings for a 6 h exposure period. The degree of erythema and oedema was assessed at 24 and 48 h after dressing removal. For the preliminary challenge procedure, two animals that had received induction applications of solvent only on days 0, 7, and 14 were treated with 0.5 ml of each of a 75% solution and undiluted test material.. The results of these tests were used to determine the concentrations used in the main study.

In the main study, 0.5 ml aliquots of undiluted test material on a cotton patch were applied to the clipped intact skin on the left flanks of twenty animals, and held in place with an occlusive dressing for six hours. This procedure was repeated on days 7 and 14 of the study. Approximately 24 h after each induction dose, the degree of erythema and oedema was assessed. A control group (10 animals) followed the same procedure except that a blank patch was applied. At day 28, challenge doses of 0.5 ml of undiluted test material and a 75% solution on a cotton patch were applied to the shorn right flanks of all animals, and held in place under occlusive dressings for 6 h. The treatment sites were washed and assessed for signs of skin irritation (Draize) at 24 and 48 h after removal of the dressings.

The induction phase of the main study recorded no irritation. During the challenge phase, scoring recorded no irritation at 24 or 48 h. Body weight gains were unaffected by treatment. No concurrent positive controls were used, but the validity of this testing protocol was routinely determined by the testing facility, and appendices were provided detailing appropriate responses obtained in tests using a positive and negative control and using this protocol and strain. Under the conditions of this study, Predator 300 Insecticide was considered not to be a skin sensitiser to guinea pigs.

3.2.20 CHA 7110

Summary of acute toxicity of CHA 7110 (480 g/litre EC formulation)

Species	Strain	Sex	Study Type	Vehicle	Outcome	Reference
Rat	SD	M F M+F	Oral	undiluted	LD50: 205 mg/kg LD50: 350 mg/kg LD50: 293 mg/kg	Dreher (1995a)
Rat	SD	M+F	Dermal	undiluted	LD50: >4000 mg/kg	Dreher (1995b)
Rat	SD	M F M+F	Inhalation	undiluted	LC50: 2160 mg/m^3 LC50: 2320 mg/m^3 LC50: 2260 mg/m^3	Blagden (1995)

Rabbit	NZ white	M+F	Eye Irritation	undiluted	Moderate eye irritant	Kuhn (1996b)
Rabbit	NZ white	M+F	Skin irritation	undiluted	Moderate skin irritant	Dreher (1995c)
Rabbit	NZ white	M+F	Skin irritation	undiluted	Slight skin irritant	Kuhn (1996a)
Guinea pig	Dunkin-Hartley	F	Skin sensitisation	aqueous	Not a skin sensitiser	Dreher (1995d)

Dreher DM (1995a) CHA 7110: Acute oral toxicity test in the rat. Safepharm Laboratories Ltd, UK Project no: 545/67. Report dated 24 April 1995. Cheminova Agro A/S report CHA Doc No: 26-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-1, TSCA Subpart B, Section 798.1175). The study was conducted between 24 November and 28 December 1994.

The undiluted test material (CHA 7110; an emulsifiable concentrate formulation containing chlorpyrifos at 480 g/litre; Cheminova Agro A/S; certified concentration of active ingredient 45.4%; batch no. 359-KMA-30) was given once to male and female Sprague-Dawley rats (Charles River UK Ltd; approximately 10-14 weeks old at the start of the main study) by oral gavage. In a range-finding study, one animal/sex/dose was tested at doses of 50, 100, 200, 500 mg/kg (dose volume 0.05-0.47 ml/kg), and deaths and overt signs of toxicity were recorded 30 minutes, and 1, 2, and 4 h after dosing and then daily for five days. Based on the results of the range-finding study, the main study consisted of three groups of animals (5/sex/dose) given doses of 245, 350, or 500 mg/kg, in the same manner as the range-finding study (dose volume 0.23-0.47 ml/kg). Deaths and overt signs of toxicity were recorded 30 minutes, and 1, 2, and 4 h after dosing and then daily for 14 days. Individual bodyweights were recorded prior to dosing on Day 0 and on days 7 and 14, or at death. At the end of the dosing period, all surviving animals were killed, and all animals, including those that died during the study, were subjected to gross pathological examination, including opening of the abdominal and thoracic cavities. No tissues were retained.

Results

In the range-finding study, both animals given 500 mg/kg died, at 1 h (male) and 2 days (female). No other deaths were recorded. Signs of intoxication included ataxia, fasciculations, hunched posture, lethargy, increased salivation, laboured respiration, occasional body tremors, splayed gait, and increased lacrimation. At 50 and 100 mg/kg, clinical signs were confined to hunched posture, ataxia and decreased respiratory rate.

In the main study, deaths occurred at all dose levels. The incidence of mortality in males was 4/5, 2/5, and 5/5, and in females was 1/5, 3/5, and 3/5, at 245, 350, and 500 mg/kg, respectively. No males survived beyond 1 day at 500 mg/kg. Common clinical signs of intoxication were similar to those reported in the range-finding study, but surviving animals recovered four to ten days after dosing. Additional or isolated signs of toxicity were ptosis, noisy respiration, red/brown stains around the eyes, pallor of the extremities, chromodacryorrhea, and pilo-erection. In animals killed *in extremis* or that died

during the study, common necropsy findings were haemorrhagic lungs, dark liver, dark kidneys, and slight haemorrhage and/or sloughing of the gastric mucosa. No abnormalities were noted at necropsy in animals killed at the end of the study.

Under the conditions of this study, the acute oral median LD50 (with 95% confidence limits) of the 45.4% EC formulation in Sprague-Dawley rats was 205 (38-1092) mg/kg in males, 350 (208-589) mg/kg in females, and 293 (167-513) mg/kg in all animals.

Dreher DM (1995b) CHA 7110: Acute dermal toxicity (limit test) in the rat. Safepharm Laboratories Ltd, UK Project no: 545/68. Report dated 24 April 1995. Cheminova Agro A/S report CHA Doc No: 27-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-2, TSCA Subpart B, Section 798.1100). The study was conducted between 28 December 1994 and 11 January 1995.

The undiluted test material (CHA 7110; an emulsifiable concentrate formulation containing chlorpyrifos at 480 g/litre; Cheminova Agro A/S; certified concentration of active ingredient 45.4%; batch no. 359-KMA-30) was applied dermally to a single group of male and female Sprague-Dawley rats (5/sex; Charles River UK Ltd; approximately 10-14 weeks old at the start of the main study). The test sites were clipped areas on the back and flanks of each animal. The applied dose was 4000 mg/kg, with a dose volume of 3.69 ml/kg. Surgical gauze was applied over the test area and semi-occluded with self-adhesive bandage for the 24-h exposure period. Animals were observed for mortality or signs of overt toxicity at 30 minutes, and 1, 2, and 4 h after dosing, and then daily for 14 days. After the 24-h contact period, the bandage was carefully removed and the treated skin wiped with cotton wool moistened with distilled water. The test sites were examined for evidence of primary irritation and scored according to the method of Draize. Individual bodyweights were recorded prior to dosing on Day 0 and on days 7 and 14. At the end of the dosing period, all animals were killed and subjected to gross pathological examination, including opening of the abdominal and thoracic cavities. No tissues were retained.

Results

No deaths were reported. In the main study, no clinical signs were noted in males. Hunched posture and lethargy were noted in all females one day after dosing, with hunched posture, lethargy and decreased respiration noted two days after dosing. No signs were reported later than two days after dosing. Very slight erythema (score 1) was noted in all males after 1 day, and in 4/5 males after 2 days. Very slight erythema was observed in 4/5 females for 1-2 days. Small superficial scattered scabs (3 animals), crust formation (2 animals), and desquamation (3 animals) was noted in females, but none of these signs persisted beyond 5 days. No signs of skin irritation were observed past day 6 in this study. No abnormalities were noted at necropsy.

Under the conditions of this study, the acute dermal LD50 for the 480 g/litre chlorpyrifos formulation in Sprague-Dawley rats was > 4000 mg/kg.

Blagden SM (1995) CHA 7110: Acute inhalation toxicity study. Four-hour (nose only) in the rat. Safepharm Laboratories Ltd, UK Project no: 545/69. Report dated 3 April 1995. Cheminova Agro A/S report CHA Doc No: 28-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-3). The study was conducted between 12 January 1995 and 2 February 1995.

Three groups of male and female young adult Sprague-Dawley rats (5/sex; Charles River UK Ltd) were exposed (nose-only) to the undiluted test material (CHA 7110; an emulsifiable concentrate formulation containing chlorpyrifos at 480 g/litre; Cheminova Agro A/S; certified concentration of active ingredient 45.4%; batch no. 359-KMA-30) as a vapour atmosphere for a period of 4 h. The test material was aerosolised using a glass concentric jet nebuliser, then introduced into the exposure chamber using a continuous supply of compressed air. The chamber atmosphere was sampled for concentration analysis at regular intervals during the exposure period. All animals were observed for clinical signs at hourly intervals during exposure, immediately after removal from restraining tubes after exposure, one hour after termination of exposure, and then daily for 14 days. Individual bodyweights were recorded on the day of exposure, and days 7 and 14. At the end of the study, all animals were killed and subjected to internal and external macroscopic examination. The mean achieved atmosphere concentrations were 1170, 2000, and 3360 mg/m^3 (nominal concentrations of 5500, 8200, and 15400 mg/m^3).

Results

Mortality was 4/5 males and 5/5 females at 3360 mg/m^3, 2/5 males and 1/5 females at 2000 mg/m^3, and 1/5 males and 0/5 females at 1170 mg/m^3. With the exception of two males found dead on day one of the study at 3360 mg/m^3, the other deaths were a result of animals being killed *in extremis*. During exposure, wet fur and decreased respiratory rates were commonly observed. Following exposure, hunched posture, lethargy, ptosis, ataxia, pallor of the extremities and piloerection were seen in all groups. At the high exposure level, the surviving animals on day one after exposure continued to show severe signs of toxicity. In addition to the signs listed above, exophthalmos, dehydration, fasciculations and increased lacrimation were also observed. Common abnormalities noted at necropsy were associated with the lungs, and included swelling, abnormal redness, pallor, dark patches, and dark foci. Incidents of darkening, pallor or patchy pallor of the liver and pallor of the kidneys were noted. No abnormalities were detected in surviving animals at the end of the study.

Under the conditions of this study, the acute inhalation LC50 (95% confidence limits) for the 480 g/litre chlorpyrifos emulsifiable concentrate CHA7110 in Sprague-Dawley rats was 2160 mg/m^3 (1310-3580) in males, 2320 mg/m^3 (1880-2870) in females), and 2260 mg/m^3 (1800-2840) in all animals.

Dreher DM (1995c) CHA7110: Acute dermal irritation test in the rabbit. Safepharm Laboratories Ltd, UK Project no: 545/70. Report dated 24 April 1995. Cheminova Agro A/S report CHA Doc No: 25-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989

(also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-5, TSCA Subpart E, Section 798.4470). The study was conducted between 14 December 1994 and 21 December 1994.

The undiluted test material (0.5 ml; CHA 7110; an emulsifiable concentrate formulation containing chlorpyrifos at 480 g/litre; Cheminova Agro A/S; certified concentration of active ingredient 45.4%; batch no. 359-KMA-30), was applied to clipped skin areas on the back of New Zealand White rabbits (3/sex; David Percival Ltd, UK; approximately 12-20 weeks old at the start of the study) under a gauze patch. Surgical gauze was applied over the test area and secured in position with a strip of surgical adhesive tape, and then the trunk of each rabbit was wrapped in an elasticised corset. After the 4-h contact period, the corset and patches were carefully removed and the treated skin wiped with cotton wool soaked in 74% Industrial Methylated Spirits. One hour after removal of the patches, and approximately 24, 48, and 72 h after patch removal, the test sites were examined for evidence of dermal irritation and scored according to the method of Draize.

Results

Well-defined erythema (score 2) was observed in all animals at every inspection interval up to and including 72 h. This erythema was generally accompanied by other signs, including loss of skin elasticity, haemorrhage of the dermal capillaries, and crust formation. These signs, along with the erythema, had disappeared by 7 days. Moderate (score 3; raised approximately 1 mm) to severe oedema (score 4; raised more than 1 mm and extending beyond the area of exposure) was observed in all animals from 1 h through 48 h. At 72 h, severe (1 animal) and moderate (3 animals) oedema was still seen, and the oedema has subsided to slight (score 2; edges of area well defined by definite raising) in two animals. No oedema was observed at 7 days. The sum of 24 and 72 h scores (S) was 62, and the Primary irritation Index (S/12) was 5.2. According to the Draize scoring methodology, the test material was classified as a severe irritant. The interpretation of these findings according to EEC Council Directive 67/548/EEC gave overall total (mean) scores of 36 (2.0) for erythema/eschar formation, and 57 (3.2) for oedema.

Under the conditions of this study, the 480 g/litre emulsifiable concentrate test material, CHA7110 was considered to be a moderate skin irritant in rabbits.

Kuhn JO (1996a) CHA 7110 Final Report: Primary dermal irritation study in rabbits. Stillmeadow Inc, USA, study no. 2585-95. Completion date 6 March 1996. Cheminova Agro A/S report CHA Doc No 56-CYF [Cheminova; Submission]

This study was conducted in compliance with GLP principles of the US EPA FIFRA 40 CFR 160, Japan Maff Notification 59 Nohsan 3850, and the OECD Annex 2, C(81)30 and designed to satisfy US EPA Guidelines (FIFRA Section 81-5). The study was conducted between 14 December 1994 and 21 December 1994.

The undiluted test material (0.5 ml; CHA 7110; an emulsifiable concentrate formulation containing chlorpyrifos at 480 g/litre; Cheminova Agro A/S; batch no. 359-KMA-30), was applied to clipped skin

areas on the trunk of young adult New Zealand White rabbits (3/sex; Ray Nicholls Rabbitry, USA) under a gauze patch. Surgical gauze was applied over the test area and secured in position with a semi-permeable dressing, and then secured on the edges with strips of tape. After the 4-h contact period, the wrapping and patches were carefully removed and the treated skin wiped with a clean cloth. Thirty minutes after removal of the patches, and approximately 24, 48, and 72 h after patch removal, the test sites were examined for evidence of dermal irritation and scored according to the method of Draize. For each animal, all of the erythema and oedema scores through 72 h were added, and the sum was divided by 4 to obtain an individual irritation score. The Primary Irritation Index was obtained by calculating the mean of the irritation scores.

Results

Very slight (score 1) erythema was observed in all animals at 30 minutes, and at 24 and 48 h. A single animal had well-defined erythema (score 2) at 24 h. Five animals had well-defined erythema at 72 h, all animals displayed well-defined erythema at day 7, and very slight erythema was seen in all animals at day 10. No erythema was observed on day 14. Very slight (score 1) and/or slight (score 2) oedema was observed in 5/6 animals at 24 h, 4/6 animals at 48 h, and 6/6 animals at 72 h. This effect persisted in 5/6 animals at day 7, and in 1/6 animals at day 10, but no oedema was observed on day 14. The individual Primary Irritation scores ranged from 1.50 to 2.50, and the Primary Irritation Index was 2.0.

Under the conditions of this study, the 480 g/litre emulsifiable concentrate test material, CHA7110 was considered to be a slight skin irritant in rabbits.

Kuhn JO (1996b) CHA 7110 Final Report: Primary eye irritation study in rabbits. Stillmeadow Inc, USA, study no. 2584-95. Completion date 6 March 1996. Cheminova Agro A/S report CHA Doc No 57-CYF [Cheminova; Submission]

This study was conducted in compliance with GLP principles of the OECD (Annex 2, C(81)30), Japan MAFF, Notification no. 59 Nohsan 3850, and US EPA FIFRA(40 CFR Part 160) and designed to satisfy US EPA Guidelines (Section 81-4).

The eye irritation potential of the test material (0.1 ml; CHA 7110; an emulsifiable concentrate formulation containing chlorpyrifos at 480 g/litre; Cheminova Agro A/S; batch no. 359-KMA-30), was assessed in young adult New Zealand White rabbits (3 animals/sex; Ray Nichols Rabbitry, USA), by instilling the test substance into the conjunctival sac of the right eye, with the left eye remaining untreated and serving as control. The test material was similarly introduced into the right eye of another group of three males, and the treated eyes of these animals were washed with deionised water 30 seconds after treatment. Assessment of ocular damage/irritation was made approximately 1, 24, 48, and 72 h and 4, 7, and 10 days following treatment, according to the numerical evaluation of Draize.

Results

In the 'unwashed' eyes, scattered or diffuse areas of corneal opacity (score 1) were noted in all animals, and this finding persisted for up to day 4 in two males, and for 48 h in the other animals. The opacities covered more than 75% of the cornea in all of these animals at some stage in the observation period. No iridial irritation was reported. Conjunctival redness was seen in all animals. This redness was severe

(score 3; diffuse beefy redness) in 5/6 animals on at least one occasion, and persisted at this level of severity for between 24 h and 4 days. At the 72 h inspection, the conjunctival redness was slight in 3/6 animals (with some blood vessels definitely injected; score 1), moderate in 1/6 animals (diffuse crimson coloured; score 2), and diffuse beefy red in 2/6 animals. Conjunctival redness persisted for up to 7 days, but was not observed at day 10. Conjunctival chemosis, ranging in severity from any swelling above normal (score 1) to swelling with lids about half closed (score 3) was observed between 1 h and 7 days after treatment. At 72 h, the chemosis score was 1 in 1/6 animals, score 2 in 1/6 animals and score 0 in 4/6 animals. Discharge (ranging from slight (score 1) to discharge with moistening of the lids and hairs and considerable area around the eyes (score 3) was observed in all animals between 1 and 72 h.

In animals that had their eyes washed after treatment, corneal opacity (severity score 1) was observed in 2/3 animals, and this effect persisted until the 48 h inspection, but disappeared by 72 h. Some conjunctival redness (score 1-2), chemosis (score 1-3) and discharge (score 1-3) was observed, but these effects were generally less severe than those seen in the unwashed eyes, and the findings disappeared more quickly in the washed eyes.

The maximum average irritation score was 31.3 in non-washed eyes, and 16.0 in washed eyes. At 72 h, the mean scores were 12.7 (unwashed) and 2.7 (washed).

Under the conditions of this study, the 480 g/litre emulsifiable concentrate test material, CHA7110 was considered to be a moderate eye irritant in rabbits.

Dreher DM (1995d) CHA 7110: Magnusson & Kligman maximisation study in the guinea pig. Safepharm Laboratories Ltd, UK Project no: 545/72. Report dated 24 April 1995. Cheminova Agro A/S report CHA Doc No: 29-CYF. [Submission 11470; Cheminova Agro A/S]

This study was conducted in compliance with GLP principles of the UK Department of Health 1989 (also OECD (OCDE/GD(92)32), EEC (87/18/EEC and 88/320/EEC), and US FDA (40 CFR Part 160, 40 CFR Part 792, and 21 CFR Part 58)) and designed to satisfy US EPA Guidelines (FIFRA Section 81-6, TSCA Subpart E, Section 798.4100). The study was conducted between 21 November 1994 and 26 December 1994.

The skin sensitisation potential of the test material (CHA 7110; an emulsifiable concentrate formulation containing chlorpyrifos at 480 g/litre; Cheminova Agro A/S; batch no. 359-KMA-30), was assessed in albino Dunkin-Hartley guinea pigs (David Hall Ltd, UK; 8-12 weeks old at the start of the study). Males were used for the topical induction range-finding test, and females were used for all other phases of the study. The test material was freshly prepared as follows:

Intradermal induction: 0.1% w/v in distilled water and 0.1% w/v in a mixture of Freund's Complete Adjuvant plus distilled water (1:1).

Topical induction: 25% v/v in distilled water.

Topical challenge: 10% and 5% v/v in distilled water.

Determination of the concentration, homogeneity and stability of the test material preparations was not conducted. No concurrent positive control group was used in this study, but an historical positive control response of between 39 and 100% was reported in four studies using the same source of animals (with a variety of positive control substances) conducted at a similar time to this study, and so the test system appears to be suitable for determining the skin sensitisation potential of compounds in guinea pigs.

Range-finding studies: The concentration of test material to be used at each stage of the main study were determined in preliminary tests as follows:

Intradermal induction: Four animals were intradermally injected with preparations of test material (0.1, 0.5, 1, and 5% w/v in distilled water), and the highest concentration that did not cause local necrosis, ulceration, or systemic toxicity was selected for this stage of the main study.

Topical induction: Two guinea pigs (intradermally injected with Freund's Complete Adjuvant seven days earlier) were treated with undiluted test material and three preparations of the test material (75, 50, and 25% v/v in distilled water). The highest concentration causing only mild to moderate dermal irritation after a 48-h occlusive exposure was selected for this stage of the main study.

Topical challenge: Four preparations of the test material (5, 10, 25, and 50% v/v in distilled water) were applied to the flanks of two guinea pigs and occluded for 24 h. These guinea pigs had been treated identically to control animals of the main study, up to day 14. The highest non-irritant concentration of the test material and one lower concentration were selected for this stage of the main study.

Main study: Thirty guinea pigs were used for the main study (twenty test and ten control). Body weights were measured at the beginning and end of the study.

Induction: A row of three injections were made to the clipped shoulder region of each animal. In test animals, the injections were:

i) Freund's Complete Adjuvant FCA) plus distilled water 1:1
ii) a 0.1% w/v dilution of test material in distilled water
iii) a 0.1% w/v dilution of test material in a 1:1 preparation of FCA plus distilled water.

In control animals, the induction procedure was identical to that used in test animals, except that the injections were as follows:

i) FCA plus distilled water 1:1
ii) distilled water
iii) 50% w/v formulation of distilled water in a 1:1 mixture of FCA plus distilled water

One week after injections, the same area on the shoulder region was clipped, and treated with a topical application of the test material (25% v/v in distilled water). The test material (dose volume not stated) was applied to saturation on filter paper, held in place by a strip of surgical adhesive tape, covered with an overlapping sheet of aluminium foil, then further secured with an elastic bandage for 48 h. The degree of erythema and oedema were quantified according to the method of Draize at 1 and 24 h after removal of the patches.

Challenge: On day 21 of the study, the undiluted test material (dose volume not stated) was applied to saturation to the clipped right flank of each animal on a square of filter paper held in place by a strip of adhesive surgical tape, and occluded in a similar manner to the induction phase of the study for 24 h. The test material was also applied to a separate skin site on the clipped right flank at a 5% v/v concentration in distilled water, and the vehicle alone similarly applied to the clipped left flank, and occluded. After removal of the dressing, the challenge sites were swabbed with cotton wool soaked in distilled water to remove residual material. Evaluation of the skin sites was made at 24 and 48 h after removal of the dressing.

Results

Very slight (score 1) to well-defined (score 2) erythema, and/or very slight (score 1) to slight (score 2) oedema were observed in all test animals 1 h following topical induction. By 24 h after induction, some erythema was still present in 14/20 animals (score 1-2), but oedema had disappeared in all but one animal (score 1). Some desquamation was also observed in 4/20 animals at the 24 h inspection. No skin irritation findings were observed in control animals after the induction phase of the study.

After the challenge phase of the study, no erythema or oedema was observed in any treated or control animals at either 24 or 48 h.

Under the conditions of this study, the 480 g/litre emulsifiable concentrate test material, CHA7110, was not a skin sensitiser in guinea pigs.

3.3 Acute Toxicity of 3,5,6-trichloro-2-pyridinol (TCP)

Gerbig CG & Emerson JL (1970a) Oral median lethal dose (LD50) determination of 3,5,6-trichloro-2-pyridinol in mice. The Dow Chemical Company report HH-240, dated 10 June 1970. [Dow; Submission 238, volume 5; A3162/5, B43]

Groups of Swiss mice (Young adult; Laboratory Supply Company, USA; 15-19 g body weight; Cox strain; 15/sex) were given a single oral dose of TCP (Dow; reference 238-11-112; 100 mg/ml suspension in 0.5% hydroxypropylmethylcellulose, [METHOCEL]; purity not stated) by gavage. Dose levels were 354, 445, 562, 708, and 891 mg/kg, and mortality at these doses was 6/15, 10/15, 14/15, 15/15, and 15/15 in males, and 3/15, 11/15, 12/15, 15/15, and 15/15 in females, respectively. The oral LD50 values (95% confidence limits) for mice were 380 mg/kg (333-433 mg/kg) for males and 415 mg/kg (367-469 mg/kg) for females. Toxic signs included tremor, flaccid paralysis, dyspnoea and exophthalmia. Deaths occurred within 1-2 hours of dosing, with the development of a rigor mortis-like rigidity of the whole body within seconds following death. Surviving animals were normal in appearance and activity within 24 h of dosing.

Gerbig CG & Emerson JL (1970b) Oral median lethal dose (LD50) determination of 3,5,6-trichloro-2-pyridinol in the rat. The Dow Chemical Company report HH-239, dated 5 June 1970. [Dow; Submission 238, A3162/5, B43]

Groups of Sprague-Dawley rats (Laboratory Supply Company, USA; weight 78-96 g; 10/sex) were given a single oral dose of TCP (DOW; reference 238-11-112; 100 mg/ml suspension in 0.5% METHOCEL; purity not stated) by gavage. Mortality was 5/10, 7/10, 7/10, 8/10, and 10/10 in males at 794, 891, 1000, 1120, and 1260 mg/kg and 4/10, 7/10, 8/10, and 9/10 at 794, 891, 1120, and 1260 mg/kg. The oral LD50 values (95% confidence limits) for rats were 794 mg/kg (709-889 mg/kg) for males and 870 mg/kg (758-1009 mg/kg) for females. Toxic signs included flaccid paralysis, dyspnoea and mild hypersalivation. Deaths occurred within 4 hours of treatment and were followed within seconds by a rigor mortis-like rigidity of the body. Surviving animals recovered within 24 h of dosing.

Gerbig CG & Emerson JL (1970c) Determination of oral minimum lethal dose of 3,5,6-trichloro-2-pyridinol in beagle dogs. The Dow Chemical Company report HH-253, dated 16 July, 1970. [Dow; Submission 238, A3162/5, B43]

Three Beagle dogs (source not stated; 8.35-12.1 kg weight) received gelatine capsules containing TCP (Dow; reference 238-11-112; purity not stated). Dog 1 received 2 doses of 1000 mg/kg TCP an hour apart, dog 2 received 4 doses of 500 mg/kg TCP at hourly intervals and dog 3 received 8 doses of 50 mg/kg TCP at hourly intervals. Toxic signs included emesis within 1 hr of dosing for the 500 and 1000 mg/kg doses and within 3 h of the 50 mg/kg dose. Other toxic signs were ptyalism, watery diarrhoea, slight transient depression of the gag reflex and transient mydriasis. There were no deaths.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1 Dietary Studies

4.1.1 Mouse

Crown S, Weiss A, Nyska A & Waner T (1984) Pyrinex. Toxicity in dietary administration to mice for two weeks: a range-finding study. Life Science Research Israel, Inc. Conducted 21 December 1986 to 6 January 1987. LSRI Report no: MAK/109/PYR, 4 March 1987. Makteshim Chemical Works report no: R-4389. [Makteshim; Submission 11471; reference 22]

This study was conducted in compliance with EPA GLP regulations 40 CFR 160, and a QA Statement was issued for this study. To determine the dose levels for a 13-week dietary study in mice, a two-week dose-ranging study was conducted using young adult CD-1 mice (Charles River UK), where Pyrinex Technical (Makteshim; 96.3% chlorpyrifos; Batch 58900.9) was incorporated into a commercially available powdered rodent diet (Altromin 1321N) and administered to groups of 12 animals/sex at dietary concentrations of 0 (control), 75, 150, 300, 600, and 1200 ppm for two weeks. An additional group (12 animals/sex) was sacrificed prior to initiation of treatment for measurement of baseline cholinesterase activity. Homogeneity and stability of the prepared diet was assessed. Animals were examined daily for treatment-related signs, and body weights, food consumption, and achieved dosage was determined weekly. At the dietary concentrations listed above, the dose levels of test material were 0, 14.3-18.5, 30.1-32.4, 56.4-66.6, 88.7-103.8 and 127.9-181.1 mg/kg/d, respectively. Plasma, erythrocyte and brain cholinesterase determinations were made at the completion of the study, and all animals were subjected to necropsy examinations.

At 1200 ppm, 1/12 males and 6/12 females died or were sacrificed *in extremis* during the study. A number of other animals died following blood collection procedures. Treatment-related clinical signs, including tremor, ocular opacity, ocular staining, hunching and lacrimation were mainly confined to animals in the 1200 ppm group. Marked reductions in body weights and food consumption were observed in animals treated at 600 ppm and above.

In males, cholinesterase activity was reduced at all doses of test material. At 75 ppm, plasma cholinesterase activity was reduced by >95%, erythrocyte cholinesterase activity by almost 40%, and brain cholinesterase activity by about 50%. The inhibition of erythrocyte cholinesterase activity was not dependent upon dose, with approximately 35% inhibition seen at all dose levels. Plasma and brain cholinesterase activity was reduced in a dose-related manner, with plasma cholinesterase inhibition of 99% and brain cholinesterase inhibition of about 89% at the high dose level. Similar patterns of cholinesterase inhibition were seen in females.

Based on the results of this study, dose levels of 5, 50, 200, 400, and 800 ppm were recommended as suitable for a 13-week dietary study in mice.

Davies DB, Tollett JT & Lomax LG (1985) Chlorpyrifos: A four-week dietary study in CD-1 mice. The Dow Chemical Company, study No. HET-K-044793-068, dated May 23, 1985. QA. [Dow submission 238, A3162/5 B43; Dow submission 11462, reference 55]

Male and female CD-1 mice (Charles River, Portage, MI, USA) were given chlorpyrifos (95.7%, Dursban F, Ref.: AGR 214637, Lot No.: MM820905-610) in the diet at 0 and 15 ppm (40/sex/group), with 20 animals/sex/group sacrificed after one week, and the remaining 20 animals/sex/dose were treated for a total period of 4 weeks. Doses equated to 2.7 and 3.4 mg/kg for males and females, respectively. Clinical signs (daily, 5/7), body weights (weekly), food consumption (weekly) and cholinesterase activity (photometric method, week one and termination) in plasma, RBCs and brain were measured. The animals were subjected to gross pathological examination at termination and organs weights were recorded. Appropriate statistical tests were applied to the data.

There were no significant in-life clinical signs or unscheduled deaths. Food consumption was unaffected by treatment with chlorpyrifos, but the body weight gain of male mice was decreased by 25% at the end of the 4-week study period. Organ weight analysis was unremarkable and there were no significant differences between groups. Pathology findings at necropsy were within normal limits for all groups. At one week, plasma cholinesterase activity was depressed by 88 to 91% and RBC cholinesterase was depressed by 40 to 53%. At 4 weeks, plasma cholinesterase activity was depressed by 91% and RBC cholinesterase was depressed by 53%. Brain cholinesterase activity was unaffected by treatment at either assay period. There was no sex difference in the changes observed in plasma and erythrocyte cholinesterase activity.

4.1.2 Rat

Crown S, Nyska A & Waner T (1984) Pyrinex technical. Two-week range-finding study in dietary administration to rats. Life Science Research Israel, Inc. Conducted 9-23 October

1984. LSRI Report no: MAK/057/a/PYR, 29 November 1984. Makteshim Chemical Works Report no: R-3644 [Makteshim; Submission 11471; reference 21]

No GLP or QA statements were issued regarding this study. In a 2-week dietary range-finding study in rats (CD; Charles River UK; 4 weeks old at arrival), used to establish dose levels for a 13-week dietary study, technical chlorpyrifos (Pyrinex Technical; Makteshim; 95.5% purity; batch 760210) was incorporated into a commercially-available powdered rodent diet (Altromin 1321N) and administered to six groups of animals (5/sex/dose) at dietary concentrations of 0 (controls), 10, 30, 84, 240, and 694 ppm for 14 days. These dietary concentrations were calculated to be equivalent to achieved dose levels of 0, 1.4-1.9, 4.1-5.2, 11.7-13.7, 34.3-39.4, and 65.8-94.8 mg/kg/d, respectively. The suitability of the mixing procedure was verified by analysis of dietary samples from a trial mix. Rats were inspected twice daily for mortality or treatment-related effects. Food consumption, body weights, and achieved dosages of test material were determined weekly. Prior to sacrifice, blood samples were obtained for cholinesterase determination, and at the study termination, all animals were killed and subjected to necropsy examination.

At the high dose (694 ppm), clinical signs of intoxication were reported, consisting of irritability, hunching, tremor, ataxia, urogenital staining, pigmented orbital secretion, failure to groom, and proneness. No treatment-related clinical signs were observed at other dose levels. A single male from the 30 ppm group died as a result of blood collection procedures, while at 694 ppm, one female was found dead, and a second female was killed *in extremis*. No mortality was observed at other dose levels. Body weights were reduced in males and females by about 35% compared with controls at 694 ppm at the 8 and 15 day examinations, while at 240 ppm, body weights in females were reduced by about 12% compared with controls at day 8 of the study. Food consumption was also reduced at 694 ppm (up to 50%).

Dose-related, biologically-significant decreases in blood cholinesterase activity were seen at all test doses, and inhibition ranged from 42% at 10 ppm to 93% at 694 ppm, in males. Blood samples were not obtained from high-dose females due to their poor condition. The major findings at necropsy were small spleen, reduced fat pads, and thinning of the posterior musculature in high-dose males and females.

On the basis of these findings, the dose levels selected for the 13-week study (LSRI study MAK/058/PYR) were 0.5, 10, and 100 ppm.

Liberacki AB, Breslin WJ, Dittenber DA & Quast JF (1990) Chlorpyrifos: Two week dietary probe study in Sprague-Dawley Rats. Dow Chemical Company Study HET K 044793-090, dated 16 April 1990 [Dow; Submission 11462, reference 54]

This study was conducted in compliance with the GLP Standards of the US EPA (40CFR160, 1983), the Japan MAFF (59 Nohsan, Notification 3850, 1984), and the OECD (1982).

The test material (chlorpyrifos technical; Dursban F Insecticide; Dow; AGR 273801; stated purity 98.5%) was added to Purina Certified Rodent Chow #5002 and given to male and female Sprague-Dawley rats (7/sex/dose; Charles River, USA; 4 weeks old) for two weeks at targeted dose levels of 0, 5, or 10 mg/kg/d. The diets were prepared once prior to the start of the study by serial dilution of the test material/feed concentration premix, which was also prepared once prior to the start of the study.

Reference samples (1/dose/sex/mix, plus the premix) were retained and stored, and analyses of the test diets to determine the concentration of test material and homogeneity were performed once during the conduct of the study.

All animals were observed daily for evidence of treatment-related effects on behaviour, and these observations included animal movement and palatability of diet. Body weights and feed consumption were recorded at 3-4 day intervals. Blood was obtained via orbital sinus puncture for plasma and RBC cholinesterase activity determinations, after which time a complete necropsy was conducted on all animals. The brain and adrenals were removed and weighed, and half of the brain was used for cholinesterase determinations.

Results

The dietary concentrations in the test diets were analysed and found to be within 92.6 and 104.8% of the targeted concentrations, and homogenous throughout. All animals survived the test period, and no treatment-related signs were observed in test groups. Feed consumption, body weights, and gross pathological findings were similar in control and treated groups. Dose-related decreases in plasma, RBC and brain ChE activity were observed in test groups. The depression of brain cholinesterase activity displayed a strong dose-dependency, with reductions in activity of 40% in males and 43% in females at 5 mg/kg/d, and 73% and 68% in females at 10 mg/kg/d. For plasma and RBC cholinesterase activity, the reductions were about 58% and 75% in males, and 65-70% and 73% in females, regardless of the dose.

Statistically-significant increases in absolute and relative adrenal weights (about 20% compared with controls) were observed in females only at 10 mg/kg/d.

Under the conditions of this study, plasma, RBC and brain cholinesterase activities were markedly reduced in rats following dietary administration for 14 days at doses of 5 and 10 mg/kg/d, in the absence of clinical signs of intoxication.

4.1.3 Monkey

Coulston F, Golberg L, Abraham R & Benitz FK (1971) Final report on safety evaluations and metabolic studies on Dowco 179 (IN 151). Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York. Report Date March 18, 1971. Sponsor: Dow Chemical USA. Unpublished [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

A summary report only was supplied. Two rhesus monkeys were given doses of 2 mg/kg Dowco 179 (purity and batch details not provided) by stomach tube for three consecutive days. Blood samples were taken at intervals of 24 h and cholinesterase activity was measured. No change in behaviour or clinical signs of cholinergic stimulation were observed in either monkey during the three-day observation period. A sharp decrease in plasma cholinesterase activity was observed 24 h after the initial dose (to 17-46% of control), with slightly greater reductions after the second and third doses (to 6-23% of control). Erythrocyte cholinesterase activity decreased slightly following the first dose (to 85-95% of control), with greater reductions after the second and third doses (to 66-89% of control).

4.1.4 Dog

Harling RJ, Barker MH, Buist DP, Crook D, Dawe IS & Anderson A (1989). Chlorpyrifos oral dose range finding toxicity study in Beagle dogs. (Final report-repeated daily dosage for 4 weeks). Huntingdon Research Centre Ltd., UK, Study completed 16 March, 1988. HRC report MBS 30/88675, dated 3 May 1989. Makteshim Chemical Works report R-4749. [Makteshim; Submission 11471; reference 24]

This study was conducted in accordance with GLP Standards of : US EPA, Title 40 CFR 160, 1983; OECD 1982; Japan MAFF, notification 3850, 1984; and UK Health 1986. Chlorpyrifos technical (stated purity 95.8%; batch 489 205; 1% w/w mixture in lactose) was administered orally via gelatine capsule to groups of purebred beagle dogs (Interfauna UK) at doses of 0 (control; lactose only), 0.01, 0.03, 0.5, and 5 mg/kg/d for 4 weeks, using 2 animals/sex/group (only 1 animal/sex/group at 0 and 0.5 mg/kg/d). The stability and homogeneity of the test mixture was analysed to confirm the test material concentration. Animals were housed individually, and received 400 g of a standard dry diet each morning, and fresh water was available *ad libitum*.

Observations were conducted for all animals throughout each day of the study for clinical signs associated with treatment, and body weights (weekly) and food consumption (daily) were also recorded during the study. Samples of venous blood were collected from all animals prior to and during the study for determination of cholinesterase activity. At the completion of the dosing regimen, animals were killed and post mortem examinations conducted, with macroscopic examination of a number of tissues (adrenals, brain, heart, lungs, kidneys, liver, pancreas, pituitary, spleen, testes or ovaries, thymus, thyroids and parathyroids, uterus or prostate). Brain cholinesterase activity determinations were made after autopsy. Samples of a range of tissues were collected and preserved, but none of the tissues were processed, as this study was designed to determine appropriate dose levels for a 13-week study in dogs.

Results

No deaths occurred during the study, and no clinical signs associated with treatment were observed. Isolated incidences of vomiting were reported in one control and one low-dose female, but these were not considered to be related to the administration of the test material. All groups gained weight steadily during the treatment period, and while there were some intra- and inter-group variations in body weight gain, these were not dose-related, and it was considered that treatment did not affect body weight gain during this study. Food consumption was similar in all groups.

Rapid inhibition of plasma cholinesterase activity was observed at 0.5 and 5.0 mg/kg/d at all sample intervals, and inhibition was both dose- and time-dependent. Compared with concurrent controls, plasma cholinesterase inhibition was about 38% within 30 minutes of administration at 5 mg/kg/d, and 30% inhibition by 1 h at 0.5 mg/kg/d. At the high-dose, plasma cholinesterase inhibition reached about 60% by 1 h, and varied between 60% and 80% from days 1 to 28, and up to about 60% during the study at 0.5 mg/kg/d.

At 0.03 mg/kg/d, plasma cholinesterase activity was reduced by up to 34% compared with pre-test mean activity, but there was substantial intra-group variation in cholinesterase determinations, probably

due to the small group sizes, including in the control group. When compared with concurrent controls, plasma cholinesterase inhibition was generally between 10-17%, though the inhibition did exceed 20% on isolated occasions. It was considered that the inhibition of plasma cholinesterase at the 0.03 mg/kg/d dose level was not biologically significant.

No biologically-significant inhibition of erythrocyte cholinesterase activity was observed until day 7, when there was a reduction of 47% compared with concurrent controls at 5 mg/kg/d. At the end of the study, the inhibition of erythrocyte cholinesterase activity was still greater than 70% at the high-dose level. At lower doses, erythrocyte cholinesterase inhibition did not reach 20% compared with concurrent controls. When compared with the pre-test means, there was an apparent inhibition of erythrocyte cholinesterase activity of about 30% at 0.5 mg/kg/d, but only on days 27-28 of the study. Due to the preliminary nature of this study, and the small animal numbers, it was considered that the inhibition of erythrocyte cholinesterase activity at 0.5 mg/kg/d was not biologically significant.

Brain cholinesterase activity was inhibited by 32% compared with concurrent controls at the end of the study at 5 mg/kg/d. No biologically-significant inhibition was noted at lower dose levels.

Group mean organ weights, and organ weights relative to body weights, were not affected by treatment, with similar results seen in control and treated groups. Macroscopic post mortem examination did not reveal any findings that were considered to be of toxicological significance.

Under the conditions of this study, no treatment-related effects were observed at 0.03 mg/kg/d, while plasma cholinesterase activity was inhibited at 0.5 mg/kg/d, and erythrocyte and brain cholinesterase activities were inhibited at 5 mg/kg/d.

4.2 Dermal Studies

4.2.1 Rabbit

Pennington JY & Edwards NH (1971) Comparison of cholinesterase depression in humans and rabbits following exposure to chlorpyrifos. The Dow Chemical Company (TA-477). [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Dursban 6 (6 lb/gallon EC, formulation M-2995, 61.5% chlorpyrifos, 35% xylene) was applied to the skin of the back and abdomen of 2 rabbits/dose at 5, 10, 25 and 50 mg/kg for 20, 4, 3, and 1 applications respectively. The applied dose was covered with occlusive patches for 12 h, and after patch removal each site was scored for irritation prior to washing. Each site was used only once. Plasma and erythrocyte cholinesterase determinations (pH-Stat method) were made pre- and at several intervals post-dose (exact time not stated).

No skin irritation was recorded. The table below indicates that both plasma and RBC cholinesterase measures were significantly inhibited by dermal application under each of the dose regimens; plasma values recovered more quickly than RBC values which were still significantly inhibited at day 40 after the 3, 4 and 20 day exposures.

Cholinesterase activity after dermal dosing of rabbits (% of predose value)*

	End of dosing	End of dosing	Day 12	Day 12	Day 40	Day 40
Dermal dosing	Plasma	RBC	Plasma	RBC	Plasma	RBC
10 mg/kg for 4 x 12 h	8	11	81	23	102	52
25 mg/kg for 3 x 12 h	2	18	100	36	103	63
50 mg/kg for 1 x 12 h	42	55	100	76	116	98
5 mg/kg for 20 x 12 h			6	10	73	25

* average of two rabbits

4.2.2 Dog

Sharp LD & Warner SD (1968) The clinical toxicity of Dursban in the dog after multiple applications of an aerosol formulation. The Dow Chemical Company. Unpublished [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

In a two-week dermal application of an aerosol formulation to dogs, five mongrels and five beagles (6-24 months old, 9-13 kg weight, 5 M and 5 F) were allotted to groups as untreated controls (2 F), vehicle (dichlorophene, Lot No: 80256) control (1 F, 2 M) and a treatment group (2 F, 3 M) receiving 0.087% Dursban (Lot No: 80137-A) in dichlorophene. Ten applications (5/7) of an aerosol spray were made to the backs of the animals (with the fur brushed against the grain) over a period of 2 weeks, with each application lasting 30 seconds. The total dose volume was not stated. The animals were observed for 8 weeks after the study commenced. Blood samples for RBC and WBC counts, PCV, Hb and differential counts were taken on days 1, 7, 14 and 28. Separate blood samples were taken pre-test and on days 1, 7, 14 and 28 to measure cholinesterase activity and for determination of ALP, ALT, AST, BUN and blood glucose. Clinical signs were recorded daily, ophthalmoscopy was performed pretest and at day 14. Body weights were measured pretest and at weeks 1, 2, 4 and 8.

There were no mortalities or abnormalities in the analysis of clinical observations, body weight gain, opthalmoscopic examination, haematology or clinical chemistry parameters. RBC cholinesterase activity was not decreased by treatment, but plasma cholinesterase activity fell from the commencement of treatment, before returning to normal at 72 days post-treatment. There were no clinical signs related to the fall in plasma cholinesterase activity.

4.2.3 Rat

Calhoun LL & Johnson KA (1988) Chlorpyrifos: 4-day dermal probe and 21-day dermal toxicity studies in Fischer 344 rats. Dow Chemical Company Study no. HET K 044793-085,086, dated 1 September 1988. [Dow; Submission 11462, reference 57]

Calhoun LL & Johnson KA (1989) Supplemental information to the report titled: Chlorpyrifos: 4-day dermal probe and 21-day dermal toxicity studies in Fischer 344 rats. Dow Chemical Company Study no. K-044793-085(S), dated 26 December 1989. [Dow; Submission 11462, reference 70]

This study was conducted in compliance with the GLP Standards of the US EPA (40CFR160, 1983),

the US FDA (21CFR58, 1978), the Japan MAFF (59 Nohsan, Notification 3850, 1984), and the OECD (1982).

To assess the potential toxicological effects of repeated dermal exposure, chlorpyrifos (stated purity 100%; Dow Chemical USA; Lot no. AGR 219646) was first tested in a preliminary 4-day study. Groups of 4 female rats (Fischer 344; Charles River USA; 9 weeks old) each received daily dermal applications of the test material in corn oil at 1, 10, 100, or 500 mg/kg for 4 days, and were killed the day after the final dose application. An additional group of 4 female rats received only corn oil, and served as controls. The application site, application technique, dose calculation, occlusion method and observations were the same as for the full 21-day study, as were the cholinesterase activity determinations, and gross necropsy examination. Body weights were recorded on days 1 and 4 of this preliminary study. Blood samples were obtained by orbital sinus puncture of anaesthetised rats immediately prior to necropsy. No tissues were retained for histopathological examination.

In the 21-day study, groups of male and female Fischer 344 rats (5/sex/dose; Charles River, USA; 11 weeks old) received dermal applications of chlorpyrifos at doses of 0.1, 0.5, or 5 mg/kg/d in a corn oil vehicle, for a total of 15 days over a 21-day period. An additional group of animals (5/sex) received corn oil only as controls. The dose volume (not stated) was claimed to be similar in all groups.

The test material was applied to the clipped skin on the back of each animal, covered by a gauze patch and held in place for approximately 6 h using an elastic bandage. After the exposure period, the bandage was removed, and the test site was wiped with a water-dampened towel to remove residual test material. The condition of the skin sites were examined daily for signs of irritation, prior to reapplication of test material. Irritation was evaluated and scored according to a modified method of Draize, as recommended by the US EPA and OECD, with a scale of 0-4 used for erythema and eschar formation, oedema, and scaling and fissuring.

All animals were observed once daily for signs of toxicity (on weekends this was limited to observation for mortality). Animals were weighed before their first exposure, and approximately weekly thereafter, and food consumption was recorded weekly.

Immediately prior to necropsy, a Functional Observational Battery (FOB) was conducted. Observations were first made on muscle tone, tremor, haircoat condition, salivation, lacrimation, urine staining, and faecal staining, and then the animals were placed individually in an observation box for approximately 20 seconds, and observations were made on deviations from normal locomotion, and on responsiveness to touch and pain.

Cholinesterase activity (ChE) in plasma, erythrocytes and brain homogenate was determined, along with the following haematological parameters: haematocrit, haemoglobin concentration, erythrocyte count, total leucocyte count, and platelet count. Differential leucocyte counts were obtained from stained blood smears from all rats.

Clinical chemistry analyses were conducted on the serum collected from all rats at terminal sacrifice, and the following parameters were measured: alanine aminotransferase activity (ALT), aspartate aminotransferase activity (AST), urea nitrogen, alkaline phosphatase activity (AP), glucose, creatinine

activity, total protein, albumin, globulin, cholesterol, triglycerides, calcium, and phosphorous.

Urine was collected from rats during the final week of exposure, and the following parameters were measured: bilirubin, glucose, ketones, blood, pH, protein, and urobilinogen. The colour and appearance of the urine was also noted, and determinations of specific gravity and sediment were conducted.

All surviving animals were killed on the day following the last application, and examined for gross pathological findings. Weights of adrenals, brain, liver, kidneys and testes were recorded, and a complete set of tissues was collected from each animal and preserved. The adrenals, brain, kidneys, liver, peripheral nerve (sciatic), skin, and spinal cord (cervical, thoracic, and lumbar areas) from all control and high dose animals, and a few selected tissues that had gross pathological findings, were prepared for histopathological examination.

Results

Stability, homogeneity and test diet concentration analyses were conducted. Stability and homogeneity were about 100%. Test doses ranged from 88-99% of the target concentrations in the 4-day study, and 79-100% of the target concentrations in the 21-day study.

In the 4-day study, the dermal application of the test material did not produce dose-related skin irritation. No signs of irritation were seen at 100 or 500 mg/kg/d, while 2 animals in each of the 1 and 10 mg/kg/d groups displayed very slight erythema (score 1) and or very slight oedema (score 1). No significant effects on body weight, in-life clinical observations, or gross pathological examinations were noted at any dose. Marked reductions in plasma and erythrocyte ChE activity were observed at doses of 10 mg/kg/d and above, with plasma ChE inhibited by up to 98%, and RBC ChE inhibited by up to 75% at 500 mg/kg/d.

In the 21-day study, all rats survived until the scheduled termination of the study. Throughout the course of the study, a red-brown exudate was observed around the eyes of most female animals, and also in males on isolated occasions. This effect was seen in control and treated groups with similar incidence. No other clinical findings were noted, and no signs of skin irritation were reported. Mean feed consumption and body weight values were similar between control and treated groups.

Results of the FOB tests did not reveal any significant findings in treated groups compared with controls. At 5 mg/kg/d, two females had reductions in muscle tone, but in the absence of any other effects, these findings were considered to be incidental. No statistically-significant changes in mean clinical chemistry, haematology, or urinalysis parameters were observed at any dose level, compared with controls. Slight decreases in plasma cholinesterase in females (18%), and plasma (7%) and RBC (11%) in males, were observed at 5 mg/kg/d, but these changes were not considered to be treatment related, and were consistent with historical control values for these parameters from this testing facility. Body weights and organ weights were unaffected by treatment.

Gross and microscopic pathological examinations did not reveal any findings associated with treatment.

Under the conditions of this 21-day dermal study, no treatment-related effects were observed at doses up to 5 mg/kg/d. Plasma, RBC and brain cholinesterase activities were not inhibited at 5 mg/kg/d for 21

days, but in the 4-day range finding component of this study, decreases in plasma ChE activity (45%) and RBC ChE activity (16%) were observed at 10 mg/kg/d, in the absence of clinical signs of intoxication.

4.3 Inhalational Studies

4.3.1 Rat

Newton PE (1988) A 5-day nose-only inhalation toxicity study of chlorpyrifos technical (Pyrinex) in the rat. Bio/Dynamics Inc, USA. Project no: 88-8057; 23 December 1988. [Makteshim; Submission 11471; reference 10]

This study, conducted in accordance with the US EPA GLP requirements (FIFRA 40 CFR Part 160), was a range-finding study to determine the cholinesterase inhibition in Fischer 344 rats (Charles River Breeding Laboratories, USA) as a result of nose-only inhalation exposure to technical chlorpyrifos (Makteshim USA; 95% purity) for 6 h/day for 5 days at a target concentration of 20 ppb as a respirable vapour. Ten animals/sex were treated with the test material, and a group of control animals (10/sex) received air only. Individual animal observations were made immediately prior to initiation of treatment, and again prior to sacrifice. Clinical chemistry analysis of cholinesterase activity was made immediately prior to sacrifice.

Analysis of the chlorpyrifos concentration confirmed that animals received exposure concentrations of 23 ppb chlorpyrifos (calculated to be approximately 0.34 mg/m^3). No mortality was reported during the study. A range of clinical signs were seen during the study, including chromodacryorrhea, matted coat, and soft stools, but the incidence of these effects was similar in control and treated groups, and so were not considered to be related to treatment. Plasma, brain, and erythrocyte cholinesterase activity in males, and erythrocyte and brain cholinesterase activity in females, was unaffected by treatment. A statistically-significant, 43% reduction in plasma cholinesterase was observed in females treated with chlorpyrifos, compared with control females. This effect was considered to be related to treatment.

Under the conditions of this study, the nose-only exposure of female rats to chlorpyrifos concentrations of 0.34 mg/m^3 (23 ppb) for 6h/day, for 5 days, resulted in a significant decrease in plasma cholinesterase activity. No effect on erythrocyte or brain cholinesterase activity was seen in females, and plasma, erythrocyte and brain cholinesterase activity was unaffected in males.

Landry TD, Dittenber DA, Lomax LG, Calhoun LL & Morabito P (1986) Chlorpyrifos: 2 week nose-only vapor inhalation exposure study in Fischer 344 rats. Laboratory report HET K-44793-81, 10 June 1986 [Dow; Submission 11462, reference 66: Submission 4033, A3162/12, B 67, & submission 238, part 2 vol 1. A3162/5 Box 42]

A Quality Assurance Statement for this study indicates that the study was conducted in accordance with US FDA and EPA GLP regulations. This study was conducted as a dose range-finding study for a subsequent 13-week inhalational study in rats. Chlorpyrifos (Agricultural Products Division of Dow Chemical USA; 99.7% purity; Lot AGR 219646) was administered to female Fischer 344 rats (6-8 weeks of age; 6 animals/exposure concentration) at concentrations of 0 or 12 ppb (equivalent to 0 or

172 µg/m^3) as a time weighted average for 6 hours/day, 5 days/week, for 2 weeks, via nose-only exposure. Animals were acclimatised to laboratory conditions for 6 weeks prior to exposure to chlorpyrifos. Animals were exposed for 3 consecutive days prior to sacrifice, at which time samples were obtained for determination of plasma, erythrocyte, and brain cholinesterase activity. A range of clinical laboratory determinations were made on blood and plasma samples collected on the last day of chlorpyrifos exposure, namely haematocrit, haemoglobin concentration, erythrocyte count, total leucocyte count, platelet count, differential leucocyte count, blood urea nitrogen, alanine aminotransferase activity, aspartate aminotransferase activity, alkaline phosphatase activity, glucose, total protein, albumin, globulins. Similarly, urinalysis was performed for measurement of the following parameters: bilirubin, glucose, ketones, blood, pH, protein, urobilinogen, specific gravity. All animals were observed for clinical signs of intoxication after each exposure period. Gross pathologic examination was conducted on all animals, and tissues were preserved in fixative, but no histopathological examinations were conducted during this study. Body weights were determined weekly during the study.

Results

No clinical signs of intoxication or treatment-related changes in body weights were reported during the study. All rats survived until the scheduled termination. Clinical chemistry examination did not reveal any treatment-related change in haematology or urinalysis parameters, including cholinesterase activity. Slight, but statistically-significant ($p<0.05$) increases in alkaline phosphatase and aspartate aminotransferase activity, and decreases in globulin and total protein, were reported in animals exposed to chlorpyrifos. These effects were not large, and were generally similar to historical control values from the testing facility, and were not considered to be toxicologically significant. Organ and body weights were not affected by treatment.

Under the conditions of this study, the nose-only inhalational exposure of female Fischer 344 rats to chlorpyrifos (99.7% purity) at a concentration of approximately 172 µg/m^3, for 6 h/day, 5 days/week, for 2 weeks, did not result in any treatment-related adverse effects, and plasma, erythrocyte, and brain cholinesterase activity was unaffected by treatment.

Torkelson TR (1965) Chronic inhalation of atmospheres containing 0,0-diethyl-0,3,5,6-trichloro-2-pyridyl ester of phosphoric acid (Dursban) Dow Chemical Company study A1A-193, dated 2 February 1965 (Summary Only) [Dow; Submission 11462, reference 74]

Torkelson TR (1968) Results of repeated inhalation of vapours of 0,0-diethyl-0-3,5,6-trichloro-2-pyridyl ester of phosphoric acid (Dursban). The Dow Chemical Company. File No:T35.12-44793-6, dated 19 December, 1968. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Rats (5/group) were exposed to a vapour containing 0.007 mg/m^3 Dursban for 7 hours/day for a total 16 days, while in a gas jar. A control group was left untreated. Plasma cholinesterase was measured pretest and after 1, 2, 6, 9 and 14 treatments.

There were no clinical signs induced by treatment. The group mean plasma cholinesterase values recorded by the control rats were consistently and significantly lower (up to 66% reduced) than those seen in the treated rats. This may have been related to the initial allocation of the rats to groups, and no meaningful conclusions could be drawn from this study. Not of regulatory standard.

Kenny TJ, Coombs DW, Hardy CJ & Crook D (1988) Chlorpyrifos inhalation toxicity in the rat. 14-day preliminary dose range finding study. Huntingdon Research Centre Ltd, UK, conducted 22 June 1987 to 6 July 1987. HRC Report MBS 21/871329, 18 March 1988. Makteshim report R-4684. [Makteshim; Submission 11471; reference 27]

This study was conducted in compliance with GLP standards of the US EPA 40 CFR 160, OECD, and Japanese MAFF notification 3850. In order to establish dose levels for a 90-day inhalation study in rats, this dose range finding study was performed, with the test material (chlorpyrifos technical; Makteshim Israel; 96.8% stated purity; batch 489205) administered as an aerosol by whole body exposure (six h/day, five days/week, for two weeks) to groups of animals (Wistar CrL:COBS WI BR; Charles River UK; aged 6 weeks upon arrival; 5/sex/dose) at target concentrations of 0 (air control), 10, 100, and 400 mg/m^3.

During exposure, clinical signs were recorded either as a group response where all visible animals appeared to be reacting in a similar manner, or as an individual response. After exposure, animals were examined at least twice daily for clinical signs of intoxication. Body weights and food consumption were measured daily from one week prior to treatment through to the completion of the study. For determination of erythrocyte and whole-blood cholinesterase activity, blood samples were collected from animals in the control, 10 and 100 mg/m^3 groups immediately after treatment on days 11 or 12 of the study. At the 400 mg/m^3 exposure level, surviving animals were bled prior to sacrifice on day 9 of the study.

All surviving animals were killed after the 14 day exposure period, at which time the brain was removed, with half of the brain tissue used for cholinesterase activity determinations. The macroscopic appearance of all tissues was noted, and the following organs were dissected free and weighed: lungs, liver, adrenals, heart, kidneys. A range of organs and tissues were collected, together with any abnormal lesions, and preserved for microscopic examination. No histopathological examinations were conducted on these tissues for this report.

Results

Analysis of the exposure conditions revealed that the doses of test material at the nominal doses listed above were 0, 10, 94, and 388 mg/m^3. Particle size analysis did not reveal any particles greater than 5.0 µm aerodynamic diameter, and so respirability was considered to be 100%. High-dose females were sacrificed in a moribund condition on days 5 and 8 of the study (two females in total). Exposure of all high-dose animals was terminated after 5 exposures due to the moribund condition of the animals, with rapid weight loss and deterioration in the general condition of the animals observed, and all surviving animals were sacrificed on day 9. No mortality was observed at other exposure levels.

During exposure, signs observed included salivation, lachrymation, hunching, and partial closure of the eyes. At 94 and 388 mg/m^3, signs also included head and whole body tremors. After the exposure period, clinical signs included red/brown staining on all parts of the body, pale eyes, and yellow staining of the urogenital region. At 94 and 388 mg/m^3, the signs of intoxication included tremors (facial, fore and hind limb, and whole-body), extreme agitation, and convulsions. The severity of these effects at the high-

dose level resulted in the animals being killed for humane purposes.

At 388 mg/m^3, animals lost weight rapidly between commencement of treatment and until treatment was suspended at day 5. At day 5, body weights were reduced by about 24% compared with controls, in both males and females. At 94 mg/m^3, body weight reductions reached about 10% in males and about 16% in females, compared with controls. At these dose levels, the reduction in body weights was considered to be treatment-related. At 10 mg/m^3, body weight gain was similar to that seen in control animals. Food consumption was similar in controls and low dose animals, but at 94 and 388 mg/m^3, food consumption was reduced in a dose-related manner during the study.

Plasma, erythrocyte, and brain cholinesterase activities were markedly decreased at all doses. At 10, 94, and 388 mg/m^3, plasma cholinesterase activity in males was inhibited by 78, 86, and 88%, respectively, compared with controls. In females, the corresponding percentage inhibition was 91, 93, and 95%, respectively. Erythrocyte cholinesterase inhibition at 10, 94, and 388 mg/m^3 in males was 78, 58, and 75%, respectively, and in females was 60, 80, and 73%, respectively. Brain cholinesterase inhibition was 50% at 10 mg/m^3, about 70% at 94 mg/m^3, and about 72% at 388 mg/m^3, in males and females.

Macroscopic examination revealed a number of findings that occurred only sporadically in both control and treated groups, including minimal hydronephrosis, enlarged cervical nodes, and pale spleen, and hence were not considered to be related to treatment. Symmetrically enlarged adrenals were observed in 1/5 males and 5/5 females at the high-dose, and in 1/5 females at 94 mg/m^3, compared with a zero incidence of this effect in other groups. Examination of the forestomach revealed findings in high-dose animals, including thickening, whiteness, invagination, depressions, and congestion, and no such effects were observed at other dose levels. The adrenal and forestomach effects were considered to be related to treatment with the test material.

Statistically-significant ($p<0.05$) increases in absolute group mean adrenal weights were seen in high-dose males and females, while in females at 94 mg/m^3 an increase in adrenal weight (not statistically significant) was also observed, and this effect may be related to treatment.

Decreased absolute group mean weights were also seen in heart (high-dose males only), kidneys (high-dose females only), and liver (mid-dose females only), and increased liver weights were seen in high-dose females only. As these changes were generally slight, were not consistent between the sexes, and/or were not related to dose, they were not considered to be treatment-related.

No NOEL was demonstrated in this study, based on the significant inhibition of plasma, erythrocyte, and brain cholinesterase activities at doses of 10 mg/m^3 and above, in males and females. Clinical signs of toxicity (tremors, salivation, lachrymation), decreases in food consumption, and decreases in body weight were observed at doses of 94 mg/m^3 and above, but these effects were not observed at 10 mg/m^3. Increased adrenal weights (94 and 388 mg/m^3), and forestomach effects including thickening and congestion (388 mg/m^3) were observed, with no treatment-related pathology observed at 10 mg/m^3.

Streeter CM, Lomax LG, Landry TD & Dittenber DA (1987) Chlorpyrifos: 2-week whole-body vapor inhalation toxicity study in Fischer 344 rats. Dow Chemical Company Study HET K 044793-078, dated 17 February 1987. [Dow; Submission 11462, reference 58]

Landry TD, Streeter CM & Lomax LG (1985) Chlorpyrifos: 2-week vapor inhalation toxicity study in Fischer 344 rats. Protocol. Dow Chemical Company AA-714, dated 5 December 1985. [Dow; Submission 11462, reference 65]

This study was conducted in accordance with GLP requirements of the US FDA (Federal Register, 22 December 1978, Vol 43, No 247, 59986-60025), US EPA, FIFRA (Federal Register, 29 November 1983, Vol 48, No 230, 53946-53969), and Pesticide Assessment Guidelines (Subdivision F).

This study was conducted to assess the effect of repeated whole-body inhalational exposure to chlorpyrifos (Dow Chemical Company; Dursban R; lot: AGR 219646; stated purity 100%) in rats (male and female Fischer 344; 6-8 weeks of age; Charles River, USA), and also to select doses for a 13-week inhalation study.

Rats (6/sex/exposure concentration) were exposed to time-weighted average concentrations (nominal) of 0, 1, or 5 ppb (0, 0.014, or 0.072 mg/m^3) chlorpyrifos vapour for 6 h/day, 5 days/week for two weeks. Exposure-chamber atmospheres were sampled to determine the actual exposure concentrations (3 analyses/day; 1.5-2 h sampling times). Chamber distribution was shown to be homogeneous. Two control groups were used to assess normal baseline cholinesterase activity. Body weights were determined weekly, and at the end of the exposure period, and samples were obtained from each rat for plasma, RBC and brain cholinesterase (ChE) activity determinations. Gross pathological examinations were conducted on each animal. Weights of brain, liver, kidneys, and adrenals were recorded. A complete set of tissue samples were obtained and preserved, but no histopathological examination was performed. All animals were observed after each exposure period for clinical signs of toxicity.

Results

The mean daily time weighted average (TWA) analysed concentrations were 0.7 and 5 ppb, respectively, at the nominal concentrations of 1 and 5 ppb, respectively.

There were no treatment-related clinical signs of toxicity or changes in body weights during the study, and all rats survived until the end of the study.

Statistically-significant decreases in plasma cholinesterase activity were seen in males and females at 5 ppb, with reductions of 18 and 45% compared with control group A (n = 6). RBC cholinesterase activity was also significantly reduced at this dose level, with reductions of 8-9% compared with control group A. Brain ChE activity was not significantly decreased.

When the test groups were compared with the second control group (group B; n = 6), significant decreases in plasma (15% and 47%, males and females, respectively), RBC (11% and 15%, males and females, respectively) and brain (8% in females) cholinesterase activity were observed.

No statistically-significant differences were determined between the two control groups, and so the test group cholinesterase activities were compared with the combined control group values. When this was done, statistically-significant decreases in plasma (16% and 46%, males and females), RBC (10% and

13%, males and females) and brain (7%, females only) cholinesterase activity were observed.

At 0.7 ppb, a statistically-significant decrease in plasma cholinesterase activity (10% reduction) was also seen in females. The study authors stated that this apparent decrease was due to a lower than expected plasma cholinesterase activity in a single female, with a value considerably less than the group mean for this enzyme. However, the individual animal data were not provided in this report.

The reductions in brain and RBC cholinesterase activity at 5 ppb did not reach 20% when compared with any of the control groups, and these effects were not considered to be of toxicological significance. The dose-dependent reduction in plasma cholinesterase activity was considered to be treatment-related and toxicologically significant at 5 ppb, based on the reduction in activity of about 45% in females. The statistically-significant decrease in activity at 0.7 ppb in females compared with the combined control group was considered to be incidental to treatment.

No treatment-related effects were noted on body weights or organ weights, and no lesions were observed at gross pathological examination.

Under the conditions of this study, reductions in plasma, RBC and brain cholinesterase activities were observed at 5 ppb (approximately 0.072 mg/m^3). The inhibition of plasma cholinesterase activity was >20% in females at this exposure concentration. No significant treatment-related effects were observed at 0.7 ppb (approximately 0.01 mg/m^3).

4.3.2 Rabbit

Pennington JY & Edwards NH (1971) Comparison of cholinesterase depression in humans and rabbits following exposure to chlorpyrifos. The Dow Chemical Company (TA-477). [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Four humans and three rabbits were exposed for 5 minutes to chlorpyrifos formulation M-2995 (61.5% chlorpyrifos, 34.5% xylene), lot 042 7032 via an ULV cold aerosol fog generator delivering 3.8 litres/h. The humans were exposed at a distance of 8 m wearing plastic coveralls but with heads and hands exposed for 2 subjects, and heads, hands and arms exposed for 2 subjects. The rabbits were exposed at a distance of 8 m at 1.3 m height (2 rabbits) or 0.8 m height (1 rabbit). Plasma and erythrocyte cholinesterase determinations were made pre- and at several intervals post-dose.

For the humans, air sampling was conducted using 2 shoulder height filters, 3.7 cm^2 in area, one of which measured passive skin deposition and the other sampled air at 1.0 litres/minute. Air samples for rabbit exposures were taken by positioning filters directly under the noses of rabbits and sampling air at 1.0 litres/minute. Exposure was terminated at 5 minutes due to the eye and lung irritation induced by the formulation.

The air sampling recorded breathing-space concentrations of chlorpyrifos of about 108 mg/litre (range 83-133) for humans and rabbits, with passive deposition accounting for 2-6% of filter load. There was no depression of plasma or RBC cholinesterase in 24 h post-exposure samples from the human subjects. At 24 h post-dose, rabbits recorded a decrease in plasma cholinesterase (up to 33%) and RBC cholinesterase (up to 12%), but by 72 h post-dose these values had recovered to near control values.

5. SUBCHRONIC TOXICITY

5.1 Subchronic studies with the TGAC

5.1.1 Mouse Studies

Crown S, Weiss A, Nyska A, Waner T & Pirak M (1987) Pyrinex technical. Toxicity in dietary administration to mice for 13 weeks. A preliminary study. Life Science Research Israel, Inc, Study Dates 11 March 1987 - 23 June 1987. LSRI Report No: MAK/105/PYR, 23 December 1987. Makteshim Chemical Works Report No: R-4671. [Makteshim; Submission 11471; reference 23]

This study was conducted in compliance with LSRI Standard Operating Procedures, designed to comply with EPA GLP Regulations, 40 CFR 160. Chlorpyrifos (Pyrinex Technical; Makteshim; 93.5% purity; Batch 58900/9) was mixed in commercially available powdered laboratory animal diet (Altromin 1321N) to provide test diets containing 0 (control), 5, 50, 200, 400, and 800 ppm of the test material. These dietary concentrations were measured to be equal to doses of 0, 0.7-1.3, 7.1-13.5, 32.4-53.4, 40.9-135.7, and 112.5-300.7 mg/kg/d, respectively. The homogeneity and stability of the test material was determined using a trial mix, and the stability and homogeneity was acceptable for the purposes of this study. Homogeneity was assessed by analysis of six spaced samples of diet from each dose group. To test for stability, the previously examined samples were reanalysed after 7-8 days and after 14 days of storage. Groups of mice (CD-1; Charles River UK; 12/sex/dose) were fed the test diet for a period of at least 13 weeks, followed by sacrifice during the 14th or 15th week of treatment. Animals were inspected twice daily for clinical signs and for mortality. Body weights and food consumption were determined weekly. Blood samples were obtained from all surviving mice prior to terminal sacrifice, and brains were dissected from 4-12 mice/group at terminal sacrifice, for cholinesterase determination.

All animals were subject to gross necropsy, with all external surfaces, body orifices, and the contents of cranial, thoracic and abdominal cavities studied. The brains, hearts, kidneys, livers, spleen and testes were weighed. Histopathological examinations were conducted on a range of tissues from all animals, namely adrenals, bone (sternum including marrow + tibia femoral joint), duodenum, eye and optic nerve, jejunum, kidneys, liver, lungs, lymph nodes (cervical, mesenteric, abnormal), spleen, stomach (fundus, pylorus), and uterus. In addition, histopathologic examinations were conducted on a further set of tissues from control and high-dose groups, and for all animals dying or killed during the study, namely aorta, brain, caecum, colon, epididymides, gall bladder, Harderian glands, heart, ilium, mammary glands, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerve, seminal vesicles, skeletal muscle, skin, skull, testes, thymus, thyroids, tongue, trachea, urinary bladder. All tissues that displayed abnormalities were also examined microscopically.

Results

Treatment-related mortality occurred in 4 males and 2 females at 800 ppm, and in 2 females at 400 ppm. A number of deaths were also recorded at 200 ppm, but these were attributed to causes unrelated to treatment, namely water bottle malfunction and injuries inflicted by cage mates. Ocular

opacity was reported in 3/12 males and 7/12 females at 800 ppm, and in 1/12 females at 400 ppm. Statistically-significant increases in urogenital staining were seen in males at 400 ppm (9/12) and 800 ppm (11/12) compared with 1/12 in controls. A range of clinical signs occurred at doses of 200 ppm and above, including tremor, lachrymation, hunching, lack of grooming, and orbital staining, but the incidence of these effects was low (1-3/group) and not dose-dependent.

Statistically-significant decreases in group mean body weight was observed in males at 800 ppm throughout the study, with reductions of 10-15% compared with controls. A lesser reduction in group mean body weight was also seen in males at 400 ppm during the study, but this reduction (about 5% compared with controls) only reached statistical significance on a few occasions, and was not considered to be biologically significant. In females, a similar pattern was observed for body weights, with a reduction in group mean weight of up to 10% in high-dose females (often statistically significant), and a lesser reduction (occasionally statistically significant) in females at 400 ppm. Food consumption was reduced at 800 ppm in the first few weeks of the study, and again during the last two weeks of the study, but food consumption patterns in the remainder of the test was similar at all dose levels.

Plasma cholinesterase activity was markedly decreased at all test dose levels in males and females compared with controls, ranging from decreases of 36% in females and 45% in males at 5 ppm, to 95-99% in males and females at doses of 50-800 ppm. These decreases in activity were highly statistically significant ($p<0.001$). No statistically-significant decreases in erythrocyte cholinesterase activity were observed in males, though decreases of 21% and 26% were seen at 50 and 800 ppm, respectively. In females, statistically-significant decreases in erythrocyte cholinesterase activity were measured at doses of 50 ppm and above, but there was no dose relationship observed for this effect. Brain cholinesterase activity was markedly inhibited at doses of 200 ppm and above in males, and at doses of 50 ppm and above in females, with a strong dose dependency seen for this effect.

Group mean cholinesterase inhibition (expressed as a percentage cf. untreated control values)

Dose (ppm)	5	50	200	400	800
Plasma - male	45%***	95%***	98%***	99%***	99%***
Plasma - female	36%***	97%***	98%***	99% ***	99% ***
Erythrocyte - male	34%(+)	21%	16%	12%	26%
Erythrocyte - female	11%	57%***	44%**	48%**	44%**
Brain - male	43%(+)	14%*	80%***	85%***	87%***
Brain - female	3%(+)	28%***	58%***	84%***	88%***

*p<0.05
**p<0.01
***p<0.001
(+) cholinesterase activity increased compared with controls

On occasion, absolute organ weights varied slightly from controls, and this difference reached statistical significance at a number of doses, for a number of organs. However, there was no consistent dose relationship observed for these effects, and they were not considered to be related to treatment. Statistically-significant changes in organ weights relative to body weights were also observed. In males and females, most of these findings occurred infrequently, and were not dependent upon dose, and so were not considered to be treatment-related. In females, statistically-significant increases in group mean relative liver weights were reported at doses of 200, 400, and 800 ppm. The changes were largest at the high dose, with an increase of approximately 15% compared with controls.

Urogenital staining was observed at doses of 200 ppm and above in males killed at terminal sacrifice, and also in high-dose females at this time, and this effect was consistent with the treatment-related clinical signs reported during the study. Some ocular effects were also reported, with ocular opacities occurring in a single high-dose male and in one female at 400 ppm and four females at 800 ppm. Pthisis bulbi (shrinking of the eyeball) was also seen in two high-dose females. During macroscopic examination, a range of other findings were reported sporadically, often with a single incidence, at a range of dose levels; these effects included distended gall bladders, congested lungs, enlarged ovaries, and enlarged lymph nodes. As the incidences of these findings were very low, and there was no dose-relationship, they were not considered to be related to treatment.

For animals that died or were killed during treatment, a number of findings related to treatment were observed during microscopic pathological examination. These findings were mainly confined to the 400 and 800 ppm groups, with instances of thymic atrophy, depletion of lymphocytes in the spleen, lipogenic pigmentation in the adrenal cortex, ocular keratitis and cataracts, and inflammatory ulceration of the glandular stomach mucosa. Some of these lesions may be associated with the general poor condition of the animals due to the toxicity of the test material. At doses of 200 ppm and below, a sporadic incidence of some findings were observed, but these effects were not considered to be related to treatment due to the lack of a dose-response relationship for these findings, and their low incidence.

Microscopic pathological examination of animals killed at terminal sacrifice revealed a number of findings in adrenal gland, eye, and uterus that may be associated with treatment. These findings have been outlined below.

Adrenal effects: In the adrenal gland, changes were generally seen in the adrenocortical cells from the inner layers of the cortex, with multinucleated cellular formations, pyknotic nuclei, finely vacuolated cytoplasm, and cytoplasmic pigmentation, possibly ceroid. These findings were associated with treatment at doses of 200 ppm and above in females and at 400 ppm and above in males, and the incidence was related to dose. At doses of 5 and 50 ppm (and 200 ppm in males), these adrenal effects were not considered to be related to treatment as the incidence at the lower dose levels was similar to the control incidence.

Adrenal Gland: Microscopic pathology, terminal sacrifice

Finding	Sex	Dose (ppm)					
		0	5	50	200	400	800
Cortex: lipogenic pigmentation, occasional, focal:	M	1/12	2/12	2/12	1/9	3/12	2/8
	F	0/11	1/12	1/12	2/10	3/10	1/11
Cortex: lipogenic pigmentation, multiple foci:	M	0/12	0/12	0/12	0/9	2/12	3/8
	F	0/11	0/12	1/12	5/10	5/10	10/11
Cortex: lipogenic pigmentation, occasional or multiple foci	M	1/12	2/12	2/12	1/9	5/12	5/8
	F	0/11	1/12	2/12	7/10	8/10	11/11
Accessory adrenocortical tissue	M	1/12	1/12	0/12	0/9	3/12	2/8
	F	0/11	1/12	0/12	0/10	0/10	0/11
Subcapsular: cell hyperplasia	M	1/12	0/12	3/12	0/9	2/12	0/8
	F	1/11	1/12	2/12	1/10	1/10	4/11

Ocular effects: Acute or subchronic keratitis was observed in two males and four females at 800 ppm, and in a single female at 400 ppm. At 800 ppm, a cataract was observed in a single female and phthisis bulbi in two females. The study authors suggested that these ocular effects may have been associated with the irritant properties of the test material, following contact with the eyes, and this was a plausible explanation.

Uterine effects: In a single high-dose female, denser endometrial stroma, with reduced stromal ground substance and closely packed stromal cells, was observed. Due to the isolated nature of this finding it was not possible to attribute the lesion to treatment.

A range of other findings were also reported following microscopic examination, including focal necrosis and mononuclear cell infiltration of the liver, para-ovarian cyst, slight subchronic nephritis and pulmonary vascular congestion. As the incidences of these and other sporadic findings occurred at a low incidence and were not dependent upon dose, they were not considered to be related to treatment.

A single incident of malignant lymphoma was reported in a high-dose female at terminal sacrifice. The incidence of this lesion in all other groups was zero, and no other neoplastic lesions were reported in this study. Given the isolated nature of this finding, it was considered that there were no treatment-related neoplastic findings in this report.

Under the conditions of this study, a NOEL was not established, with marked and statistically-significant inhibition of plasma cholinesterase in males and females at the low dose of 5 ppm. The LOEL for this study, based on the plasma cholinesterase inhibition, was 5 ppm, calculated to be equal to 0.7 mg/kg/d. No other treatment-related effects were reported at the low dose level.

Marked and statistically-significant, dose-related inhibition of brain cholinesterase activity was observed at doses of 50 ppm and above. The NOEL for brain cholinesterase inhibition was 5 ppm (equal to 0.7 mg/kg/d), based on this inhibition of activity at 50 ppm (equal to 7.1 mg/kg/d).

5.1.2 Rat

Szabo JR, Young JT & Granjean M (1988) Chlorpyrifos: 13-week dietary toxicity study in Fisher 344 rats. Jackson Research Centre, Health and Environmental Sciences - Texas. Laboratory study No.: TXT: K-044793-071. Report dated December 28, 1988. [Dow; Submission 11462, reference 68. Reviewed by PMRA]

The following study assessment report was obtained from the Pest Management Regulatory Agency of Canada (PMRA) under the auspices of the OECD Ad Hoc Exchange Program. The Canadian review was completed on 14/1/93, and has been incorporated with minimal textual change. An independent assessment of the original data has not been conducted by Australian regulatory authorities. ***Additional Australian regulatory conclusions and comments are enclosed in square brackets [ ].***

GLP: Yes (US EPA, OECD, and Japanese MAFF standards). Test Period: 03 July 1985 - 04 October 1985 (13 weeks). Test Materials: Dursban F*, AGR 214637, Lot No. MM820905-610 supplied by the Agricultural Products Test Chemical Repository, The Dow Chemical Company, purity of 95.7%.

Stability of test material in the diet was determined. Test Animals: Male and female rats CDF Fischer-344, obtained from Charles River Laboratories Inc. Kingston, N.Y. approximately 28 days of age, weights not given.

Treatment: Groups of rats (10 rats/sex/group) were fed diets containing chlorpyrifos at dose levels of 0, 0.1, 1.0, 5.0 and 15 mg/kg body weight/day for 13 weeks. Rats were housed individually in stainless steel, wire bottom, suspended cages. Cages were placed in rooms with controlled temperature (75 degrees $\pm$ 5 degrees F) and a 12 hour light/12 hour dark cycle. Purina Certified Rodent Chow #5002 diet and drinking water were available to the animals ad libitum. Each lot of diets used was analysed for nutritional values and contaminants.

Parameters: Animals were observed at least once daily (including weekends) for signs of toxicity and mortality. Functional observational batteries were conducted on all rats on day 8, day 30 and day 87. Food consumption was recorded weekly. Water intake was not determined. Body weights were recorded weekly.

Blood samples were collected from the orbital sinus after 90 days for males and 91 days for females and examined for the following haematology parameters: packed cell volume, haemoglobin, total red and white blood cells, platelets, differential white blood cells (100 cells), the morphologies of erythrocytes, leucocytes and platelets.

Blood samples for erythrocyte and plasma cholinesterase determinations were drawn on days 43 and 90 for males and on days 44 and 91 for females. One half of the brain, upon necropsy, was used to determine brain ChE activity. Clinical chemistry determinations were obtained on fasted rats at the end of the experiment and included alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), total protein (TPRO), albumin (ALB), and glucose. Globulin was calculated. Urine samples were collected after 92 days on surviving rats and tested for specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood and urobilinogen.

At termination all surviving rats which had been fasted overnight were necropsied and examined for gross pathology. The following organs were removed and weighed: brain, heart, liver, kidneys, thymus, testes (males), ovaries (females). Ratio of the organ weights to fasted terminal body weights were calculated. Eyes were also examined in situ immediately after sacrifice. Tissues and organs from animals of control and high-dose group were histopathologically examined (those marked with an asterisk were examined in rats given doses of 0.1 and 1.0 mg).

Adrenal glands*	Liver*	Skeletal muscle
Aorta	Lungs	Skin
Bone	Mammary gland	Small intestine
Bone marrow	Mediastinal lymph node	Spinal cord
Brain	Mediastinal tissue	Spleen
Caecum	Mesenteric lymph node	Stomach
Cervix	Mesenteric tissue	Testes
Coagulating gland	Nasal tissue	Thymus
Epididymis	Ovaries	Thyroid gland
Oesophagus	Oviducts	Tibial nerve
Eyes	Pancreas	Tongue
Gross lesions*	Parathyroid glands	Trachea
Heart	Pituitary gland	Urinary bladder
Harderian/lacrimal gland	Prostate	Uterus
Kidneys*	Salivary gland	Vagina
Large intestines	Sciatic nerve	
Larynx	Seminal vesicle	

Results

Clinical signs of toxicity: Two male rats, one from the control and the other from the 0.1 mg/kg bw/day dose group lost significant weight on day 42 of the study. Observations were done weekly. However, the following week these male rats appeared healthy. Among the female rats, two (one from the 5 mg/kg bw/day and the other from the 15 mg/kg bw/day) were seen to have eye damage resulting from orbital bleeding procedures.

Mortality: There were no deaths reported.

Functional observational battery: Male rats fed 15 mg/kg bw/day had urine staining only at the 87 day observation interval (7/10). Increased frequency in the number of rats experiencing urine staining at each time interval was observed in the female rats fed 15 mg/kg bw dose: on day 8 (1/10), on day 30 (3/10), and on day 87 (8/10). Urine staining was also observed on day 87 only, in female rats (2/10) fed 1 and 5 mg/kg bw/day.

Food consumption: Food consumption was slightly higher (not statistically significant) in male rats of the 15 mg/kg bw/day dose group when compared with the controls.

Body weight and body weight gains: Following the first week of treatment and throughout most of the recorded time intervals in the study, body weight and body weight gain were significantly lower in male rats of the 15 mg/kg bw dose group when compared with the respective controls. No changes in body weight or weight gain were seen in the females of the 15 mg/kg bw dose group compared to the control group.

Hematology: (see attached tables) A significant decrease was seen in the red blood cell (RBC) count in males of the 5 mg/kg bw/day. Significant decreases in RBC count were seen in both the female and male rats fed 15 mg/kg bw/day of chlorpyrifos. No histopathological changes were seen in the bone

marrow or the spleen. A significant increase (dose-related) in the platelet count was seen in male rats at both the 5 and 15 mg/kg bw/day dose groups. No changes in the platelet count were observed in female rats. A slight increase (dose-related) of the white blood cell (WBC) count though not statistically significant was seen in male rats at all doses. No changes in WBC was observed in female rats. A significant decrease in the packed cell volume (% PCV) was seen in female rats at the high-dose group, though none was observed in male rats.

Urinalysis: (see attached tables) Statistically-significant increases of the specific gravity in the urine was observed in the female rats of the 15 mg/kg bw dose group. A slight increase (not statistically significant) in the urinary specific gravity was observed in the female rats at the 5 mg/kg bw/day dose group and in the males at the 5 and 15 mg/kg bw/day dose groups. All other parameters (pH, protein, sugar, ketones, bilirubin, blood and urobilinogen) were comparable to control.

Clinical chemistries: (see attached tables) Statistically significantly lower ALP and ALT (not dose-related) activity values were recorded for male rats fed 1, 5, and 15 mg/kg bw/day. No changes in ALP and ALT were observed in the female rats. Significant decreases in the total protein and globulin concentration were seen In male rats of the 5 and 15 mg/kg bw dose group, while no changes were seen among the female rats. A significant decrease in the albumin concentration was seen in male rats only, at the 15 mg/kg bw dose.

A significant decrease in the serum glucose concentration (not dose-related) was seen in female rats of the 1, 5 and 15 mg/kg bw dose group. No changes in the serum glucose concentration was seen in male rats. A slight increase (not statistically significant) in the blood urea nitrogen (BUN) was seen in female rats at the 15 mg/kg bw/day dose, however this was attributable to two female rats of the group (outliers) having higher BUN values than the rest of the group. A statistically-significant increase in the kidney weight (absolute weight) was seen in the high-dose group of female rats (of which the two outliers mentioned above and another female rat had higher kidney weights than the rest) when compared to the control.

Cholinesterase activity: (see attached tables) Significant dose-related decreases in the plasma ChE was seen in male rats fed 1, 5 and 15 mg/kg bw/day at both time intervals, 43 and 90 days. Significant dose-related decreases with regards to the erythrocyte activity was observed in male rats fed 1, 5 and 15 mg/kg bw/day at 43 days, and in those fed 5 and 15 mg/kg bw/day at 90 days. Brain ChE activity was significantly lower and dose-related in male rats fed 5 and 15 mg/kg bw/day (measured only at the termination of study).

[Statistically] significant dose-related decreases in the plasma ChE was seen in female rats fed 0.1, 1, 5 and 15 mg/kg bw/day at 44 days and in female rats fed 1, 5 and 15 mg/kg bw/day at 91 days. In female rats fed 0.1 mg/kg bw/day, the plasma ChE activity decreased by 15% after 44 days and was statistically significant (alpha = 0.05), whereas the decrease after 91 days was only 9% but was not statistically significant. At the higher dose of 1 mg/kg bw/day, the plasma ChE activity was inhibited by 81-82% at both time intervals denoting an abrupt and more severe inhibition. Significant dose-related decreases were also seen in RBC (erythrocyte) ChE activity at both time intervals in female rats fed 1, 5 and 15 mg/kg bw/day. Brain ChE activity was significantly lower and dose-related in female rats fed 5 and 15 mg/kg bw/day at 94 days (after less than 1 day of fasting prior to sacrifice).

Terminal body weight, organ and relative organ weights: (see attached tables) Statistically significantly higher relative brain and heart weights were identified in male rats fed 15 mg/kg bw/day though not in female rats. A significant increase in the absolute weight of the kidney was seen in female rats fed the highest dose (15 mg/kg bw/day) but not in male rats. A statistically-significant increase was seen in female rats fed 0.1 mg/kg bw/day with regards to the absolute and relative heart weight. No additional weight effects in female rats were seen at the higher doses which seems to indicate that this effect was biologically insignificant.

Gross Pathology: Perineal soiling was seen in female rats (7/10) and male rats (1/10) fed 15 mg/kg bw/day. Other observations such as periocular hemorrhage, phthisis bulbi and cloudy cornea were attributed to orbital sinus bleeding procedures. Cysts were observed in female rats at dose levels of 1 mg/kg bw/day (1/10), 5 mg/kg bw/day (1/10) and 15 mg/kg bw/day (2/10).

Histopathology: In male rats, histological changes were seen in the adrenals; very slight vacuolation of cells consistent with fatty change in the zone fasciculata was seen in 8/10 male rats at the mid dose range (5 mg/kg bw/day), while all rats at the highest dose levels were affected - 5 cases with slight vacuolation and the remaining 5 cases were moderately affected. The adrenals of the female rats were not affected.

Very slight histological changes in the liver such as aggregates of mononuclear (predominantly Iymphoid) cells, multifocal, in both the female and male rats were observed in all dose groups (females: control 4/10; 0.1 mg/kg bw/day 3/10; 1 mg/kg bw/day 1/10; 5 mg/kg bw/day 3/10; and 15 mg/kg bw/day 1/10) (males: control 1/10; 0.1 mg/kg bw/day 2/10; 1 mg/kg bw/day 0/l0; 5 mg/kg bw/day 2/10; and 15 mg/kg bw/day 0/10). Liver architecture altered secondary to diaphragmatic hernia was observed only in the female rats in the control and high-dose group: control 2/10; and 15mg/kg bw/day 1/10.

Very slight histological changes in the kidneys consisting of aggregates of mononuclear (predominantly lymphoid) cell, corticomedullary junction, in the male rats were observed in the control as well as in most of the dose groups. No histopathological changes in the kidneys were observed in female rats. Very slight degeneration (with or without inflammation) of the myocardium of the heart was seen in male rats in both the control and high-dose groups (the intermediate dose groups were not analysed) at comparable rates: control - 2/10; 15mg/kg bw/day - 1/10. The myocardium of the female rats were not affected.

Inflammation of the lacrimal/harderian gland were observed in both male and female rats but were secondary effects of orbital bleeding. One female in the high-dose group was seen to have a focal inflammation (subacute to chronic) of the tongue muscle.

Conclusion: Chlorpyrifos affected mainly the cholinesterase activity (dose-related effects) by reducing ChE levels in the plasma, and in the erythrocytes starting at doses of 1 mg/kg bw/day and in the brain starting at 5 mg/kg bw/day. Histological changes consisting of fatty vacuolization of the adrenal zone fasciculata with increasing severity was observed in male rats fed 5 mg/kg bw/day (8/10 slight) and 15 mg/kg bw/day (5/10 slight and 5/10 moderate). Body weight and body weight gain was lower in males fed the highest dose level (15 mg/kg bw/day) while none was observed in females.

Among the clinical chemistry parameters, ALP and ALT was significantly lower in males fed 1, 5 and

15 mg/kg bw/day, whereas these parameters were unchanged in females. Glucose levels were significantly lower in females fed 1, 5 and 15 mg/kg bw/day. [Decreases in the above clinical chemistry parameters were not considered to be toxicologically significant.]
The NOEL, under the conditions of this study (subchronic dietary administration during 13 continuous weeks) was 0.1 mg/kg bw/day for both female and male rats based on erythrocyte cholinesterase depression. The NOAEL was considered to be 1 mg/kg bw/day based on brain cholinesterase depression. [The NOEL was 0.1 mg/kg/d, based on significant inhibition of plasma and erythrocyte cholinesterase activity at doses of 1 mg/kg/d and above. Decreases in plasma cholinesterase activity in females at 0.1 mg/kg/d (15% at 44d and 9% at 91d) were not considered to be toxicologically significant. The NOEL for the inhibition of brain cholinesterase activity was 1 mg/kg/d, based on significant inhibition at doses of 5 mg/kg/d and above.]

Summary: Groups of 10 rats/sex/group were fed diets containing chlorpyrifos at dose levels of 0, 0.1, 1.0, 5.0 and 15 mg/kg bw/day for 13 weeks. Statistically significantly reduced body weight and body weight gain were seen only in male rats fed 15 mg/kg bw/day. The number of female rats fed 15 mg/kg bw/day showing urine staining increased at each time interval; 1/10 on day 8, 3/10 on day 30 and 8/10 on day 87. Perineal soiling was seen in 7 out of 10 female fed 15 mg/kg bw/day compared to 1/10 with male rats of the same dose group.

Chlorpyrifos significantly affected the haematologic parameters (decreased RBC and increased platelets) in male rats at both the 5 and 15 mg/kg bw/day dose. Statistically-significant decreases in the RBC and PCV parameters were seen in the female rats fed 15 mg/kg bw/day. Specific gravity in the urine was increased (statistically significant) in the female-rats fed 15 mg/kg bw/day.

The following parameters in clinical chemistries at dose levels of 1, 5 and 15 mg/kg bw/day were significantly affected: for male rat, decreased ALP, decreased ALT and for female rat, decreased serum glucose concentration. Total protein and globulin levels were significantly reduced in male rats fed 5 and 15 mg/kg bw/day. A significant decrease in the albumin level was seen in the male rats fed 15 mg/kg bw/day.

Significant dose-related decreases in plasma cholinesterase (ChE) were seen in female and male rats fed 1, 5 and 15 mg/kg bw/day. Statistically-significant, dose-related decreases in the erythrocyte cholinesterase activity was observed in female rats fed 1, 5 and 15 mg/kg bw/day at both time intervals (43 and 90 days). Male rats showed significant decreases (dose-related) at 43 days for doses of 1, 5 and 15 mg/kg bw/day and at 90 days only at 5 and 15 mg/kg bw/day. Significant dose-related decreases in brain ChE was seen in both female and male rats fed 5 and 15 mg/kg bw/day.

Relative heart and brain weights (relative to body weights) were statistically significantly increased in male rats of the highest dose group. Kidney weight (absolute weight) was statistically increased in female rats of the high-dose group. Increasing severity of fatty vacuolization of the adrenal zone fasciculata was observed in male rats fed 5 mg/kg bw/day (8/10 slight) and 15 mg/kg bw/day (5/10 slight and 5/10 moderate), while no effects were seen in female rats.

The NOEL, under the conditions of this study (subchronic dietary administration during 13 continuous weeks) was 0.1 mg/kg bw/day for both female and male rats based on erythrocyte [and plasma]

cholinesterase depression. The NOAEL was considered to be 1 mg/kg bw/day based on brain cholinesterase depression.

Synopsis: Five groups, each consisting of 10 male and 10 female CDF Fischer-344 rats, were fed diets containing chlorpyrifos at concentrations of 0, 0.1, 1.0, 5.0 and 15.0 mg/kg bw for 13 weeks. The NOEL was 0.1 mg/kg bw/day in both males and females, based on erythrocyte cholinesterase depression and the NOAEL was 1 mg/kg bw/day based on brain cholinesterase depression.

At 1mg/kg bw/day, significant decreases ($P<0.05$) in clinical chemistry parameters (ALP and ALT for males only, and glucose for females only) were seen. Significant dose-related decreases in cholinesterase activity (plasma ChE for both sexes, erythrocyte ChE for females at both time intervals, and erythrocyte ChE for male rats at 43 days only).

At 5 mg/kg bw/day, in addition to the changes seen at 1 mg/kg bw/day, there was significant decreases ($P<0.05$) in the total protein and globulin (clinical chemistry) for male rats only, and brain ChE was significantly inhibited for both sexes. Moreover erythrocyte ChE activity in male rats was significantly decreased ($P<0.5$) at both time intervals (43 days and 90 days) compared to the lowest dose group. There was a significant decrease ($P<0.05$) in RBC and a significant increase ($P<0.05$) in platelets for male rats only. There was increased number of males (8/10) with slight fatty vacuole formation of the zona fasciculata of the adrenals compared to the control.

At 15 mg/kg bw/day, in addition to the changes seen at the lower doses, there was a significant decrease ($p<0.05$) in albumin (clinical chemistry) in male rats only. In addition, there was increased severity of fatty vacuole formation of the zone fasciculata of the male adrenals (5/10 slight and 5/10 moderate) compared to the previous dose. Significant reduction ($P<0.05$) in body weight and body weight gain was also seen in male rats. In female rats, there was a significant decrease ($P<0.05$) in RBC and packed cell volume (hematology), and a significant increase ($P<0.05$) in the specific gravity of their urine. The absolute kidney weight was increased in female rats, while relative brain and heart weights were increased in male rats. An increased number of female rats showing urine staining was seen at each time interval: 1/10 on day 8; 3/10 on day 30; and 8/10 on day 87. On day 87 only, 7/10 male rats showed urine staining. A higher dose-related incidence in the number of females exhibiting perineal soiling was seen compared to the control and lower dose groups (0/10, 0/10, 0/10, 1/10 and 7/10).

Addendum to Study Review: Chlorpyrifos: 13-week dietary toxicity study in Fischer-344 rats".

This addendum was in response to EHD's comments of Canada's draft review of the above study on October 26, 1992 in which it was suggested that "statistically-significant observations on some parameters may not be treatment-related and perhaps these observations should not be detailed in the Summary and Synopsis sections of the review". The following study summary, which was prepared based on the original review of M. Loos's (19/09/91) and appeared in S.Ma's "Summary of Toxicity Data for Chlorpyrifos" (5/08/92), now forms part of the evaluation report of the above study.

13-Week Feeding Study in Rats - Summary and Conclusions

Ten CDF Fischer-344 rats/sex/group were fed diets containing chlorpyrifos (purity 95.7%) at concentrations [doses] of 0, 0.1, 1, 5, or 15 mg/kg bw/day for 13 weeks. Plasma and erythrocyte

cholinesterases were depressed at 1 mg/kg bw/day and above. Depression of brain cholinesterase activity occurred at 5 and 15 mg/kg bw/day. The NOEL was 0.1 mg/kg bw/day in both males and females based on plasma and erythrocyte-cholinesterase depressions and the NOAEL was 1 mg/kg bw/day based on brain cholinesterase inhibition. At the highest dose (15 mg/kg bw/day), additional treatment-related effects consisted of a decrease in bodyweight and bodyweight gain (males), increased fatty vacuolization of the adrenal zone fasciculata (males), and changes in hematology and clinical chemistry parameters (decreased RBC in both sexes; increased platelets and reduced serum total protein, albumin, globulin, alanine transaminase and alkaline phosphatase in the males; decreased serum glucose and increased urinary specific gravity in the females).

[Australia: The NOEL was 0.1 mg/kg bw/day in both males and females based on plasma and erythrocyte-cholinesterase depressions. The NOEL for brain cholinesterase inhibition was 1 mg/kg bw/day.]

TABLE 1

Changes (statistically-significant or slight changes) in the different parameters measured in the male rat					
PARAMETERS	**DOSE GROUPS (MG/KG BW/DAY)**				
	0	0.1	1	5	15
Hematology					
RBC $X10^6$	8.54	8.63	8.49	8.20*	8.05*
(S.D.)	(0.19)	(0.27)	(0.43)	(0.15)	(0.19)
Platelets $X\ 10^s$	691.60	670.40	678.80	732.60*	756.80*
(S.D.)	(29.81)	(20.30)	(46.92)	(24.15)	(22.28)

- Difference from control mean statistically significant; alpha = 0.05, two-sided.
- ( ) Standard deviation.

TABLE 1 (CONT)

Changes (statistically-significant or slight changes) in the different parameters measured in the male rat					
PARAMETERS	**DOSE GROUPS (MG/KG BW/DAY)**				
	0	**0.1**	**1**	**5**	**15**
WBC $X\ 10^3$	6.50	6.09	6.63	7.24	7.59
(S.D.)	(0.94)	(1.76)	(1.07)	(0.97	(0.71)
PVC (%)	no changes were observed				
Urinalysis					
Specific gravity	1.085	1.086	1.087	1.104	1.102
(S.D.)	(0.014)	(0.023)	(0.018)	(0.016)	(0.019)
Clinical chemistry					
ALP u/l	99.0	93.79	90.15*	88.34*	89.78*
(S.D.)	(4.08)	(6.86)	(9.82)	(7.61)	(6.09)
ALT (u/l)	67.60	60.11	57.66*	49.69*	57.71*
(S.D.)	(11.56)	(5.25)	(5.99)	(4.92)	(7.85)
Total protein (g/dl)	5.76	5.43	5.46	5.19*	5.17*
(S.D.)	(0.39)	(0.16)	(0.17)	(0.17)	(0.70)
Globulin (g/dl)	2.42	2.16	2.22	1.82*	2.05*
(S.D.)	(0.34)	(0.11)	(0.14)	(0.55)	(0.77)
Albumin (g/dl)	3.34	3.27	3.24	3.37	3.12*
(S.D.)	(0.13)	(0.13)	(0.10)	(0.56)	(0.31)
Glucose (md/dl)	no changes were observed				

BUN (mg/dl)	no changes were observed				
Cholinesterase activity					
Plasma ChE (43 d)	0.618	0.602	0.308*	0.149*	0.129*
(S.D.)	(0.031)	(0.034)	(0.025)	(0.010)	(0.006)
% inhibition		3%	50%	76%	79%
Plasma ChE (90 d)	0.640	0.574	0.267*	0.135*	0.106*
(S.D.)	(0.059)	(0.045)	(0.015)	(0.007)	(0.013)
% inhibition		10%	57%	78%	83%
Erythrocyte (43 d)	2.10	2.01	1.41*	1.08*	1.12*
(S.D.)	(0.42)	(0.15)	(0.20)	(0.28)	(0.36)
% inhibition		4%	33%	49%	47%
Erythrocyte (90 d)	1.33	1.59	1.13	0.79*	0.73*
(S.D.)	(0.33)	(0.59)	(0.36)	(0.34)	(0.29)
Brain (90 d)	11.37	11.26	11.08	6.79*	4.31*
(S.D.)	(0.44)	(0.27)	(0.39)	(0.94)	(0.27)
% inhibition			3%	40%	62%
Absolute organ weight Heart, Kidney weight (g)	no changes were observed				
Relative organ weights (g/100g) (relative to body weight)					
Relative brain weight	0.636	0.639	0.660	0.665	0.682*[A]
(S.D.)	(0.035)	(0.031)	(0.019)	(0.021)	(0.030)
Relative heart weight	0.286	0.283	0.292	0.290	0.301*[A]
(S.D.)	(0.008)	(0.012)	(0.010)	(0.013)	(0.016)

* Difference from control mean statistically significant; alpha = 0.05, two-sided.
( ) Standard deviation
A Probably due to change in body weight

TABLE 2

Changes (statistically-significant or slight changes) in the different parameters measured in the female rat					
PARAMETERS	**DOSE GROUPS (MG/KG BW/DAY)**				
	0	0.1	1	5	15
Hematology					
RBC X 10^6	7.87	8.08	8.23	8.00	7.51*
(S.D.)	(0.79	(0.14)	(0.16)	(0.16)	(0.15)
Platelets x 10^3	no changes were observed				
WBC x 10^3	no changes were observed				
PCV (%)	40.20	40.30	40.75	40.25	38.80*
	(1.30)	(0.79)	(0.54)	(1.23)	(0.75)
Urinalyses					
Specific gravity	1.072	1.080	1.080	1.093	1.116*
(S.D.)	(0.020	(0.016)	(0.016)	(0.017)	(0.046)
Clinical chemistry					
ALP (u/l)	no changes were observed				
ALT (u/l)	no changes were observed				
Total protein (g/dl)	no changes were observed				
Globulin (g/dl)	no changes were observed				
Albumin (g/dl)	no changes were observed				
Glucose (mg/dl)	122.59	118.13	112.71*	104.76*	111.31*
	(6.87)	(6.91)	(6.41)	(5.45)	(12.59)
BUN (mg/dl)	18.02	19.01	18.96	19.56	23.96

	(1.20)	(2.17)	(1.85)	(1.74)	(12.60)
Cholinesterase activity					
Plasma ChE (44 d)	2.974	2.518*	0.539*	0.196*	0.154*
(S.D.)	(0.268)	(0.300)	(0.063)	(0.017)	(0.011)
% inhibition		15%	82%	93%	95%
Plasma ChE (91 d)	3.312	3.006	0.619*	0.199*	0.130*
(S.D.)	(0.209)	(0.395)	(0.132)	(0.022)	(0.013)
% inhibition		9%	81%	94%	96%
Erythrocyte (44 d)	1.89	1.70	1.19*	0.97*	0.79*
(S.D.)	(0.29)	(0.26)	(0.24)	(0.14)	(0.19)
% inhibition		10%	37%	49%	58%
Erythrocyte (91 d)	1.81	1.84	1.16*	0.93*	0.88*
(S.D.)	(0.53)	(0.24)	(0.18)	(0.27)	(0.24)
% inhibition			36%	49%	51%
Brain (91 d)	11.91	11.84	11.77	7.08*	4.18*
(S.D.)	(0.34)	(0.46)	(0.64)	(0.30)	(0.20)
% inhibition			2%	41%	65%
Absolute organ weights					
Heart weight (g)	0.563	0.615*	0.586	0.567	0.585
(S.D.)	(0.038)	(0.038)	(0.041)	(0.046)	(0.025)
Kidney weight (g)	1.289	1.357	1.319	1.347	1.392*
(S.D.)	(0.061)	(0.064)	(0.071)	(0.042)	(0.079)
Relative organ weights (g/100g) (relative to body weight) Relative brain weight	no changes were observed				
Relative heart weight	0.344 (0.016)	0.365* (0.011)	0.358 (0.020)	0.341 (0.023)	0.350 (0.010)

.* Difference from control mean statistically significant; alpha = 0.05, two-sided.
( ) Standard deviation

Beatty SC (1964) Results of 90-day dietary feeding studies of 0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate in rats. No study numbers found. Dated February 24, 1968. [Dow; Submission 11462, reference 67]

Beatty SC & McCollister DD (1971) Results of 90-day dietary feeding studies of 0,0-diethyl-0-3,5,6-trichloro-2-pyridyl phosphorothioate in rats. Revised from 1964. A1A TBRATS AM 1-17 [Dow; submission 238, part 2 vol 1. A3162/5 Box 42, Submission 11462, reference 71]

Male and female rats (10/sex/group, 7-8 weeks old, strain and source not stated) were started on diets containing 0, 0.001, 0.003, 0.03 or 0.1% chlorpyrifos (0, 10, 30, 300, and 1000 ppm, respectively; source and purity of chlorpyrifos not stated). The group receiving 0.1% chlorpyrifos were discontinued after 4 weeks due to the severity of the toxicity at this level. At 90 days, 5 rats/sex/dose were sacrificed for plasma, RBC and brain cholinesterase determination. An additional 6 rats/sex/dose were initiated as interim sacrifice groups of 3/sex/dose for sacrifice at days 14 and 41 for plasma, RBC and brain cholinesterase determination. Animals were weighed twice weekly for 4 weeks and weekly thereafter. Haematological values (HOT, Hb, WBC) were obtained from 5 female rats at 0, 0.03 and 0.01% doses at 90 days. At terminal sacrifice (90 days) selected organs were weighed from all groups and portions processed for histopathology from the control and 0.03% groups, and serum samples (all groups) processed for limited clinical chemistry (BUN, ALP). No details were provided on the dietary stability, concentration, or homogeneity of the test material.

Rats (both sexes) started on a diet containing 0.1% chlorpyrifos (1000 ppm) were sacrificed (4 weeks) because of a dramatic weight loss and high mortality. Their condition was characterised by tremors, moist bloody noses, emaciation, ulceration of the cornea and motor coordination problems, with profound depression of cholinesterase activity in all three compartments.

At 0.03% (300 ppm), tremors were observed in only a few rats, and plasma cholinesterase activity was zero for these animals. Slight growth retardation and cloudy swelling in the livers were seen in both sexes, but were more pronounced in the females. Probably as a consequence of the grow retardation, the relative weights of the lung, liver and kidney of females were all significantly elevated (approx. 15%) while the absolute weights of these organs were normal. There were no treatment-related variations observed in the organ weight analysis for males at this dose level.

Plasma cholinesterase activity (in both sexes) was reduced in a dose-related manner and was significantly lower than controls at all dose levels, with the animals from the 0.001% dose level (10 ppm) registering a residual activity (47%) of the control value at day 90. Red blood cell cholinesterase activity was similarly significantly reduced at all dose levels, although not in dose-related manner, and was 61% of control value at the lowest treatment dose at day 90. Brain cholinesterase displayed a clear dose-related depression and a clear NOEL at the lowest dose of 0.001% (10 ppm) in both sexes, but depression at the 0.003% level (30 ppm) at 90 days was only marginally significant (M 81%, F 78%). Haematology recorded a small reduction in total leucocytes compared to control at 0.01% (13% reduction) and 0.03% (22% reduction), however there were no other adverse haematological or clinical chemistry findings, but very few parameters were examined. No histopathological lesions were found that could be linked to treatment with chlorpyrifos. The NOEL for brain cholinesterase inhibition was 0.001% (LOEL 0.003%). This was estimated to be equivalent to a NOEL of 1 mg/kg/d with an LOEL estimated to be 3 mg/kg/d. No NOEL for RBC or plasma cholinesterase inhibition was established, with effects seen at 0.001% (1 mg/kg/d) and above.

Anon (1968) Short term (subacute) dietary administration - rats organophosphate insecticide Dursban. Final Report. Hazleton Laboratories. Project No. 174-114, dated November 15, 1968. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

This study was designed to establish a NOEL for 4 and 13 week dietary intake of chlorpyrifos. Charles River rats (10/sex/group) were exposed to chlorpyrifos (Dursban, 97.5% ai) in the diet at levels of 0 and 0.3 mg/kg/d for 13 weeks (groups 1 & 2) and 1.0, 3.0 and 10 mg/kg/d for 4 weeks (groups 3, 4 & 5). At the end of the 4-week exposure period, animals in groups 3-5 were allowed to recover from treatment for three weeks, after which time they were given chlorpyrifos in the diet, at doses of 0, 0.03 and 0.1 mg/kg/d, respectively, for 13 weeks. Dosages were adjusted weekly on the basis of group mean body weights and food consumption. Tail-tip blood samples were taken for plasma and RBC cholinesterase measurement at 1, 2, 4, 8 and 13 weeks for groups 1 & 2, and at weeks 1, 2, 4, 5 and 6 for groups 3, 4 and 5. Brain cholinesterase was measured at week 13 for groups 1 & 2. After the 3 week recovery period, groups 3, 4 & 5 were fed diets achieving a chlorpyrifos intake of 0, 0.03 and 0.1 mg/kg/d respectively for a further 13 weeks in a 2nd phase of the experiment. Tail-tip blood samples were taken for RBC and plasma cholinesterase measurements at weeks 1, 2, 4, 8 and 13 weeks of this 2nd dosing phase, and the animals were then sacrificed for brain cholinesterase estimation.

There were no deaths during the study. In the first phase of the study, animals from groups 4 and

especially 5 (ie. 3.0 & 10 mg/kg/d) showed weight loss, reduced food consumption, a hunched and thinning appearance with occasional tremors, although these signs disappeared during the recovery period. There was a clear dose-response for RBC and plasma cholinesterase inhibition with no NOEL evident after 4 weeks intake. This effect was still present at week 13 when Groups 1 & 2 were sacrificed, but brain cholinesterase was not affected by treatment. Samples from the end of the recovery period (week 6) recorded a return of plasma and RBC cholinesterase activities to control value in groups 3, 4 & 5.

Cholinesterase activity (_pH/h) after 4 weeks dietary intake

	Male		Female	
Dose (mg/kg/d)	**RBC**	**Plasma**	**RBC**	**Plasma**
0	0.84	0.54	0.98	1.8
0.3	0.65	0.40	0.67	1.6
1.0	0.31	0.38	0.28	1.1
3.0	0.21	0.31	0.16	0.89
10.0	0.18	0.30	0.13	0.63

Phase 2 of the study revealed no consistent effect of 13-weeks intake of 0.03 mg/kg/d on any of RBC, plasma or brain cholinesterase in either sex, but 0.1 mg/kg/d significantly reduced RBC and brain cholinesterase in males, and RBC and plasma cholinesterase in females.

Cholinesterase activity (DpH/h) after 13 weeks dietary intake

	Male			Female		
Dose (mg/kg/d)	**RBC**	**Plasma**	**Brain**	**RBC**	**Plasma**	**Brain**
0	0.77	0.31	2.0	0.52	1.9	1.2
0.03	0.75	0.37	1.9	0.56	1.8	1.3
0.1	0.67	0.31	1.7	0.47	1.7	1.2

The use of these animals in phase 1 of the study makes interpretation of this data uncertain, however the observed NOEL for plasma, RBC and brain cholinesterase activity was 0.03 mg/kg/d based on significant inhibition of one or more of these activities at 0.1 mg/kg/d in one or both sexes.

Coulston F, Golberg L, Abraham R & Benitz FK (1971) Final report on safety evaluations and metabolic studies on Dowco 179, Section I.D.1. (IN 151). Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York. Report Date March 18, 1971. Sponsor: Dow Chemical USA. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Albino Sprague-Dawley rats (90-100g) were administered DOWCO 179 (20/sex/dose) in the diet at 0, 0.03, 0.15 or 0.75 mg/kg/d for 6 months. Diets were prepared weekly and clinical signs recorded daily. An interim sacrifice group of 5 animals/sex/dose were examined at 3 months. Necropsies and histopathological examination were reported in a limited manner (summary incidence) for 5 animals/sex in the control and high-dose groups after terminal sacrifice. Limited haematology (RBC count, Hb, HCT, WBC count) and serum chemistry (glucose, sodium, potassium and ALT concentrations) was reported for 5 animals/sex/dose at 4 and 6 months for haematology and 6 months for clinical chemistry. Brain

cholinesterase was determined at scheduled sacrifice, while plasma and erythrocyte cholinesterase determinations were recorded for 3 animals/sex/dose from the control and high-dose groups at 3, 5, 7 and 16 weeks, and on 3 animals/sex/dose in all dose groups at 6 months.

The formulated diets were within 10% of the nominal values at each assay. A total of 17 rats died or were sacrificed (moribund) during the study. The main cause of death (16 animals) was chronic murine pneumonia, and while the occurrence of mortality was spread among the groups, it was slightly higher in the high-dose group. One male at 0.75 mg/kg/d was sacrificed with paralysis of hind legs and tail. There were no treatment-related changes to body weight gain, food consumption, haematology or clinical chemistry values. The limited histopathological report did not record any treatment-related findings. Cholinesterase inhibition occurred at the high-dose (0.75 mg/kg/d) in both red blood cells (50%) and plasma (65%), but there was no inhibition of cholinesterase activity in the brain at any dose. The NOEL in this study was 0.15 mg/kg/d based on inhibition of plasma and erythrocyte cholinesterase activity at 0.75 mg/kg/d.

***Cholinesterase activity*[$]**

		Plasma		RBC	
Sex	**Weeks**	**control**	**0.75 mg/kg/d**	**Control**	**0.75 mg/kg/d**
Male	3	0.5±0.2	0.4±0.2	1.3±0.6	1.1±0.4
	5	0.4±0.1	0.4±0.1	1.5±0.5	1.0±0.4
	7	0.4±0.1	0.5±0.1	0.8±0.2	0.7±0.0
	16	0.4±0.1	0.1[*]	1.2±0.7	1.5[*]
Female	3	0.5±0.2	0.5±0.2	1.5±0.4	1.0±0.2
	5	0.6±0.2	0.4±0.1	0.8±0.1	0.8±0.2
	7	0.8±0.1	0.5±0.2	1.2±0.4	0.9±0.3
	16	0.8±0.1	0.1±0.1	1.6±0.4	0.8±0.1

[*] insufficient data for computation

[$] µmoles acetylcholine hydrolysed/min/ml (expressed as mean and standard deviations for three animals per group)

Cholinesterase activity*

	Dose (mg/kg/d)	Plasma	RBC	Brain 3 months	Brain 6 months
Male	0	0.4±0.1	1.9±0.2	208±5	203±5
	0.03	0.6±0.1	2.2±0.3	215±2	202±6
	0.15	0.7±0.1	3.0±0.1	207±9	204±6
	0.75	0.3±0.1	0.9±0.0	205±5	193±4
Female	0	1.1±0.3	1.9±0.0	201±3	182±8
	0.03	1.6±0.3	2.3±0.3	202±0.6	192±0.7
	0.15	1.4±0.1	2.8±0.1	182±11	193±9
	0.75	0.5±0.1	1.0±0.2	191±1	199±3

*µmoles acetylcholine hydrolysed/min/ml (mean and standard deviation) in plasma and RBC at 6 months, and brain at 3 and 6 months (µl CO_2/30 min, mean and standard error)

Crown S, Gur E, Nyska A & Waner T (1985) Pyrinex technical toxicity in dietary administration to rats for 13 weeks. Life Science Research Israel Ltd., conducted 3 March 1985 to 16 June 1985. LSRI report MAK/058/PYRA, 30 October 1985. Makteshim report R-3682. [Submission 11471; Makteshim; reference 26]

The study protocol was in accordance with OECD Test Guideline No. 408, and OECD GLP recommendations. This study was designed to test the toxicity associated with the dietary administration of technical chlorpyrifos (Pyrinex Technical; Makteshim; 95.5% purity; batch not stated) to rats (CD, remote Sprague-Dawley; Charles River UK; four week old on arrival; 20/sex/dose) at dietary concentrations of 0 (control), 0.5, 10, and 200 ppm for 13 weeks. The test material was mixed into a commercially available powdered laboratory animal diet (Altromin 1321N), and the feed was analysed for homogeneity, stability and test material concentration. At the dietary concentrations listed above, the minimum-maximum achieved doses of test material were calculated to be equal to 0, 0.030-0.078, 0.601-1.541, and 13.053-30.677 mg/kg/d, respectively.

All animals were inspected once or twice daily for signs of ill-health or other treatment-related signs, and a close examination of all animals was conducted weekly. Body weights and food consumption were measured weekly, and opththalmoscopic examinations were conducted on all animals prior to treatment, and on control and high-dose animals after 12 weeks of treatment. Laboratory investigations were conducted on 10 animals/sex/group, with these animals selected prior to the commencement of treatment. Urine and blood samples were collected during weeks 14 and 13, respectively, with rats fasted overnight prior to bleeding.

The following haematological estimations were performed: packed cell volume (PCV), haemoglobin (Hb), red cell count (RBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), total white cell count (WBC total), platelet count, differential WBC count.

Clinical chemistry parameters were measured, namely total protein, albumin, globulin, urea, creatinine, sodium, calcium, inorganic phosphorous, chloride, alkaline phosphatase, total bilirubin, glucose, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, potassium, plasma cholinesterase activity.
The following urinalysis parameters were measured: volume, pH, specific gravity, protein, glucose,

ketones, blood pigments, bilirubin, urobilinogen, epithelial cells, leucocytes, erythrocytes, organisms, casts, crystals, other abnormal constituents.

At the completion of the dosing regimen, animals were killed and post mortem examinations conducted, with macroscopic and microscopic examinations conducted on a number of tissues. Samples of tissues from control and high-dose animals (* indicates all groups examined) were collected and preserved, then examined using light microscopy, namely: adrenals, aorta, bone, brain, caecum, colon, duodenum, eyes, heart, ilium, jejunum, kidneys*, liver*, lungs*, lymph nodes, mammary gland, oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, skeletal muscle, spinal cord, spleen, testes, thymus, thyroid, trachea, urinary bladder, uterus, any tissue showing macroscopic abnormalities*.

Results

No unscheduled deaths occurred during the study, and no clinical signs associated with treatment were reported. Low incidences of orbital/nasal staining and hair loss were observed, but these findings were seen in all groups including controls, and were common findings in laboratory rats. Statistically-significant reductions in group mean body weights were seen at 200 ppm in males (weeks 1-7) and females (weeks 1-3). In males, the reductions in weight in weeks 1-4 (about 10% compared with controls) was highly statistically significant ($p<0.001$; t-test), while in weeks 5 and 7 the reduction in weights (5-7%) was less so ($p<0.01$ and 0.05, respectively). In females, the reductions in weight at weeks 2 and 3 (8-9% compared with controls) was highly significant ($p<0.001$). At the high dose, these reductions in body weights were considered to be related to treatment. Group mean body weights were unaffected by treatment at 0.5 or 10 ppm. The reduction in body weights was not attributed to palatability of the diet, as food consumption was increased in high-dose males and females for most of the study, often by 10-15% compared with controls.

Statistically-significant reductions in PCV, Hb, and haematocrit were observed in males and females at 200 ppm. No other significant haematological findings were reported. Statistically-significant inhibition of plasma cholinesterase activity was seen in females at 10 and 200 ppm, and in males at all test doses. In females, there was no dose-relationship for this effect, with cholinesterase activity higher at 200 ppm (57% inhibition) than at 10 ppm (91% inhibition), compared with controls. In males, however, the reduction in plasma cholinesterase activity was dose-related, with activity reduced by 22%, 37%, and 72% at 0.5, 10, and 200 ppm, respectively, at 12 weeks. No NOEL was demonstrated for this effect in males.

Statistically-significant increases in bilirubin were noted in males at 10 and 200 ppm, though the values were similar at both doses.

For other clinical chemistry parameters, there were a variety of increases and/or decreases that reached statistical significance, including increases in AP, decreases in ASAT, decreases in gamma glutamyl transferase, and increases and decreases in urea. None of the findings were considered to be related to treatment or toxicologically significant, as the changes were generally small, and/or no consistent relationship with dose was observed.

Urinalysis and ophthalmoscopy examinations did not reveal any findings that were considered to be related to treatment. Slight, but statistically significant, increases in absolute liver weights were observed

in females at 0.5 and 200 ppm, but not at 10 ppm, and absolute adrenal weights were also significantly increased in females at 200 ppm only. A reduction in absolute kidney weights was noted in males only at 200 ppm. When organ weights were calculated relative to body weights, there was a slight but significant increase in liver weights in females at 200 ppm, but no other significant effects were noted. It was possible that this increase in liver weight in females was related to treatment, but this finding was not accompanied by pathological findings, and no such effect was seen in males, so it was likely that the increases in absolute and relative liver weights were incidental.

Macroscopic pathological examination revealed a number of isolated findings, including congested thymus, pale jejunal contents, enlarged adrenals, and slight hydronephrosis, but in the absence of a dose relationship for these effects, they were not considered to be treatment-related. No treatment-related findings were observed at microscopic examination. A range of common changes were observed, both in control and high-dose animals, with generally only a single incidence for most findings, and no dose-response relationships observed.

Under the conditions of this study, no mortality or significant clinical signs were observed at any dose level. At 200 ppm (approximately 13 mg/kg/d), reduced body weights and reduced RBC, haematocrit and Hb, and increased food consumption were observed. Biologically and statistically-significant reductions (>20% compared with controls) in plasma cholinesterase activity were observed in males at 12 weeks at doses of 0.5 ppm and above (approximately 0.03 mg/kg/d and above), while in females, cholinesterase activity was reduced at doses of 10 ppm and above (approximately 0.6 mg/kg/d). The LOEL for this study was 0.5 mg/kg/d, calculated to be equal to 0.03 mg/kg/d, based on the inhibition of plasma cholinesterase at all test doses in males.

Corley RA, Landry TD, Calhoun LL, Dittenber DA & Lomax LG (1986) Chlorpyrifos: 13-week nose-only vapor inhalation exposure study in Fischer 344 rats. Dow Chemical Company Laboratory report Code HET K 044793-077 13 Nov 1986 [Dow; Submission 11462, reference 59; Submission 4033, A3162/12, B67]

This study was conducted in accordance with GLP requirements of the US EPA (Title 40 Code of Federal regulations Part 160, 1983), the Japanese Ministry of Agriculture, Forestry, and Fisheries (Notification 3850, 1984), and the OECD, 1982. Groups of Fischer 344 rats (10/sex/exposure concentrations; approximately 13-weeks old at initiation of treatment) were exposed nose-only to 0, 5.2, 10.3 or 20.6 ppb (0, 75, 147 or 296 µg/m^3) chlorpyrifos (100% purity; Dow Chemical Company, USA; lot AGR 219646) for six hrs/d, five days/week for 13 weeks. Exposures were conducted using 44-litre ADG design chambers, with airflow maintained at approximately 25 litres/min. Determinations of chlorpyrifos chamber concentrations were made three times daily (weekly for the control chamber), with analysis confirming that actual chlorpyrifos concentrations were consistent with the nominal concentrations of test material (0, 5, 10, and 20 ppb).

Body weights were determined weekly, and all animals were observed after each exposure period for signs of treatment-related effects. On the day after the last exposure, necropsies were performed on all animals, major organs (adrenals, brain, testes, liver, kidneys, lungs) were weighed, and samples of tissues were preserved for histopathological examination. These tissues were: adrenals, aorta, auditory sebaceous glands, bone, brain, caecum, cervix, coagulating glands, epididymides, oesophagus, eyes,

heart, kidneys, lacrimal/harderian glands, large intestine, larynx, liver, lungs, mammary gland, mediastinal lymph node, mediastinal tissues, mesenteric lymph node, mesenteric tissues, nasal tissues, oral tissues, ovaries, oviducts, pancreas, parathyroid glands, peripheral nerve, pituitary, prostate, salivary glands, seminal vesicles, skeletal muscle, skin, small intestine, spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina. Histopathological examination was performed on samples from the control and high-dose groups only. Pathology was also performed on all animals that died during the study.

Immediately prior to sacrifice, haematological parameters were measured for all animals, namely: haematocrit, haemoglobin, erythrocyte count, total leucocyte count, and platelet count. Differential cell counts were performed on animals from control and high-dose exposure groups. Prior to necropsy, urinalysis was performed for all animals for the following parameters: bilirubin, glucose, ketones, blood, pH, protein, urobilinogen, specific gravity. At necropsy, blood samples were obtained from all animals for serum clinical chemistry analysis of the following parameters: urea nitrogen, alanine aminotransferase activity, aspartate aminotransferase activity, bilirubin, alkaline phosphatase activity, glucose, total protein, albumin, globulins, calcium, phosphorous, sodium, potassium, chloride. In addition to routine clinical chemistry, determinations were conducted for plasma, erythrocyte, and brain cholinesterase activity from all animals.

Results

No treatment-related mortality was observed during the study. Clinical signs were confined to red staining around the eyes and nares during the first month of the study, possibly associated with the stress associated with the confinement in the exposure tubes, but this effect was not considered to be related to chlorpyrifos exposure, as it was observed in control groups also, and was not seen in the final two months of the study. No statistically-significant changes in body weight were observed in any treatment group during the study, and no clinical signs of intoxication were reported in treated animals.

A slight, but statistically-significant, decrease in RBC count was observed in females in all treatment groups, compared with controls. This effect was not considered to be treatment-related, as the magnitude of the effect was small, and there was no dose relationship demonstrated. No other changes in haematology parameters were reported in treated animals.

No statistically-significant differences in urinalysis parameters were seen between control and treated animals. Clinical chemistry examination revealed statistically-significant increases in urea nitrogen in males at 5 and 20 ppb chlorpyrifos. In the absence of any consistent dose-response relationship for this increase, and as no adverse histopathological findings were associated with this effect, the reported change in urea nitrogen was not considered to be related to treatment with chlorpyrifos. Similarly, an isolated, very slight, but statistically-significant increase in sodium seen in females at 5 ppb only was not considered to be related to chlorpyrifos administration. No effect on plasma, erythrocyte or brain cholinesterase activity was seen at any dose level tested.

Pathological examination did not reveal any changes in organ weights, or organ weight/body weight ratios, in animal treated with chlorpyrifos, and no treatment-related effects were noted following gross examination of treated animals. Histopathological examination did not reveal any effects associated with the administration of chlorpyrifos in high-dose animals.

Under the conditions of this study, no adverse, treatment-related effects were seen in rats exposed to chlorpyrifos by nose-only inhalation, at doses up to 20.6 ppb (296 µg/m^3), for six h/day, five days/week, for 13 weeks.

Newton PE (1988) A thirteen week nose-only toxicity study of chlorpyrifos technical (Pyrinex) in the rat. Bio/dynamics Inc, USA, Project no: 88-8058, 14 November 1988. Makteshim Chemical Works Report R-4750. [Makteshim; Submission 11471; reference 28]

This study was conducted in accordance with US EPA GLP guidelines, and test guidelines 82-4. Chlorpyrifos technical (Makteshim-Agan, USA; Lot 489205; stated purity 95%) was administered to Fischer 344 rats (Charles River, USA; 7 weeks old at initiation of treatment; 15/sex/dose) by nose-only exposure for six hours/d, five days/week, for thirteen weeks at target concentrations of 0 (control), 5, 10, and 20 ppb (0, 0.07, 0.14 and 0.28 mg/m^3 respectively). Exposures were initiated over a four-day period beginning 18 April 1988, and terminated over a four-day period ending 20 July 1988. In-life observations were conducted twice daily for mortality and gross signs of toxicity, and all animals were examined for clinical signs of intoxication during daily exposure periods. Detailed physical examinations were conducted pretest and weekly thereafter, and ophthalmoscopic examinations were performed pretest and at study termination. Body weights and food consumption were measured weekly and at termination.

Blood samples were collected from animals pretest (14 males /10 females; control only) and at week 8 and termination (at least 10 animals/sex/group) for laboratory studies. Haematology parameters measured were: haemoglobin concentration, haematocrit, erythrocyte count, platelet count, mean corpuscular volume, mean corpuscular haemoglobin concentration, prothrombin time, activated partial thromboplastin time, total and differential leucocyte counts. The following clinical chemistry parameters were measured: total protein, albumin, globulin, urea, creatinine, sodium, calcium, inorganic phosphorous, chloride, cholesterol, alkaline phosphatase, total bilirubin, glucose, alanine aminotransferase, aspartate aminotransferase, plasma cholinesterase activity, erythrocyte cholinesterase activity, lactic acid dehydrogenase, blood urea nitrogen, triglycerides, albumin/globulin ratio, potassium, creatine phosphokinase, gamma glutamyl transpeptidase. Brain cholinesterase activity was measured following terminal sacrifice. Urinalysis was not performed.

Complete post mortem examinations were conducted on all animals. External surface, all orifices, cranial cavity, carcass, external and sectioned surfaces of the brain and spinal cord, nasal cavity and paranasal sinuses, the thoracic, abdominal and cervical tissues and organs were examined. At the completion of the dosing regimen, animals were killed and post mortem examinations conducted, with a number of tissues weighed (adrenals, brain, heart, lungs, kidneys, liver, spleen, testes or ovaries). Samples of tissues were collected and preserved, then examined using light microscopy. Lungs were examined for all animals, and the following tissues were examined for control and high-dose animals only: adrenals, aorta, bone and bone marrow, brain, eyes, heart, intestine (caecum, colon, duodenum, ilium, jejunum, rectum), kidneys, larynx, liver, lungs, lymph nodes, nasopharyngeal tissues, oesophagus, ovaries, pancreas, pharynx, pituitary, prostate, salivary gland, seminal vesicles, skin, spinal cord, spleen, stomach, testes, thymic region, thyroids and parathyroids, trachea, urinary bladder, uterus, gross lesions, tissue masses.

Results

At 10 ppb, a single female was killed in a moribund condition, but no other treatment-related mortality was observed at any dose level. A number of animals died accidentally following blood collection procedures. The moribund animal exhibited excessive lacrimation, anogenital staining, mucoid nasal discharge, and dried material on the fur. However, these signs, and a range of other findings including soft stools and chromodacryorrhea were seen in many animals at all dose levels including controls. These effects were not considered to be related to treatment, but probably occurred as a result of the nose-only exposure tubes used in this study. Ophthalmoscopic examinations did not reveal any ocular effects that could be related to treatment.

No treatment-related changes in group mean body weights were seen during the study, with weight gain being similar in control and treated groups. A slight increase in body weight was seen in treated males, but this finding could not be considered to be toxicologically significant. Food consumption in treated groups was occasionally lower than control values, but the changes were slight, and no consistent dose relationship was demonstrated, and so such findings were not considered to be related to treatment.

Haematological examination revealed a number of parameters that were statistically significantly different to controls, including increases in haemoglobin and mean corpuscular haemoglobin concentration (increased at all doses in males and females), erythrocyte count (increased at all doses in males and low dose females), haematocrit (increased in low and mid dose males and mid dose females), and platelets (increased in mid and high-dose males). However, the changes in these parameters were generally slight and were not consistently dose-related, and so were not considered to be treatment-related.

At the interim clinical chemistry examination, a range of findings were observed, with statistically-significant changes in some parameters, including increased alkaline phosphatase, lactate dehydrogenase, and plasma cholinesterase activities in males. Similar findings were not reported as frequently in females. These changes were not considered to be treatment-related, as there was no dose relationship, most of the findings were not seen at the high dose level, and some of the findings (increases, for example, in cholinesterase activity) were not toxicologically significant. Similarly, at final clinical chemistry examination, a range of parameters were significantly increased or decreased compared with controls. There was no consistent dose or time relationship demonstrated for these effects, and values at the high dose were generally similar to controls.

Plasma cholinesterase activity was inhibited by 23% at the high dose in males ($p<0.01$). Plasma cholinesterase activity was reduced in females at all dose levels, but there was no dose relationship for this effect, and activity was practically identical at low- and high-dose levels. Cholinesterase inhibition was seen at other doses in males, in a dose-related manner, but the inhibition was not statistically significant and the change was less than 20% compared with controls. In high-dose males, the inhibition of cholinesterase activity was considered to be treatment-related. No other changes in clinical chemistry parameters were considered to be treatment-related.

On occasion, statistically-significant changes in absolute organ weights, and organ weights relative to brain weight or body weight, were observed in treated animals. These effects were not consistently related to dose, and the magnitude of such changes relative to control values was not great. These effects were attributed to increases in body weights in high-dose animals, and were not considered to be

toxicologically significant.

No treatment-related findings were observed following pathological examination. Instances of a range of findings were reported, including minimal to moderate chronic interstitial pneumonia and bilateral degeneration of testicular germinal epithelia, but such findings were seen in control and treated groups, with no dose relationship demonstrated. Histopathological examination did not reveal any findings that could be attributed to treatment.

The NOEL for this study was 10 ppb, based on a decrease in plasma cholinesterase activity in terminal sacrifice males at 20 ppb (0.28 mg/m^3). This effect was of marginal toxicological significance, and was not accompanied by any clinical signs of intoxication. No other treatment-related effects were demonstrated in this study, with body weights and other clinical chemistry parameters unaffected by treatment. Pathological examination did not reveal any neoplastic or non-neoplastic lesions that were associated with treatment. The absence of demonstrably frank toxicity at the high-dose level in this study makes it difficult to draw any conclusions regarding the subchronic inhalational toxicity potential of chlorpyrifos.

5.1.3 Dog

Blackmore RH (1968) Oral administration - dogs. Dursban. Final Report. Hazleton Virginia report: Project No. 174-115, dated November 13,1968. The Dow Chemical Company. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Young adult beagle dogs (source not stated; 2/sex/dose) were given daily doses of chlorpyrifos orally (by capsule; purity and source not stated), at doses of 0, 0.03, 0.10, 0.30 and 1.0 mg/kg/d for 90 days. Initial (days 0, 2, 4, 8 and 16) cholinesterase measurements for these animals were discarded (inaccurate) and all subsequent estimations (days 16, 32, 64 and 90) were performed using the delta pH method. A sixth group was initiated into the study to capture these initial cholinesterase values; these animals received 1.00 mg/kg/d for days 1-18, 0.03 mg/kg/d for days 43-58, 0.10 mg/kg/d for days 78-94, interspersed with control diet for days 19-42, 59-77 and 95-124 to allow cholinesterase to return to base level. Concerns that 0.03 mg/kg/d may not be demonstrated as a NOEL prompted the initiation of a seventh group which received 0.01 mg/kg/d for days 1-32 and control diet for days 33-45. At termination, gross necropsies were performed on all animals, brain weights recorded and plasma, RBC and brain cholinesterase values measured.

Reliable cholinesterase activity measurements in dogs in groups 1-5 (days 16, 32, 64 and 90) indicated that plasma cholinesterase activity was clearly inhibited (up to 70% inhibition) in a dose-related manner at doses of 0.03 mg/kg/d and above from day 32 onwards. RBC cholinesterase activity was marginally inhibited (12%) at 0.03 mg/kg/d, but clearly inhibited (up to 60% inhibition) in a dose-related manner at doses of 0.10 mg/kg/d and above from day 16 onwards. Brain cholinesterase activity was not altered by treatment at any dose. When Dursban was administered orally for 90 consecutive days to beagle dogs of both sexes, the LOEL for plasma cholinesterase inhibition was 0.03 mg/kg/d, the NOEL for RBC cholinesterase inhibition was 0.03 mg/kg/d based on a LOEL of 0.10 mg/kg/d, and the NOEL for brain cholinesterase inhibition was 1.0 mg/kg/d.
Comment: [The data recorded for animals in groups 6 and 7 were not considered to be reliable and

were not evaluated.]

Harling RJ, Barker MH, Buist DP, Crook D, Majeed S, Gopinath C, Dawe IS & Anderson A (1989) Chlorpyrifos oral toxicity study in Beagle dogs (Repeated daily dosage for 13 weeks) Huntingdon Research Centre Inc, UK, study completed 30 June 1988. HRC Report MBS 31/88999, 24 May 1989. Makteshim report R-4950. [Makteshim; Submission 11471; reference 25]

This study was conducted in accordance with GLP Standards of the US EPA (Title 40 CFR 160, 1983) OECD 1982, Japan MAFF (notification 3850, 1984), and UK Health, 1986. A QA audit statement was issued for the study. Technical chlorpyrifos (stated purity 95.8%; Makteshim; batch 489205) as a mixture in lactose (1 part chlorpyrifos : 99 parts lactose) was administered orally to purebred Beagle dogs (Interfauna UK; 4 animals/sex/dose) by gelatin capsule once daily for 13 weeks, at doses of 0 (control), 0.01, 0.22, and 5 mg/kg/d. Control animals received lactose (500 mg/kg/d). Batches of the test mixture were analysed for homogeneity and purity. Animals were housed individually, and each received 400 g of standard dry diet daily.

During the working day, all animals were routinely checked for clinical signs. Body weights were determined weekly, food consumption was measured daily, and ophthalmoscopic examinations were performed prior to the commencement of dosing, and again during week 13 of dosing. Blood samples were collected from all animals prior to treatment and again during weeks 6 and 13 of dosing for haematological examination.

The following haematological estimations were performed: packed cell volume (PCV), haemoglobin (Hb), red cell count (RBC), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), total white cell count (WBC total), platelet count, reticulocyte count, differential WBC count, cell morphology, prothrombin time, activated partial thromboplastin time.

Clinical chemistry parameters were measured, namely: total protein, albumin, globulin, urea, creatinine, sodium, calcium, inorganic phosphorous, chloride, cholesterol, alkaline phosphatase, total bilirubin, glucose, alanine aminotransferase, aspartate aminotransferase, plasma cholinesterase activity, erythrocyte cholinesterase activity. Cholinesterase activity was measured between 3 and 24 h after dosing.

Urine samples were collected at the same intervals as blood samples, and the following parameters were measured: volume, pH, specific gravity, protein, glucose, ketones, bile pigments, urobilinogen, haem pigments, epithelial cells, polymorphonuclear leucocytes, mononuclear leucocytes, erythrocytes, organisms, renal tubule casts, other abnormal constituents.

At the completion of the dosing regimen, animals were killed and post mortem examinations conducted, with macroscopic examination conducted on a number of tissues (adrenals, brain, heart, lungs, kidneys, liver, pancreas, pituitary, spleen, testes or ovaries, thymus, thyroids and parathyroids, uterus or prostate). Brain cholinesterase activity determinations were made after autopsy. Samples of tissues were collected and preserved, then examined using light microscopy, namely: adrenals, alimentary tract, aorta, brain, eyes*, femur and articular surface*, gall bladder, heart, kidneys, liver, lungs, lymph nodes, mammary gland*, ovaries, pancreas, pituitary, prostate, salivary gland, sciatic nerve, skeletal muscle*, skin, spinal cord*, spleen, sternum, testes, thyroids and parathyroids, tongue*, trachea, urinary bladder, uterus,

vagina*, any tissue showing macroscopic abnormalities. (* preserved but not processed)

Results

There were no unscheduled deaths during the study. Clinical signs consisted of vomiting and liquid faeces in a small number of animals during the study. These findings were observed in animals from all groups, including controls, and were even seen prior to daily test material administration, on occasion. There was no clear relationship with the dose of test material administered, and the incidence of these effects was small, so they were not considered to be of toxicological significance.

There was a reduction in group mean body weights in males and females in the high-dose group, while group mean body weights in other treated animals were similar to controls. There was considerable intra-group variability in individual body weights, including controls, and high-dose males recorded a lower group mean weight than other groups even before test material administration commenced. Subsequently, statistical analysis did not reveal any treatment-related effect on body weight gain during the study. However, given that the animals of both sexes failed to gain weight at 5 mg/kg/d, it was considered that this effect was related to treatment. No effect on body weights was seen at 0.22 mg/kg/d.

During the treatment period, food consumption was reduced in males and females by 2-3% compared with controls. This finding was not considered to be biologically significant, as there was considerable variation within groups, and the change compared with controls was not large. Food consumption at other doses was similar to controls. Ophthalmoscopic examination did not reveal any findings that were considered to be treatment-related.

Haematological examination revealed a number of findings in treated groups that were statistically significantly different to controls, but none of these changes were consistently dose dependent, nor were the effects dependent upon the length of administration of the test material. These findings included increases in leucocyte and total WBC count, decrease in prothrombin time, and decrease in RBC count. These sporadic findings were not considered to be treatment-related.

Biochemical examination revealed statistically-significant decreases in albumin at all test dose levels in males and females at week 6, and in high-dose males at week 13. However, these effects were not dose or time dependent, the change compared with controls was small, and similar findings were also seen in males at all dose levels prior to initiation of treatment, and so this effect was not considered to be treatment-related. Similarly, some group mean globulin and electrolyte values were either increased or decreased on a number of occasions, but no consistent dose dependency was demonstrated, and these findings were not considered to be related to treatment.

In the urinalysis examination, no findings were considered to be related to treatment. In some isolated instances, some parameters were statistically significantly different to controls, but these findings were sporadic and considered to be incidental to treatment.

Plasma cholinesterase: Statistically-significant, dose-related decreases in plasma cholinesterase activity were observed in males and females at doses of 0.22 and 5.0 mg/kg/d. At these doses, enzyme inhibition

was as high as 52% and 74%, respectively, compared with concurrent controls, in females at 3 h post dosing on week 1. During the study, plasma cholinesterase inhibition was as high as 85% in males and females (week 6). At 0.01 mg/kg/d, plasma cholinesterase inhibition did not reach statistically or biologically significant levels in males during the study. In females, plasma cholinesterase activity was reduced by as much as 24% compared with concurrent controls at weeks 6 and 12, with this effect reaching statistical significance ($p<0.05$; Williams' Test) at week 6. As such, plasma cholinesterase was inhibited at all test dose levels in females, and there was no NOEL for this effect in this study.

Plasma cholinesterase - percent inhibition compared with concurrent controls at 3 h and 24 h post-dosing

Doses in mg/kg/d	Week 1 -3 h	Week 1 -24 h	Week 6 -3 h	Week 6 -24 h	Week 12 - 3 h	Week 12 - 24 h
Males						
0.01	-	1%	4%	11%	14%	14%
0.22	39%**	33%**	55%**	45%**	63%**	42%**
5.0	73%**	69%**	85%**	74%**	78%**	70%**
Females						
0.01	2%	8%	24%*	19%*	24%	24%
0.22	52%**	42%**	64%**	52%**	67%**	52%**
5.0	74%**	64%**	87%**	73%**	84%**	75%**

* $p<0.05$ Williams' test
** $p<0.01$ Williams' test

Erythrocyte cholinesterase: Statistically-significant, dose-related decreases in erythrocyte cholinesterase activity were observed at 0.22 and 5.0 mg/kg/d. At the high-dose level, inhibition reached 85% of concurrent control values within 6 weeks. At 0.01 mg/kg/d, inhibition of erythrocyte cholinesterase activity did not attain biological or statistical significance, but by the week 12 determination, the inhibition had reached 18% of concurrent control values in females at this dose level. The NOEL for erythrocyte cholinesterase was 0.01 mg/kg/d in males and females.

Erythrocyte cholinesterase - percent inhibition compared with concurrent controls at 3 h and 24 h post-dosing

Dose in mg/kg/d	Week 1 -3 h	Week 1 -24 h	Week 6 -3 h	Week 6 -24 h	Week 12 - 3 h	Week 12 -24 h
Males						
0.01	4%	2%	6%	1%	4%	10%
0.22	16%	10%	34%**	33%**	36%**	46%**
5.0	38%**	40%**	85%**	81%**	83%**	85%**
Females						
0.01	12%	10%	15%	9%	17%	18%
0.22	14%	15%	31%**	24%*	38%**	36%**
5.0	29%**	30%*	86%**	85%**	81%**	84%**

* $p<0.05$ Williams' test
** $p<0.01$ Williams' test

Brain cholinesterase: Brain cholinesterase activity was inhibited by 46% in males and females at the high-dose level, compared with concurrent controls. No statistically or biologically significant decreases in brain cholinesterase activity were observed at other dose levels. The NOEL for brain cholinesterase inhibition was 0.22 mg/kg/d.

Pathology: At macroscopic examination, one male and one female at 5 mg/kg/d had thickening of the duodenal and/or jejunal mucosal wall, and the male had raised red foci on the mucosal surface throughout the duodenum. The isolated nature of these findings were such that they cannot be attributed to treatment. Other findings reported following macroscopic examination were isolated in nature, and seen in both control and treated animals, and were not considered to be treatment-related. Group mean organ weights were within normal limits, and were similar in control and treated groups.

Histopathological examination was generally unremarkable. At 5 mg/kg/d, a single female had a area of papillomatous hyperplasia (pyloric) in the stomach, and one male and one female had thickened muscle coat in the duodenum. These findings were not common, but due to the isolated nature of these effects, it was not possible to attribute them to treatment.

Under the conditions of this study, there was no NOEL for inhibition of plasma cholinesterase activity, with biologically and statistically-significant reduction of activity seen in females at doses of 0.01 mg/kg/d and above. In males, plasma cholinesterase activity was reduced at doses of 0.22 mg/kg/d and above. Erythrocyte cholinesterase activity was inhibited in males and females at 0.22 mg/kg/d and above, with an NOEL of 0.01 mg/kg/d for this effect, and brain cholinesterase activity was inhibited at 5 mg/kg/d, with an NOEL for this effect of 0.2 mg/kg/d. Pathological examination did not reveal any macroscopic or microscopic findings that could be attributed to treatment.

Anon (1964) Results of 93-day dietary feeding studies of 0,0-diethyl 0-3,5,6-trichloro-2-pyridinol phosphorothioate in hounds. Dow Chemical Co., Biochemical Research Laboratory, Midland, Michigan, USA, dated 15 January, 1964. [Dow; Submission 11462, reference 69]

Beagle dogs (4/sex in control groups; 2/sex in treatment groups; approximately 7 months old; source unstated) were maintained on diets (Wayne Laboratory Chow) containing chlorpyrifos technical (estimated purity 98%, DH Croope, reference OL 10A7-2-66) for 93 days. The homogeneity and stability of the test diets was not measured. The dietary concentrations were 0 (control), 0.002 or 0.006%. Food consumption information indicated that these dietary concentrations were equivalent to doses of 0, 0.8 or 1.8 mg/kg bw/d, respectively. Originally, the high dose level chosen was 0.2%, but the animals in this group did not accept their food at the beginning of the experiment, and the concentration was changed to 0.006% after 5 days. The 0.002% group originally started the study at 0.06% concentration, but this level was changed to 0.002% after 16 days, as the animals had developed gross cholinergic signs. An additional dose level (0.02%) was also used in this study, with an 0.02% (A) group receiving the test diet for 45 days, and then placed on a control diet for the remainder of the study due to gross cholinergic signs. The 0.02% (B) group was added to the study after 45 days (without previously being dosed, maintained at this dose for 27 days, then put on a control diet for 5 days because of clinical signs of toxicity, then returned to the test diet for the remaining two weeks of the study.

Animals were weighed weekly, and observed frequently for signs of toxicity. Food consumption was measured daily. Serum urea nitrogen, alkaline phosphatase, serum alanine aminotransferase and plasma, erythrocyte and brain cholinesterase determinations were conducted. All animals were fasted overnight and weighed before examination at autopsy. The heart, lungs, liver, kidneys, spleen, brain, and testes were removed and weighed. Portions of these organs, as well as spinal cord, peripheral nerve, pituitary, thyroid, parathyroid, adrenals, aorta, lymph nodes, thymus, oesophagus, stomach, small intestine, large intestine, pancreas, gall bladder, urinary bladder, skeletal muscle, ovary, and uterus were preserved, and prepared for histopathological examination.

Results

The dietary concentrations of test material (0.2 (A), 0.2 (B), 0.006, and 0.002% were equivalent to doses of 5.8, 3.4, 1.8, and 0.8 mg/kg/d, respectively, calculated for the periods that the animals received the test diets.

No details were provided on the incidence or severity of the "cholinergic signs" observed in animals at 0.02% (described as dilated and watery eyes, loose stools, vomiting, rough coats, laboured breathing and tremors of the legs and head), and at all dose levels, animals had reduced body weights by the end of the treatment period. No significant changes in organ weights, serum urea nitrogen, alkaline phosphatase or haematological values were observed in test animals at 0.006 and 0.002%.

Dogs given chlorpyrifos at 0.2% (A) had large decreases in plasma and RBC cholinesterase activity (90-100% inhibition) and were removed from treatment after 45 days. After 41 days of recovery, the plasma and RBC ChE activity had returned to 50-80% of pre-test levels. Similar large reductions in cholinesterase activity were also seen in the 0.2%(B) animals. At 0.006%, plasma cholinesterase activity was inhibited throughout the treatment period, while RBC ChE activity was also inhibited, but had begun to return to pre-test levels by the end of the study in some animals. Brain ChE was significantly inhibited in both females after 93 days at this dietary concentration.

Plasma and RBC ChE activity was also inhibited at 0.002%, and brain ChE activity was reduced in one female at this concentration.

Under the conditions of this study, significant plasma, RBC and brain cholinesterase activity reductions were observed in dogs given chlorpyrifos in the diet for 93 days, at doses from 0.8 to 5.8 mg/kg/d. The study design and level of reporting in this study do not make it suitable for regulatory purposes.

5.1.4 Monkeys

Coulston F, Golberg L, Abraham R & Benitz FK (1971) Final report on safety evaluations and metabolic studies on Dowco 179, Section I.E.1 (IN 151). Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany, New York. Report Date March 18, 1971. Sponsor: Dow Chemical USA. Unpublished [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Fourteen rhesus monkeys were divided into groups of 2M+2F, 2M+1F, 2M+1F and 2M+2F receiving, respectively, 0, 0.08, 0.40 and 2.0 mg/kg/d DOWCO 179 (purity not specified) in 1% aqueous gum

tragacanth by stomach tube for a period of 6 months. The test material was prepared weekly. Clinical observations were recorded daily and body weights monthly. RBC and plasma cholinesterase were recorded at 0, 1, 3, 5, 8, 16 and 24 weeks, and on 4 monkeys sacrificed at 12 weeks. Limited clinical chemistry and haematology were recorded at 8, 16 and 24 week. Brain cholinesterase and biphenyl hydroxylase activity were recorded for all animals at necropsy. Gross pathology was recorded for 1M+1F/dose after sacrifice at 24 weeks, and limited histopathology of liver and kidney was reported for 2, 1, 1, and 2 animals at 0, 0.08, 0.4 and 2.0 mg/kg/d, respectively.

Results

One animal at 2.0 mg/kg/d was found dead at 18 weeks due to infection unrelated to treatment. There were no treatment-related changes in body weight gain, food consumption, clinical observations, haematology, clinical chemistry or histopathological examinations. Plasma cholinesterase activity was depressed at all dose levels, erythrocyte cholinesterase activity was depressed at 0.4 and 2.0 mg/kg. There was no dose-related depression in mid-brain cholinesterase activity at either 12 or 24 weeks, although the single 2.0 mg/kg/d animal recorded 85% of the single control value at 24 weeks.

Mean Cholinesterase activity in monkeys

Dose (mg/kg/d):-	**0**	**0.08**	**0.40**	**2.00**
plasma 16 weeks	5.97	4.4 (26%)	1.65 (72%)	1.80 (70%)
plasma 24 weeks	4.40	3.8 (14%)	1.70 (61%)	2.00 (55%)
RBC 16 weeks	9.50	8.25 (13%)	6.5 (32%)	2.63 (72%)
RBC 24 weeks	8.63	7.55 (13%)	6.3 (27%)	5.35 (38%)

[1] numbers in brackets are percentage inhibition cf. control
(n = 2-3) receiving Dowco 179 (µmol/min/ml)

A LOEL of 0.08 mg/kg/d was seen for plasma cholinesterase inhibition at 16 weeks but this concentration was a NOEL at 24 weeks. A NOEL of 0.08 mg/kg/d was seen for RBC cholinesterase inhibition at 16 and 24 weeks and a NOEL of 0.4 mg/kg/d for brain cholinesterase inhibition. However, this study was not deemed adequate for regulatory purposes due to the low numbers (sometimes one) of animals tested at each point.

5.1.5 Chickens

Stevenson GT (1965) The effects of phosphorothoic acid : 0,0-diethyl-3-3,5,6-trichloro-2-pyridyl ester, K-44, 739-7 upon blood plasma cholinesterase in chickens. The Dow Chemical Company. Exp. No: 3-807-10, dated 18 May 1964, Report dated 11 January, 1965. [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

White leghorn chickens (number/group not clear) were given chlorpyrifos (purity/batch not stated) in drinking water at 1 ppb, 1 ppm and 100 ppm for up to 84 days. Another group commenced on 1000 ppm were terminated after one week due to weight loss. Animals (5/group) were sacrificed for plasma cholinesterase determination on each of days 7, 14, 21, 28, 31 and 84 days. Only plasma cholinesterase was reported, due to technical difficulties with the blood samples.

Significant inhibition (54% decrease) of plasma cholinesterase was recorded on day 84 at 100 ppm.

5.2 Subchronic Studies with Metabolites

5.2.1 Rat

Beatty SC (1964) Results of 90-day dietary feeding studies of 3,5,6-trichloro-2-pyridinol in rats. No study numbers found. Dated July 9, 1964. [Dow; submission 939 part 3 vol 1]

Rats (10/sex/group, 7-8 weeks old, strain not stated) were started on diets containing 0, 0.01, 0.03, 0.1, 0.3 or 1.0 % TCP (3,5,6-trichloro-2-pyridinol; 0, 100, 300, 1000, 3000, and 10000 ppm, respectively). Animals were weighed twice weekly for 4 weeks and weekly thereafter, while food consumption was only recorded for the first month. Haematological values (HCT, Hb, WBC) were obtained from 5 female rats at 0, 0.3 and 1.0 % doses at 90 days. At terminal sacrifice (90 days) selected organs were weighed from all groups and portions processed for histopathology (tissues but not groups were specified), and serum samples (all groups) processed for limited clinical chemistry (BUN, ALP). Differences from control values were calculated using the Fischer "t" test.

There were three deaths during the study at 0.01% (1M & 1F) and 0.1% (1F), but these were not considered to be related to treatment. There were no treatment-related effects at 0.01, 0.03 or 0.10% TCP on body weight gain, mortality, food consumption, haematology values, BUN, ALP, and average body and organ weights. Bodyweight gain was reduced in males and females at 1.0% TCP, resulting in some increases in relative organ weights in this group, although absolute organ weights were not altered. Food consumption was reduced in females at 1.0%. The author states that diuresis was observed in males and females at 0.3 and 1.0% throughout the study and that the 1.0% group also had dry bloody noses for the first month; however no clinical observations were provided. Based on bodyweight gain reduction in both sexes, the NOEL for TCP was 0.10% in the diet for 90 days (1000 ppm or approx. 50 mg/kg/d).

Comment: [This report was accompanied by an undated and unauthored pathology report from International Research and Development Corporation, which contained a summary of histopathological lesions seen in the "controls and D.1 dosage level" of a rat 90-day study identified as D63-38278-2. This report was incomplete (table 1 was missing), and inaccurate (dosage group D1 received 0.1%, but the quoted animal numbers correspond to 1.0 %), and the main study has no identifying number.]

Barna-Lloyd T & Szabo JR (1985) Sodium 3,5,6-trichloro-2-pyridinol: 3-month rat dietary toxicity study. The Dow Chemical Company, report code (TXT:K-065999-099), dated July 16, 1985 [Dow; submission 939 part 3 vol 1]

Fischer-344 (CRL) rats (15/sex/group) were fed 0, 10, 30 or 100 mg/kg bw/d sodium-TCP (3,5,6-trichloro-2-pyridinol; batch MM 730142, AGR-178247, 92.1% purity) in their diets for 91 days. Diets were prepared freshly every week and dietary stability of sodium-TCP was satisfactory. Clinical signs were recorded daily and food consumption and body weight were recorded weekly.

Blood was collected from 10 animals/group at 0 and 100 mg/kg/d on days 84 (M) and 85 (F), and additionally from all males on day 90 for a repeat estimation of prothrombin times only. The following

haematological estimations were performed: packed cell volume (PCV), Hb, red cell count (RBC), MCHC, MCV, total white cell count (WBC total), platelet count, differential WBC count, prothrombin time, activated partial thromboplastin time.
Clinical chemistry parameters were measured at scheduled necropsy, namely: BUN, AP and ALT. Urine samples were collected prestudy (day-2), mid-study (day 42) and pre-necropsy (day 89). The following parameters were measured: pH, specific gravity, protein, glucose, ketones, bilirubin, urobilinogen and blood.

At scheduled necropsy, fasted animals were killed and post mortem examinations conducted, with organ weights recorded for brain, heart, kidneys, liver, testes or ovaries and thymus. Samples of tissues were collected and preserved from control and high-dose animals, then examined using light microscopy, namely adrenals, aorta, bone and bone marrow, brain, caecum, cervix, coagulating gland, colon, epididymis, eyes, heart, kidney, liver, lungs, mammary gland, mediastinal tissue, mesenteric lymph nodes, mesenteric tissue, ovaries, oviduct, pancreas, parathyroid, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicle, skeletal muscle, skin, small intestine, spinal cord, spleen, stomach, testes, thymus, thyroids, tongue*, trachea, urinary bladder, uterus, vagina, any tissue showing macroscopic abnormalities (from all dose groups). Appropriate statistical methods were applied to all data collected.

Results

Dietary analysis revealed good agreement between targeted and achieved dosages. The dietary concentration was varied to allow for increasing body weight, and ranged from ca. 85, 250 and 825 ppm at day 0, through to ca. 160, 460 and 1540 ppm at day 84 for the 10, 30 and 100 mg/kg/d groups respectively. Food consumption values of the treated groups were the same as or higher than controls, especially in the 30 and 100 mg/kg/d males in the latter half of the study. Significantly increased bodyweight gains were observed in 30 mg/kg males from day 48 onwards, and at day 90 the values were 281, 294, 303 and 288 g for the 0, 10, 30 and 100 mg/kg/d male groups respectively.

Although no data were presented, the study authors state that a dose-related staining and matting of the perineal haircoat was seen throughout the study, often in 100 mg/kg females, sometimes in 30 mg/kg females, occasionally in 100 mg/kg males and rarely in 10 mg/kg females. The effect was thought to be treatment-related, but of unknown aetiology and toxicological significance.

There were no treatment-related changes in haematology values; a significant elevation of prothrombin time in high-dose males at day 84 was not apparent at the repeat assay time on day 90. Blood chemistry values were similarly unaffected by treatment, other than a dose-related trend to lower ALP values in treated males which achieved statistical significance at 100 mg/kg/d (107.9 vs 95.2 for 0 vs 100 mg/kg/d). Urinalysis parameters were normal, slight and variable differences in the urine specific gravity of 30 and 100 mg/kg males on day 89 not being considered significant.

Absolute and relative (to bodyweight) liver and kidney weights and absolute heart weights were significantly increased in 100 mg/kg males and relative liver and kidney weights were significantly increased in 100 mg/kg females. There were increases in absolute heart, liver and kidney weights and relative brain and testis weights for the 30 mg/kg males. While some of these changes were thought to reflect the increased bodyweight of the 30 mg/kg/d males, the relative liver and kidney weights were

affected in both sexes at the 100 mg/kg/d dose, and this probably reflects an effect of treatment. There were no gross or histopathological findings due to treatment.

Absolute (abs) and relative (rel) organ weights for male and female rats at necropsy

Dose (mg/kg/d)	Body Weight (g) M/F	Liver (abs) M/F	Liver (rel) M/F	Kidney (abs) M/F	Kidney (rel) M/F
0	255/157	7.05/4.30	2.76/2.74	1.94/1.26	0.76/0.80
10	267/158	7.52/4.33	2.82/2.73	2.05/1.27	0.77/0.80
30	275/159	7.78*/4.40	2.83/2.77	2.15*/1.27	0.78/0.80
100	263/153	7.66*/4.40	2.91*/2.88*	2.13*/1.28	0.81*/0.84*

* statistically significantly difference from control mean

Based on the increases in relative liver and kidney weights, the NOEL for sodium-TCP was 30 mg/kg/d in the diet for 90 days.

Comment: [The NOEL determination assumes that the staining and matting of the perineal haircoat in higher dose groups of sodium-TCP-treated rats and the increased bodyweight gain, food consumption and resulting increased absolute and relative organ weight particularly in the 30 mg/kg males were not of toxicological significance].

5.2.2 Dog

Copeland JR (1964) Results of 93-day dietary feeding studies of 3,5,6-trichloro-2-pyridinol in beagle hounds. The Dow Chemical Company, File T35.12-38278-3, signed July 29, 1964 [Dow; Submission 939, vol 2]

Six-month-old beagle dogs (2 animals/sex/group) were administered TCP (3,5,6-trichloro-2-pyridinol; 95.5%) in the diet at levels of 0, 26 and 80 mg/kg/d for a period of 93 days. The animals were observed "frequently", food consumption was recorded daily, and body weights weekly. Limited clinical chemistry (BUN, ALP and bromosulfophthalein retention) and haematology parameters (Hct, RBC, WBC, Hb, WBC diff) were measured pre-test and at week 11. At terminal sacrifice organ weights were recorded for lungs, heart, liver, spleen, kidney, brain and testes. Samples from these and other tissues were prepared for histopathological examination, but these results were not reported except for unusual findings. Additionally, while a full set of tissues were examined for controls, histopathology was limited to liver and kidney at 26 mg/kg/d, and liver only at 80 mg/kg/d.

Results

Serum alkaline phosphatase activity was elevated (2 to 4 times control) in both treatment groups. Haematological findings did not show any effect of treatment. Liver damage (focal lymphocytic infiltrate or swelling and coarse granularity of parenchymal cells) was observed in 1 control M, 1M and 1F at 26 mg/kg/d, and in 2M and 1F receiving 80 mg/kg/d TCP. Organ weight analysis was equivocal, with absolute liver weights decreasing in a dose-related manner in both sexes, while relative liver weights increased in males but not females. Testes weights were increased in males at both doses. Brain weights were unaffected by treatment in females but increased in males at both doses.

Absolute (abs) and relative (rel) organ weights* for male and female dogs at necropsy

Dose mg/kg/d	Body Weight M/F	Liver (abs) M/F	Liver (rel) M/F	Testes (abs)	Testes (rel)	Brain (abs) M/F
0	11.2/8.35	350/276	3.12/3.32	11.85g	0.11	78.3/75.6
26	9.55/6.90	325/231	3.38/3.34	17.10g	0.18	86.4/75.8
80	8.80/7.40	308/214	3.52/2.90	17.46g	0.20	82.2/78.3

* Average of two animals. Organ weights relative to body weight. Organ weights in grams. Body weights in kg.

This study was inadequate to establish clear NOELs for toxicity. However the liver effects in both sexes at both doses, and the brain and testes weight increases in males at both doses were considered toxicologically relevant, and 26 mg/kg/d was regarded as a LOEL for TCP in this study.

Comment: [This study has shortcomings in design, methodology and reporting. The relative lack of positive findings was of equivocal significance because of the small numbers of animals in each group and the incomplete histopathology examinations. Two dogs received a varying dose regime due to problems with palatability (0.6% for nine days, 0.2% for nine days, 0.4% for ten days, 0.2% for the last nine weeks). No data were evaluated for this group.]

Emerson JL & Gerbig CL (1970) 91 day toxicology study in beagle dogs treated with 3,5,6-trichloro-2-pyridinol. The Dow Chemical Company (Human Health R&D Center, report no.: 263, dated August 26, 1970). [Dow; Submission 939, vol 2]

Six-month old beagle dogs (3 animals/sex/group; Hazleton Research Animals, Indiana) were administered TCP (3,5,6-trichloro-2-pyridinol; ref: 238-11-112, purity not stated) in the diet at levels of 1, 3, 10 or 30 mg/kg/d of TCP for a period of 91 days, while 4 dogs/sex served as controls. The animals were observed daily, routine physical examinations were conducted initially and terminally, food consumption was recorded daily and body weights weekly. Clinical chemistry (BUN, ALP, AST, serum proteins, blood glucose, total bilirubin) and haematology parameters (clotting parameters, ESR, RBC count, Hct, Hb, WBC diff count, WBC count, reticulocyte count, MCH, MCHC, MCV) were measured on days -34, -18, 0, 14, 29, 59 and 91. Additionally, ALP, ALT and ASP were measured on day 36 for dogs in the 10 and 30 mg/kg/d groups. Urinalysis was conducted initially and terminally on all dogs, and at 1 and 2 months on the 30 mg/kg/d group. Urinalysis included determinations of colour, turbidity, pH, specific gravity, protein, ketones, bilirubin, blood, glucose and microscopic examination of sediment.

At terminal sacrifice, organ weights were recorded for heart, liver, spleen, kidney, adrenals, thyroids, brain, pituitary and gonads; these data and organ/brain weight ratios were stated to be statistically analysed (anova); however this analysis was not presented. Histopathology findings were stated to have been reported for livers of all animals, and the following organs/tissues from control and high-dose animals only: salivary gland, skin, spleen, stomach, testes, thyroid, trachea, urinary bladder, uterus, gross lesions, heart, kidneys, liver, lungs, mammary gland, mesenteric lymph node, muscle (skeletal), nerve, sciatic, oesophagus, ovaries, pancreas, pituitary adrenals, aorta, brain (3 levels), intestine (5 levels), epididymis, eyes, gall bladder.

Results

There were no unscheduled deaths. No data relating to clinical signs were presented; the study authors state that there were no overt signs of toxicity during the test period. The study authors state that emesis resulting from ascarid infection was observed in one animal from each treatment group; all dogs were wormed with piperazine citrate on days 37 and 41. Body weights and body weight gains were not affected by treatment. Haematology and urinalysis parameters were not affected by treatment. Clinical chemistry parameters were generally unaffected by treatment; however ALP, ALT and ASP were elevated (2-10 times control values) from day 14 or 28 in all animals at 30 mg/kg/d. One male at 10 mg/kg/d recorded elevated ALP, ALT and ASP throughout the study including pretest; this animal may have been developing hepatitis. Organ weights were generally unaffected by treatment; however adrenals in high-dose males were almost 20% lighter than controls, and liver weights in both sexes were slightly increased at 1 mg/kg/d, but then declined in a dose-related manner to 94% and 73% of control values in 30 mg/kg/d males and females respectively. Additionally, female organ weights included a column for testes weights which were presumed to be actually ovarian weights. These weights were highly variable ranging from 2.74-26.01 g, and possibly reflect the variable time of onset of sexual maturity in the female dogs.

Mean body, adrenal and liver weights in males and females at necropsy.

Dose (mg/kg/d)	Body Weight (kg) M/F	Adrenals (g) M/F	Liver (g) M/F
0	11.7/9.88	1.06/0.97	353/311
1	10.0/9.95	1.02/0.84	376/327
3	11.4 / 10.8	0.95 / 0.99	357 / 312
10	10.7 / 9.44	0.97 / 0.98	341 / 284
30	10.8 / 9.10	0.86 / 0.90	333 / 227

The presentation of gross pathology findings and microscopic findings was limited to a summary of unusual findings for each animal. There were no dose-related findings in either the gross or microscopic pathology findings presented. The only significant toxicity resulting from TCP intake in this study would appear to be liver effects evidenced by elevated ALP, ASP and AST, and decreased liver weight at 30 mg/kg/d. The NOEL for toxicity was 10 mg/kg/d.

6. CHRONIC TOXICITY

6.1 Mouse

6.1.1 Dietary Administration for 78 Weeks

Gur E, Nyska A & Waner T (1991) Pyrinex technical oncogenicity study in the mouse. Life Science Research Israel Ltd., Project MAK/106/PYR, completed 6 February, 1991. Final LSRI report undated. Makteshim Chemical Works report: R-4985. [Makteshim; Submission 11471; reference 30]

This study was conducted in accordance with test guidelines of the US EPA 83-2, and OECD 451, and in compliance with GLP standards 40 CFR Part 160. To investigate the carcinogenicity potential of Pyrinex technical (chlorpyrifos; stated purity 95.9%; Makteshim; batch 289318), the test material was

mixed in maize oil and incorporated into a commercially available powdered animal diet (Altromin 1321N) to produce dietary concentrations of 0 (control), 5, 50, and 250 ppm. These dietary levels were calculated to be equal to dose ranges of 0.7-1.1, 6.1-11.6, and 31.7-55.1 mg/kg/d in males, and 0.7-1.2, 6.6-12.3, and 33.6-61.7 mg/kg/d in females. All diets were prepared weekly, and the stability and homogeneity of the test diet was analysed in a trial mix. Checks for test material concentrations were made at approximately monthly intervals during the first 6 months of the study, and approximately bimonthly thereafter.

The test diets were administered to groups of mice (CD-1; Charles River UK; 3 weeks old on arrival; 64 animals/sex/dose) for at least 79 weeks, with terminal sacrifice undertaken during weeks 80-82. Animals continued to receive treated diets until scheduled necropsy. An additional 12 males/group were designated as protocol spares. These animals were treated in an identical manner to mice in the main groups, and were used as replacement animals when animals had to be replaced for reasons unrelated to treatment (eg. excessive aggression). All unused protocol spares were discarded after 10 weeks of treatment.

Each animal was weighed weekly for the first 13 weeks of the study, and fortnightly thereafter, while food consumption was measured weekly for 13 weeks, and monthly thereafter. Animals were examined daily for clinical signs of intoxication, and a careful examination, including palpation, was conducted weekly. Blood smears were prepared from all mice at 12 and 18 months for differential white cell counts (neutrophils, lymphocytes, eosinophils, monocytes, normocytes) and these were examined for control and high-dose animals only. Plasma, erythrocyte, and brain cholinesterase determinations were conducted using 5 animals/sex/dose at 9 and 18 months.

All animals were subjected to gross pathological examination, including the examination of all external surfaces, cranial, thoracic, abdominal and pelvic cavities, and carcass. A large number of organs and tissues from all animals were fixed for histopathological examination, with the following tissues examined microscopically from control and high-dose animals and all animals dying or killed during treatment, unless indicated by an *, in which case all dose groups were examined: abnormalities, adrenals, aorta, bone and bone marrow, brain, epididymides, eyes*, gall bladder, harderian glands, heart, intestine (caecum, colon, duodenum, ilium, jejunum, rectum), kidneys*, liver*, lungs*, lymph nodes, mammary glands, oesophagus, ovaries, pancreas, pituitary, prostate, salivary glands, sciatic nerves, seminal vesicles, skeletal muscle, skin, skull, spinal cord, spleen, stomach, testes, thymus, thyroids and parathyroids*, tongue, trachea, urinary bladder, uterus.

Results

Mortality: The survival rate in males was greatest at the high-dose, with percentage survivals of 48, 41, 58, and 77% at 0, 5, 50, and 250 ppm, respectively. In females, the corresponding survival rates were 75, 83, 73, and 72%, respectively. Thus, treatment with chlorpyrifos did not adversely affect survival in this study.

Clinical signs: A range of clinical signs were reported in all groups, including controls. A number of these findings, including ocular opacities, excessive lachrymation, and hair loss on the head, were observed at a higher incidence in the treated groups, and were probably related to treatment. However,

if the increased incidence in hair loss in males was due to the irritant nature of the test material, as proposed by the study authors, there was no proposed explanation for the absence of these findings in females. The incidence of other signs, including hypersensitivity to touch, proneness, and hunching, was low, and there was no relationship with dose, and so these effects were not considered to be related to treatment.

Incidence of clinical signs related to treatment

Observation incidence (64 animals/group)	**Males**				**Females**			
Dose (ppm)	0	5	50	250	0	5	50	250
Excessive lachrymation	2	4	7	16	0	2	0	6
Ocular opacity	0	3	8	10	3	1	3	10
Hair loss on head	0	2	4	9				
Hair loss around eyes	4	6	10	18				

Body weights: Group mean body weights were reduced in males at 250 ppm for the duration of the study, with this effect being highly statistically significantly different from controls ($p<0.001$) for weeks 1-13, and less so ($p<0.05$ - $p<0.001$) for the remained of the study. The body weights of males in this group were usually about 5% lower than controls, with this effect reaching a maximum reduction of about 10% at week 72. In females at 250 ppm, group mean body weights were statistically significantly reduced for weeks 1-3, with reductions of about 5% compared with controls. After the initial weight reduction at this dose level, the body weights in all groups of females were similar to controls. At doses of 5 and 50 ppm, no adverse treatment-related effect on body weights was observed.

Food consumption: Statistically-significant reductions in group mean food consumption was seen in males at 250 ppm during weeks 1-3 and on occasion during the study (10-15% reduction compared with controls), and at this dose level, the food consumption was generally lower than controls for the entire study (5-10% reduction). Similar reductions in food consumption were observed in high-dose females in the first two weeks of treatment, after which the food consumption was similar in all female groups. At 5 and 50 ppm, food consumption was unaffected by treatment. On occasion, statistically-significant decreases in food consumption were observed in males at 50 ppm, but these findings were isolated (weeks 6 and 21 only), and the magnitude of the effect was slight (<10% reduction compared with controls) and so these findings were not considered to be treatment-related.

Cholinesterase activity: Statistically-significant ($p<0.001$), dose-related decreases in plasma cholinesterase activity were seen in all treated groups of males and females at 42 and 78 weeks, with inhibition of activity compared with controls of about 50% at 5 ppm, and 95% or greater at 50 and 250 ppm ($p<0.05$ for males at 5 ppm at 78 weeks only). No NOEL was demonstrated for plasma cholinesterase activity in this study.

Erythrocyte cholinesterase determinations revealed inhibition of activity of over 20% at 42 weeks, and of about 30% at week 78 at 250 ppm, but at other dose levels, the erythrocyte cholinesterase determinations were variable, and considerable intragroup variation was observed. In addition, the erythrocytes were washed with saline before the cholinesterase determinations at 78 weeks, but this procedure was not followed at 42 weeks. The NOEL for RBC cholinesterase inhibition was 50 ppm.

Significant inhibition of brain cholinesterase ($p<0.001$ or $p<0.01$) was observed in males and females at 42 and 78 weeks at 250 ppm, with activity reduced by 80-86% compared with controls. At 50 ppm, decreases in activity of 43-47% were also seen in males at 42 and 78 weeks, and in females at 42 weeks only, but this finding reached statistical significance ($p<0.05$) only in the females. At 5 ppm, brain cholinesterase activity was reduced in males at 78 weeks (27% reduction), but this finding was not statistically significant, and was considered to be incidental to treatment. The NOEL for brain cholinesterase inhibition was 50 ppm.

Inhibition of cholinesterase activity: percentage inhibition compared with controls

	(n=5)	Male	Female	Male	Female	Male	Female
	Interval (weeks)	5 ppm		50 ppm		250 ppm	
Plasma	42	49%[c]	45%[c]	95%[c]	97%[c]	98%[c]	99%[c]
	78	49%[a]	50%[c]	95%[c]	96%[c]	98%[c]	98%[c]
Erythrocyte	42	1%	15%	11%	41%[b]	22%	23%
	78	-	+10%*	29%[a]	4%	31%[a]	29%[b]

	(n=5)	Male	Female	Male	Female	Male	Female
	Interval (weeks)	5 ppm		50 ppm		250 ppm	
Brain	42	8%	13%	43%	46%[a]	80%[b]	85%[c]
	78	27%	+13%*	47%	9%	86%[b]	84%[c]

[a] $p<0.05$; [b] $p<0.01$; [c] $p<0.001$ *increased activity cf. controls

Haematological examination did not reveal any treatment-related changes in parameters. No statistically-significant changes were observed in absolute organ weights, or organ weights relative to body weight.

Pathology: Macroscopic pathological examination of animals killed at terminal sacrifice revealed a slight increase in ocular opacities at 50 and 250 ppm in males and females, and this finding was attributed to the irritant qualities of the test material. In high-dose males, an increase in lung nodules was observed, with an incidence of 5/31 (16%), 4/26 (15%), 2/37 (5%), and 14/49 (28%) at 0, 5, 50, and 250 ppm, respectively. These nodules were histologically diagnosed as bronchioalveolar adenomas or carcinomas, and were within historical control ranges for these lesions. In addition to these lesions, a large range of lesions were observed in control and treated animals, with findings noted in most organs and tissues at a low incidence, and no consistent dose relationship was demonstrated for these effects. As such, they were not considered to be related to treatment.

Histopathological examination did not reveal any statistically-significant increase in the incidence of neoplasms in treated animals compared with controls. On occasion, there was a slight increase in the incidence of some neoplasms in treated groups, but there was generally no consistent dose relationship observed for these findings, and/or the incidence fell within historical control values, or the findings were sporadic. Two sets of historical control data were reported for neoplasm incidence. The historical controls values cited in this report were less than ideal, however, as one report (Control A) used the control values obtained from 24-month studies, and the second was a published report on spontaneous neoplasms from CD-1 mice from a range of testing laboratories. The use of background incidence from

24-month studies would be expected to increase the range of spontaneous tumours compared with the 78 week administration used in this study. In addition, the published paper (Control B) reports the tumour incidence as a percentage of the animals examined, while the historical data from the 24 month study, and the tumour incidence in this study, refer to tumour incidence as a percentage of the number of organs examined.

Incidence (percentage) of various tumour types in this study, and the historical control data for these lesions

Lesion	Control A	Control B	0 ppm	5 ppm	50 ppm	250 ppm
Hepatocellular adenoma and carcinoma - males	62/482 (12.9%) range (1.4-31.8%)	316/891 (15.5%)	4/59 (6.8%)	9/59 (15.2%)	8/59 (13.6%)	4/59 (6.8%)
Alveolar-bronchiolar adenoma and carcinoma - males	36/480 (7.7%) range (0-35.3%)	298/891 (33.4%)	7/59 (11.9%)	10/59 (17%)	3/59 (5.1%)	13/59 (22%)

Lesion	Control A	Control B	0 ppm	5 ppm	50 ppm	250 ppm
Malignant lymphoma - males	26/353 (7.4%) range (0-12.5%)	72/891 (8.1%)	1/59 (1.7%)	1/59 (1.7%)	5/59 (8.5%)	2/59 (3.4%)
Fibrosarcoma - females	2/432 (0.5%) range (0-1.4%)	8/890 (0.9%)	0/59	0/59	0/59	1/59 (1.7%)
Non-glandular stomach epithelium: squamous cell carcinoma - females	-	1/890 (0.1%)	0/59	0/22	0/24	1/58 (1.7%)

A range of non-neoplastic lesions were observed in treated males and females. The majority of these lesions were not attributed to treatment, as they occurred in controls at a similar incidence as treated groups, and/or there was no dose relationship demonstrated in their incidence. For a number of lesions, however, the possibility that the effects were related to treatment could not be eliminated, including effects observed in the liver (centrilobular hepatocytic fatty vacuolation, slight subchronic pericholangitis) and eyes (keratitis) at the high-dose level. The increase in incidence of hepatocytic fine and large fatty vacuolation (slight, moderate, and marked) in males was highly significant ($p<0.001$), but no such increase in incidence was observed in females. Similarly, the increased incidence of slight subchronic pericholangitis was limited to high-dose males ($p<0.05$), with the incidence in females being similar in control and treated groups. The incidence of keratitis was highly significant ($p<0.001$) at the high-dose level in the sexes combined, and to a lesser extent in males only ($p<0.05$), but the lesion was only reported in 3 females at the high-dose.

Incidence of some non-neoplastic lesions over the study period.

	0 ppm	5 ppm	50 ppm	250 ppm

	0 ppm	5 ppm	50 ppm	250 ppm
Hepatocytic fine and large fatty vacuolation - centrilobular, slight: males	2/59	3/59	1/59	10/59
Hepatocytic fine and large fatty vacuolation - centrilobular, moderate: males	0/59	0/59	2/59	3/59
Hepatocytic fine and large fatty vacuolation - centrilobular, marked: males	0/59	0/59	0/59	1/59
Histiocytic proliferation - multiple foci: males	3/59	0/59	1/59	6/59
Pericholangitis - subchronic, slight: males	11/59	8/59	7/59	18/59
Keratitis - diffuse, acute: males	0/54	0/54	1/57	0/57
Keratitis - focal or diffuse, subchronic: males	1/54	0/54	3/57	6/57
Keratitis - focal, chronic: males	0/54	0/54	0/57	1/57
Keratitis - diffuse, acute: females	0/57	0/58	0/57	0/58
Keratitis - focal or diffuse, subchronic: females	0/57	0/58	0/57	3/58
Keratitis - focal, chronic: females	0/57	0/58	0/57	0/58

Summary: In this study, the dietary administration of technical chlorpyrifos to CD-1 mice for up to 78 weeks at doses of 5, 50, and 250 ppm did not have any adverse effect on survival, and there were no increases in neoplastic findings which could be attributed to treatment at any dose level. Treatment-related clinical signs (ocular opacities, excessive lachrymation, hair loss on head) were observed at 250 ppm (approximately 31.7 mg/kg/d), and reductions in body weights and food consumption were also seen at the high-dose level. Plasma cholinesterase activity was inhibited at doses of 5ppm (0.7 mg/kg/d) and above in this study, and no NOEL was demonstrated for this effect. Brain and erythrocyte cholinesterase activities were inhibited at 50 ppm (6.1 mg/kg/d) and above, and the NOEL for these endpoints was 5 ppm (0.7 mg/kg/d). Non-neoplastic effects were observed in the livers of high-dose males (slight subchronic pericholangitis, histiocytic proliferation, and centrilobular hepatocytic fatty vacuolation), and in the eyes of high-dose males and females. No treatment-related lesions were seen at doses of 50 ppm (6.1 mg/kg/d) and below.

Based on the inhibition of plasma cholinesterase activity at all dose levels in this study, no NOEL could be established. The LOEL for plasma cholinesterase inhibition was 5 ppm (0.7 mg/kg/d).

6.1.2 Dietary Administration for Two Years

Warner SD, Gerbig CG, Strebing RJ & Molello JA (1980) Results of a two-year toxicity and oncogenic study of chlorpyrifos administered to CD-1 mice in the diet. The Dow Chemical Company, report dated March, 4, 1980. [Dow; Submission 238, A3162/5, box 43]

Mice (56/sex/group, CD-1 strain from Charles River, Michigan) were maintained on diets containing 0, 0.5, 5 and 15 ppm of chlorpyrifos (99.6%) for 105 weeks. The animals were observed daily for clinical signs, body weights recorded monthly, and daily food consumption calculated from a 3 or 4-day measurement obtained once a month. Gross pathological examination was performed on all unscheduled and scheduled deaths. This procedure recorded organ weights for brain, testes, heart, kidneys and liver, as well as collection of the standard tissues (as listed in Appendix II) with some exceptions (aorta, optic nerve, Harderian glands, sections of the head, rectum and vagina). Sections were prepared of all the collected tissues from all animals for histopathology. Haematology was limited to blood smears from tail

vein collection at terminal sacrifice.

Results

Body weights, absolute and relative organ weights were generally not significantly affected by treatment. The mean body weight of all treated groups of female mice was greater (up to 13%) than the control mean from day 84 onwards, although food consumption was comparable between all groups. Consumption of the test compound initially approximated 0.1, 1 and 3 mg/kg/d; however, higher than expected bodyweight gains rapidly decreased this level, such that at month 4, the high-dose male and female intake was 2.02 and 2.68 mg/kg/d respectively. Over the entire treatment period the mean chlorpyrifos intake was approx. 0.05, 0.5 and 1.5 mg/kg/d for both sexes.

Mortality was also generally unaffected by treatment. Treated males had slightly higher survival rates than controls (final survival rates: 39%, 46%, 50% and 48% for control, 0.5, 5 and 15 ppm respectively), whereas 15 ppm females were lower than controls only at the highest dose, (final survival rates: 46%, 59%, 46% and 38% for control, 0.5, 5 and 15 ppm respectively).

Clinical signs were presented in the form of a general summary of physical examinations only, with no behavioural observations; individual data were not presented. There were no clinical signs that indicated an effect of treatment. Gross pathology was reported in the form of a general summary of gross necropsy observations only; individual data were not presented. There were no findings that indicated an effect related to treatment. Histopathology examinations were reported as a summary table with no indication of severity of lesions or abnormalities; individual data other than positive findings were not presented. A significant increase was observed in the incidence of spindle cell hyperplasia of the adrenal gland (both sexes) in animals ingesting 0.5 ppm chlorpyrifos and in males only from the group ingesting 5 ppm chlorpyrifos. This finding was not considered to be treatment-related as there was a high background incidence in aging mice, and there was no dose-response relationship. Sciatic nerve preparations recorded vacuolation in males and females of 2/56, 3/54 in controls, 5/54, 8/55 at 0.5 ppm, 2/56, 2/52 at 5 ppm, and 6/52, 8/53 at 15 ppm. The combined incidence for this effect was 5/110 (4.5%), 13/109 (11.9%), 4/108 (3.7%), and 14/105 (13.3%), respectively, at 0, 0.5, 5, and 15 ppm. Overall, there were no histopathology findings that indicated an effect of treatment.

Neoplastic findings were presented as a summary table of incidence, a time-to tumour table and as individual findings. The lung, liver, lacrimal gland, mammary gland, skin and organs of the lymphoreticular system were the most frequent sites of proliferative lesions in both treated and control mice. The incidences of alveologenic adenomas and hyperplastic nodules in the liver were significantly elevated in the intermediate (5 ppm) male group, but not in any other treatment group. These findings were considered to be incidental. No haematology data were presented.

No NOEL was established for chronic effects due to study deficiencies. There was no clear evidence of treatment-related carcinogenicity during this study. This study was considered to be barely adequate for assessment of carcinogenicity and inadequate for chronic toxicity assessment, as it had some serious shortcomings in design, methodology and data collection. No clinical pathology was performed, including no measurement of cholinesterase activity. No clinical signs other than physical examination were reported. Individual data was not presented for several parameters including histopathology. Intestinal parasites (nematodes) were present in all groups.

6.2 Rat

6.2.1 Dietary Administration for Two Years

McCollister SB, Kociba RJ & Humiston CG (1971a) Results of two-year dietary feeding studies on Dowco 179 in rats. The Dow Chemical Company, report No.: NBT35.12-44793-21), dated September 20, 1971. [Dow; Submission 238, A3162/5 B43]

Rats (Sherman, 25/sex/dose in main group) were fed Dowco 179 (chlorpyrifos, 97.2%, Lot No. CP523-CD235C) at 0, 0.01, 0.03, 0.1, 1.0 or 3.0 mg/kg bw/d (ppm dietary levels not provided) in their diet for 2 years. Supplementary animals (at each dose level) were included to enable interim sacrifices to be carried out as detailed below.

Group Designation	Sacrifice interval: tissues collected	Number of rats/sex/dose
A	Primary: 2-year feeding (blood and brain ChE)	25
B	1-week ChE: blood	5
C	1-month ChE: blood	5
D	3-month ChE: blood	5
E	6-month ChE: blood and brain	5
F	9-month ChE: blood	5
Group Designation	**Sacrifice interval: tissues collected**	**Number of rats/sex/dose**
G	12-month ChE: blood and brain	6
H	12-month necropsy	5
I	12-month, 7-8 week recovery, necropsy ChE: blood and brain	7
J	18-month ChE: blood and brain	7
K	18-month necropsy	7

Clinical signs were recorded “frequently”, body weights were recorded twice weekly for the first 4 weeks, weekly for months 2-6, and every two weeks for the remainder of the 2 years. Food intake was recorded on group A continuously during the first 3 months, and one week in every four thereafter. Clinical pathology investigations included: haematology (PCV, Hb, RBC, WBC) and urinalyses (solids, pH, albumin, sugar, occult blood, ketones) on 5 rats/sex from the 0, 1.0 and 3.0 mg/kg/d group-A; serum levels of BUN, ALP and SGPT were measured in all rats at the 12- and 18-month sacrifices, and in all male survivors and 4-5 females at 24 months. Cholinesterase activity (pH-Stat method) was determined on blood (plasma and RBC) and brain samples as detailed in the above table. Necropsies of rats in groups A, H, I, K were conducted in a standard manner, and recorded organ weights for brain, heart, liver, kidney, spleen and testes. Portions of these organs and a standard set of tissues were preserved for histopathology. However, slides were scored only from the control and 3.0 mg/kg/d groups, except that sections from the 1.0 mg/kg/d animals were included at the 12 month necropsy. Some tissues from unscheduled deaths or from animals sacrificed when moribund were also examined histologically. Statistical evaluations of data were limited to the Students “t” test.

Results

Body weights (data presented as graphs only) were generally not significantly affected by treatment. The mean body weight of all treated groups of male and sometimes female rats was generally slightly greater (up to 10%) than the control mean from month 2 onwards, although food consumption was comparable between all groups. Consumption of the test compound was stated to be regularly monitored and to closely approximate the nominal levels in both sexes. Organ weights were not affected by treatment.

Mortality was generally unaffected by treatment. Treated males had slightly higher death rates than females (final male survival rates: 40%, 36%, 24%, 28%, 52% and 40% for control, 0.01, 0.03, 0.1, 1.0 and 3.0 mg/kg/d, respectively), whereas females recorded survival rates of 44%, 56%, 56%, 52%, 68% and 40% for control, 0.01, 0.03, 0.1, 1.0 and 3.0 mg/kg/d respectively.

No data detailing the recorded clinical signs was presented; the study authors stated that "No changes in appearance or demeanour or signs of toxicity were observed grossly in any of the rats. No evidence of a cholinergic response was noted at any time."

Clinical chemistry: Clinical pathology results were presented as individual and summary data. There were no unequivocal treatment-related differences between control and treated groups recorded for the limited haematological parameters measured, nor for the urinalyses. Clinical chemistry examination found no effect of treatment on serum levels of BUN, ALP and SGPT.

Cholinesterase activity measurements were presented as individual and summary data. Plasma cholinesterase was significantly decreased at 3.0 mg/kg/d at all assay times, ranging from about 20-40% inhibition in males, and 55-74% inhibition in females; at 1.0 mg/kg/d, plasma cholinesterase levels were less severely inhibited, but from 6 months onwards activity ranged between 18-38% inhibition (males) and 50-69% inhibition (females), when compared to controls, and these differences were statistically significant at most assay times. Plasma cholinesterase activities at 0.1, 0.03 and 0.01 mg/kg/d were slightly (males) or moderately (females) inhibited at one week, but were comparable to controls at all other assay times in males, whereas females showed variable inhibition at most assay times, and >20% inhibition at all doses at the 2 year assay, but these differences were not statistically significant at any assay time. The recovery animals (1 year treatment + 50 d control diet) showed only minor recovery of cholinesterase activity in males (still 18% inhibition versus 34% in non-recovery animals), but significant recovery in females (8% inhibition versus 64%).

RBC cholinesterase activity was severely inhibited in both sexes at the higher doses at all assay times. Inhibition at 3.0 mg/kg/d was severe (60-100% inhibition), only slightly less severe at 1.0 mg/kg/d (13-90% inhibition) and relatively unaffected at the other dose levels. Recovery animals showed full restoration of cholinesterase activity. Brain cholinesterase activity in both sexes was also significantly inhibited at all assay times by treatment at 3.0 mg/kg/d (30-53% inhibition), less inhibited at 1.0 mg/kg/d (3-16% inhibition), and comparable to controls at other doses. Recovery animals showed good restoration of brain cholinesterase activity.

Pathology: Gross pathology was reported in the form of a general summary of gross necropsy observations; individual data were presented for the unscheduled deaths only. There were no findings that indicated an effect of treatment. The limited histopathology examinations were reported as a

summary table with no indication of severity of lesions or abnormalities; individual data were not presented. The available histopathology data did not indicate any treatment-related effects.

Neoplastic findings were presented as a crude summary table of incidence in the primary 2-year feeding group A. There was no presentation of individual data and no details regarding time-to-tumour, severity, other pathology measures, and historical control incidence.

Summary: The NOEL for plasma cholinesterase inhibition was 0.1 mg/kg/d based on significant inhibition in both sexes at 1.0 mg/kg/d. The NOEL for RBC cholinesterase inhibition was 0.1 mg/kg/d based on significant inhibition in both sexes at 1.0 mg/kg/d. The NOEL for brain cholinesterase inhibition was 1.0 mg/kg/d based on significant inhibition in both sexes at 3.0 mg/kg/d. This study was considered to be inadequate for assessment of carcinogenicity, and also inadequate for assessment of chronic effects other than cholinesterase inhibition in a chronic study. The study had some serious shortcomings in data collection. The gross pathology and histopathology reports were inadequate and no clinical signs were reported. The study was inadequate to enable any assessment of possible carcinogenic effects.

McCollister SB, Kociba RJ & Keyes DG (1985) Supplement to original report titled "Results of two-year dietary feeding studies on Dowco 179 in rats." The Dow Chemical Company, report No.: NBT35.12-44793-21 Suppl), dated May 23, 1985 [Dow; Submission 238, A3162/5 B43]

This supplement provides some of the data identified as lacking/inadequate in the original study report (see preceding assessment). However the "in life" individual animal observations were very limited and do not include basic observations such as abnormal behaviour or gait. Individual body weight records, gross pathology findings and histopathology findings were included in this supplement; histopathology was limited and variable with only a small number of tissues examined consistently in each rat. The histopathology results were not presented as summary tables of incidence and severity. The conclusions of the evaluation of the original study remain unchanged by this supplement.

Crown S, Nyska A, Pirak M &Waner T (1988) Pyrinex technical. Oncogenicity study in the rat. Life Science Research Israel, Ltd. LSRI Report no: MAK/095/PYR, 18 February, 1988. Makteshim Chemical Works report no: R-4751. Guidelines OECD 451, USEPA 83-5, GLP [Makteshim; Submission 11471; reference 29]

Rats (Fischer F344, CRL UK.) were exposed to chlorpyrifos (Pyrinex technical, Makteshim, batch 58900.9, 96.1%) in the diet at 0, 0 (vehicle control), 0.2, 5 and 100 ppm (60/sex/dose) for two years. The vehicle control (vc) was maize oil at 0.04% w/w. Diets were prepared weekly and assayed regularly for stability and accuracy of dosing. Body weights were generally recorded weekly; food consumption was recorded weekly for 13 weeks and monthly thereafter. Clinical signs were recorded daily and palpation performed weekly. Cholinesterase measurements were performed pretest, and at weeks 14, 32, 45, 50 (interim sacrifice of 5M and 5F, includes brain ChE), 78 and 104 weeks (terminal sacrifice, includes brain cholinesterase for 10 M and 10 F), and generally on 10 rats/sex/dose. Haematology was performed on blood smears from 10 rats/sex from the 0(vc) and 100 ppm groups at months 12 and 18. Gross pathology was recorded for all animals at necropsy, including unscheduled deaths and early sacrifices, and this included removal of the eyes with the optic nerve and adnexa. This procedure

recorded organ weights for brain, testes, thyroids, adrenals, kidneys and liver, as well as collection of the standard tissues with some exceptions (Appendix II; exceptions: aorta, gall bladder, Harderian glands, head sections, smooth muscle, sternum, vagina and Zymbal's gland). All gross lesions were sectioned. Sections were examined from all the 0, 0(vc) and 100 ppm animals. Appropriate statistical tests were used to analyse the data.

Results

Mortality was not affected by treatment (males: 38, 55, 64, 51 and 65% survival; females 65, 87, 78, 75 and 87% survival for 0, 0(vc), 0.2, 5 and 100 ppm respectively. The incidence of clinical signs, and palpable masses (confirmed as neoplasms) was presented comprehensively, and displayed no treatment-related effects. Body weights in all the 0.2 and 5 ppm animals were comparable to controls throughout the study period. Body weights in 100 ppm males were significantly lower (ca. 5% depression) than controls at most times from week 3 through to week 94. Similarly, body weights in 100 ppm females were significantly lower (ca. 4%) than controls at most times from week 2 through to week 64. Food intake and food conversion ratios were unaffected by treatment. The achieved doses of the test compound rapidly declined during the first three months of treatment, and more slowly thereafter. During weeks 54-104 the achieved doses of the test compound in males ranged from 0.009-0.014, 0.224-0.291, and 4.81-6.18 mg/kg/d for 0.2, 5 and 100 ppm respectively. In females during the same period the achieved doses were 0.011-0.013, 0.278-0.325, and 5.83-6.82 mg/kg/d for 0.2, 5 and 100 ppm respectively. Mean consumption can be approximated to 0.012, 0.3 and 6 mg/kg/d for both sexes. Water intake (monitored in controls (vc) and high-dose animals only), was generally unaffected in males, but was consistently lower than controls in high-dose females.

ChE activity: Cholinesterase activity at 50, 78 and 104 weeks is shown in the table below expressed as a percentage reduction from the vehicle control mean.

Group mean % cholinesterase inhibition compared to vehicle controls[a]

	Plasma		**RBC**		**Brain**	
50 weeks	Male	Female	Male	Female	Male	Female
0	0	7	0[b]	31	0	35
0.2 ppm	1	4	0	42	0	14
5 ppm	15	51	0[b]	39	9	10
100 ppm	93	98	13	45	57[b]	80[b]
78 weeks						
0	0	3	27	0		
0.2 ppm	4	3	11	0		
5 ppm	28[b]	47[b]	0	0		
100 ppm	93[b]	97[b]	10	0		
104 weeks						
0	8	9	0	0	18	4
0.2 ppm	13	0	0	0	0	0
5 ppm	36	37[b]	17	11	0	0
100 ppm	95[b]	96[b]	34	18	58[b]	61[b]

a. Values the same as or higher than the vehicle control are recorded as 0 inhibition.
b. Significantly different from control $p<0.01$ or $p< 0.001$

The control values were occasionally higher or lower than the vehicle control values, but this variation was not considered to be of toxicological significance. Plasma cholinesterase activity recorded a clear dose response in both sexes at each sampling time, and the NOEL was considered to be 0.2 ppm, based on significant (>20%) inhibition relative to the control value at 5 ppm and above. RBC cholinesterase activity was not consistently inhibited at the 50 or 78 week sample times, but a dose relationship was evident at 104 weeks with males more affected than females. These differences were not statistically significant due mainly to the large standard deviations obtained. A conservative view would be that a NOEL for inhibition of RBC cholinesterase in both sexes can be set at 5 ppm based on toxicologically significant inhibition at 100 ppm; however the large variation in the data and the lack of statistical significance mitigate against an unequivocal finding, and it was considered inappropriate to set an NOEL for RBC cholinesterase inhibition in this study. Brain cholinesterase activity in both control groups declined >50% between weeks 50 and 104; brain cholinesterase activity was significantly inhibited at 100 ppm at both 50 and 104 weeks, and hence 5 ppm was considered to be the NOEL for inhibition of brain cholinesterase.

Leucocytes counts which were recorded for control (vc) and high-dose animals at weeks 53, 76 and 97 did not show any affects of treatment.

Pathology: Absolute organ weights at necropsy recorded decreased liver and kidney weights for high-dose males only, however when corrected for the lower body weights of these animals, there was no effect of treatment on relative organ weights.

Macroscopic pathology was comprehensive, and reported lesions typical of this age and strain of rat, with no effects of treatment apparent. Microscopic pathology examination was also comprehensive and included a detailed statistical analysis. The only observation that was unequivocally treatment-related was the incidence of changes in the eyes of high-dose females (cataracts and diffuse retinal atrophy), as shown below.

Eye abnormalities recorded by microscopic pathology

	0	**0 (vc)**	**0.2 ppm**	**5 ppm**	**100 ppm**
males: retinal atrophy-diffuse	4/60	0/60	3/23	1/28	3/60
females: retinal atrophy- diffuse	5/60	15/59[a]	9/60	5/58	24/60[a]
males: cataract	31/60	31/60	7/23	6/28	30/60
females: cataract	38/60	38/59	33/60	32/58	51/60[a]

a Highly significantly different from controls. $p<0.01$ or $p<0.001$

A variety of non-neoplastic and neoplastic lesions were recorded, and occasionally the incidence of these lesions displayed a positive trend with treatment. However the incidence of lesions was generally within the range of historical laboratory controls or within the range of published NTP values. There were no neoplastic lesions which recorded an unequivocal or statistically-significant dose relationship.

Summary: In this study the NOEL for plasma cholinesterase activity was 0.012 mg/kg/d (0.2 ppm), based on significant (>20%) inhibition relative to the control value at 0.3 mg/kg/d (5 ppm) and above. No treatment-related carcinogenic effects were seen at any doses. No NOEL was established for RBC

cholinesterase inhibition in this study due to data inadequacies. The NOEL for inhibition of brain cholinesterase was 0.3 mg/kg/d based on significant inhibition at the next highest dose of 6 mg/kg/d.

Young JT & Grandjean M (1988) Chlorpyrifos: 2 year dietary chronic toxicity-oncogenicity study in Fischer 344 rats. Lake Jackson Research Center, The Dow Chemical Company, Texas. Study ID: K-044793-079, dated December 23, 1988. GLP. [Dow; Submission 11462, reference 73] PMRA

The following study assessment report was obtained from the Pest Management Regulatory Agency of Canada (PMRA) under the auspices of the OECD Ad Hoc Exchange Program. The Canadian review was completed 14/1/93, and the PMRA evaluation is incorporated here with minimal textual changes. An independent assessment of the original data has not been conducted by Australian regulatory authorities. ***Australian regulatory conclusions and comments are enclosed in square brackets [ ].***

This study was conducted in compliance with the Good Laboratory Practice Standards of U.S. EPA (1983), Japan Ministry of Agriculture, Forestry and Fisheries (1984) and OECD (1982).

Test Material: Chlorpyrifos (DURSBAN*F insecticide, AGR 214637) with a purity of 98.5%. Supplied as white granular crystals by the Agricultural Products Department, Dow Chemical U.S.A., Midland, MI.

Test System: Four-week old Fischer-344 rats were purchased from Charles River Breeding Laboratory (Kingston, NY). The rats were allowed to acclimatise for approximately 2 weeks prior to the study.

The animals were housed singly in stainless-steel, suspended cages in a room designed to have controlled humidity (40-60%) and temperature (72± 5°F), and a 12-hour photocycle. Certified Rodent Chow $5002 (Ralston Purina Co., St. Louis, MO) and tap water were available *ad libitum*.

Dose Levels: Chlorpyrifos was administered to the rats in their diets at concentrations formulated to provide dose levels of 0 (control), 0.05, 0.1, 1 or 10 mg/kg bw/day.

Test diets were prepared once per week. Initial concentrations of chlorpyrifos in the diets were calculated from pretest body weights and feed consumption. Thereafter, the mean body weights and feed consumption data determined periodically over the course of the study were used to adjust the chlorpyrifos concentration in the diets to maintain constant dose levels on a mg/kg bw/day basis.

An initial premix (for the two highest doses of 1 and 10 mg/kg bw/day) was prepared by dissolving the test material in acetone and then adding the solution to ball-milled basal rodent chow. After mixing and evaporating the acetone, a 2nd premix of lower chlorpyrifos concentration (for the 0.05 and 0.1 mg/kg bw/day doses) was then prepared from the 1st premix by further ballmilling in rodent chow. The mixing-procedure used was found to result in homogeneous dispersion of test material in the feed samples. Chlorpyrifos had been shown to be stable in rodent chow for up to 42 days. Most test diets were prepared within ±20% of the target concentrations; only a few batches were found to be within ±25%.

Study Design: The rats were allocated to the various treatment groups via a computer-generated randomisation process. Sixty rats/sex/group were fed chlorpyrifos in their diets at 0 (control), 0.05, 0.1, 1, or 10 mg/kg bw/day for 24 months. Ten rats/sex/dose level were randomly designated at the start of the study for an interim sacrifice at 12 months. Interim bleedings for haematology, clinical chemistries, and plasma and erythrocyte cholinesterase activities were performed at 6, 12 and 18 months as well as at the 24-month terminal sacrifice.

Experimental animals were observed daily for mortality and signs of toxicity. All rats were given a careful clinical examination at least once weekly beginning after the 6th month. All animals were palpated for externally detectable masses at prestudy, prior to the 12-month interim kill, and monthly thereafter for the rest of the test period.

Body weights and feed consumption were determined weekly for the first 3 months and monthly thereafter. All rats were weighed but feed consumption was determined only for 20 rats/sex/group.

All clinical laboratory procedures scheduled for 6 and 12 months were performed on rats designated for the 12-month interim kill (10/sex/dose). Clinical laboratory tests at 18 and 24 months were done on rats designated for the terminal sacrifice. Blood samples for haematology and clinical chemistries were obtained by orbital sinus-puncture under light anaesthesia. Haematology determinations consisted of packed cell volume (PCV), haemoglobin concentration (HGB), erythrocyte count (RBC), total (WBC) and differential leucocyte counts, and platelet count (PLAT). Clinical biochemical determinations included urea nitrogen (BUN), alkaline phosphatase (ALP), alanine aminotransferase (ALT), glucose (GLUC), total protein (TPRO), albumin (ALB), globulin (GLOB) (calculated), creatine phosphokinase (CK), aspartate aminotransferase (AST), total bilirubin (TBIL), cholesterol CHOL), calcium (CA), phosphorus (PHOS), sodium (NA), potassium (K) and chloride (CHL).

Urine samples were obtained approximately 1-2 weeks prior to the scheduled sacrifice (at 12 and 24 months), and at 6 and 18 months from 10 rats/sex/group. Urine parameters measured included specific gravity, and a semi-quantitative estimate of bilirubin, glucose, ketones, occult blood, pH, protein and urobilinogen. Microscopy of a pooled sample was also conducted.

Plasma and erythrocyte cholinesterase activities were assayed in 10 rats/sex/group at 6, 12, 18 and 24 months. Cholinesterase activity was also measured in a 1/2 brain sample obtained at the 12-month (10 rats/sex/group) and the 24-month (20 rats/sex/group) scheduled necropsies.

All animals (interim and terminal sacrifices, unscheduled deaths and moribund kills) were necropsied and subjected to a complete gross examination. The brain, liver, kidneys, testes (males), ovaries (females) and adrenal glands were weighed. A complete list of organs and tissues were removed and preserved in neutral, phosphate-buffered 10% formalin for subsequent histopathological valuation. Histological sections of the formalin-fixed tissues from all rats in the control and top dose level groups were prepared, stained with H & E and examined microscopically. Histopathological examination of tissues from the three lower dose groups was limited to the liver, kidneys, adrenals and tissues with gross lesions at both sacrifice intervals. At the terminal sacrifice, the lungs, spleen, testes, pituitary and thyroid/ parathyroid were also microscopically examined.

Appropriate statistical tests were applied to the data generated by this study. Because numerous measurements were made and compared statistically within the same group of animals, the final interpretation of data should consider other factors such as dose-response relationships and biological significance as well as the results of statistical analyses.

Results:

Mortality: No treatment-related increase in mortality rate or significant change in the mortality pattern was observed at any dose level over the course of the study. There was no indication of excessive mortality due to a single cause in any dose group or a preponderance of unusual causes in treated animals.

Clinical Signs and Palpable Masses: There were no clinical signs for individual animals which suggest an adverse effect related to chlorpyrifos treatment. Mild, yellowish perineal staining was noted in 30-40% of the 10 mg/kg bw/day females during study months 6 - 20; but was not present in any significant amount in the high-dose males or other groups of animals.

There was no indication in the monthly palpation examination records of any excess of masses, unusual tumours or early onset in any of the treated groups compared with the control. The type and incidence of palpable cutaneous /subcutaneous "masses" noted were within the normal range of neoplasms for the Fischer-344 rat.

Food Consumption: Mean food consumption values were comparable among the control and chlorpyrifos-treated groups throughout the study period.

Body Weights: Group mean body weights and bodyweight gains for males at the highest dose level (10 mg/kg bw/day) were consistently lower than the respective control values throughout the study - body weight depression was 5% in Week 1, 7% in Week 29 and 7-9% thereafter. While group mean body weights in the 1 mg/kg bw/day males were statistically significantly decreased relative to controls at some measurement intervals, the body weight depression was <3.5% at all intervals and therefore judged to be of little biological significance. No treatment-related effects were observed at the 2 lower dose levels (0.05 and 0.1 mg/kg bw/day). Group mean body weights for females at the top dose level showed a slight (<3.5%), transient decrease compared with the controls - body weight depression was statistically significant during Weeks 8 - 53 of the study. No treatment-related effects were observed at the 3 lower dose levels (0.05, 0.1 and 1 mg/kg bw/ day).

Considering that food consumption was not reduced, the decrease in body weight of treated animals was interpreted as a direct effect of chlorpyrifos treatment.

Haematology: Results of the haematology evaluations at 6, 12, 18 and 24 months were generally comparable for all experimental groups (control and chlorpyrifos-treated). Incidence of statistically identified differences relative to controls was small and isolated. They were judged to be random variations not related to the treatment.

Clinical Chemistry and Electrolytes: Clinical chemistry and electrolytes analyses for treated rats identified a number of values at various dose levels and sampling periods as being statistically different

from the corresponding control means. However, the only parameters which exhibited consistent differences were: decreases in cholesterol, total protein and globulin values for males in the top dose group at 6, 12 and 18 months; decreases in cholesterol values at 6, 12 and 18 months, and globulin values at 6 and 12 months for the top dose females. These effects were considered to be treatment-related. All other statistical differences were either not dose-related or inconsistent over time as to occurrence or direction of change relative to control; therefore judged to be incidental and due to random variability.

Dose (mg/kg/d):-		**0**	**0.05**	**0.1**	**1.0**	**10.0**
Cholesterol (mg/dl)						
male	6 months	70.2	74.2	71.0	81.3	54.4*
	12	99.9	111	110	113	68.5*
	18	160	232	150	162	88.8*
	24	260	241	235	264	150
female	6	121	117	114	111*	97.6*
	12	148	159	154	167*	123*
	18	136	145	142	142	113*
	24	179	194	202	196	166
Total Protein (g/dl)						
male	6 months	7.89	7.93	7.94	8.01	7.50*
	12	6.63	6.69	6.52	6.62	6.11*
	18	7.02	6.80	6.57*	6.56*	6.40*
	24	6.52	6.33	6.32	6.38	6.55
female	6	7.55	7.59	7.42	7.35	7.30
	12	6.87	7.09	7.30*	7.19	7.10
	18	7.17	7.30	7.19	7.10	6.94
	24	7.04	7.32	7.13	7.21	7.08

* Statistical difference from control by the Dunnett's Test, alpha = 0.05, two-sided.

Dose (mg/kg/d):-		**0**	**0.05**	**0.1**	**1.0**	**10.0**
Globulin (g/dl)						
male	6 months	3.32	3.29	3.31	3.33	2.83*
	12	3.11	3.15	3.02	3.06	2.70*
	18	3.48	3.45	3.03*	3.12*	2.83*
	24	3.64	3.52	3.69	3.63	3.55
female	6	3.04	2.93	2.77	2.80	2.45*
	12	2.30	2.28	2.38	2.29	2.05*
	18	2.91	3.03	2.94	2.91	2.87
	24	3.30	3.36	3.34	3.45	3.48

* Statistical difference from control by the Dunnett's Test, alpha = 0.05, two-sided.

Cholinesterase Determinations:

Group mean cholinesterase (ChE) activities of treated rats, expressed as % of the respective control values for each of the sampling periods, are summarised in the following table.

Dose (mg/kg/d):-		0	0.05	0.1	1.0	10.0
Plasma ChE						
male	6 months	100	96.5	95.1	60.9*	44.2*
	12	100	94.5	97.8	28.6*	13.3*
	18	100	93.4	80.1	36.6*	22.5*
	24	100	92.4	85.5	40.0*	19.7*
female	6	100	97.9	91.0	34.6*	16.9*
	12	100	104	87.0*	13.8*	4.8*
	18	100	99.1	85.8*	30.4*	12.4*
	24	100	103	94.4	39.7*	17.9*
Erythrocyte ChE						
male	6 months	100	107	88.8	76.1*	75.6*
	12	100	107	93.2	67.4	63.1
	18	100	109	95.3	66.2*	71.0*
	24	100	105	108	86.5	73.5*
female	6	100	93.3	106	104	87.5
	12	100	88.2	109	82.2	59.5*
	18	100	114	99.7	78.0	81.9
	24	100	107	87.2	83.6	79.9
Brain ChE						
male	12 months	100	93.8*	93.1*	91.0*	42.1*
	24	100	102	100	103	44.3*
female	12	100	97.9	102	95.3*	38.9*
	24	100	101	100	96.2	42.9*

* Statistical difference from control by the Dunnett's Test or Wilcoxon's Test, alpha = 0.05, one-sided.

There was a consistent, statistically-significant depression of plasma ChE at each of the sampling periods in the 1 and 10 mg/kg bw/day males and females (range of inhibition compared with the control: 39-95%). Male erythrocyte ChE activities for the 2 top dose groups were also depressed - inhibition was 20-40% at all sampling periods except for the 1 mg/kg bw/day group at 24-month which was only 14%. Depression of female erythrocyte ChE in the 2 top dose groups was $\leq$22% at all sampling intervals with the exception of a 41% reduction at 12-month in the 10 mg/kg bw/day group. Brain ChE was measured at 12 and 24 months, and toxicologically significant (>10%) depression was noted in the 10 mg/kg bw/day group at both sampling periods - a 56-61% inhibition of brain ChE was measured among the top dose males and females.

[Australian conclusion: Plasma ChE was clearly inhibited in both sexes in a dose-related manner at all assay times. The NOEL was considered to be 0.1 mg/kg/d based on toxicologically and statistically-significant inhibition at 1.0 mg/kg/d. Erythrocyte ChE, at all assay times, was less severely inhibited than plasma ChE, with males being more sensitive than females. The dose relationship was generally weak, and the NOEL was considered to be 1.0 mg/kg/d based on toxicologically or statistically-significant inhibition in both sexes at 10 mg/kg/d. Brain ChE was clearly inhibited in a statistically and toxicologically significant manner at 10 mg/kg/d at both 12 and 24 months.]

While treatment-related depression of plasma and erythrocyte ChE activities was measured at and above 1 mg chlorpyrifos/kg bw/day, the no observed adverse effect level for ChE inhibition in this study was considered to be 1 mg/kg bw/day based on a biologically significant depression of brain ChE measured at the next higher dose level (10 mg/kg bw/day).

Urinalyses: Urine specific gravity of male rats treated with chlorpyrifos was slightly elevated at all sampling periods. The increase (over the control group) was statistically significant for the top dose group at the 6, 18 and 24-month intervals. Treated female rats showed a similar but milder elevation in urine specific gravity at the 6 and 12-month time periods, which was also statistically significant at the top dose level. In the absence of supportive renal histopathological data (significantly less renal pathology was observed in the top dose than in the control animals), this apparent dose-related change in urine specific gravity was not considered to be the result of a primary effect of chlorpyrifos treatment on kidney function, but was probably a secondary effect of altered body metabolism. All other urinary parameters in either males or females appeared to be unaffected by chlorpyrifos administration.

Organ Weights: Significant increases in absolute (+14-18%) and relative (+21- 23%) adrenal weights of the top dose males were observed at both sacrifice intervals. Significant increases in absolute (7%) and relative (9%) adrenal weights were also found in the top dose females but only at the terminal (24-month) sacrifice interval. No other changes in organ weights were considered to be treatment-related. A number of statistically-significant changes in organ weights were identified: an increase in relative brain weight of top dose males at both necropsies; a slight elevation in absolute brain weight of top dose females at 24 months; and decreases in absolute kidney and liver weights in top dose males at 24 months. None of these changes were interpreted as related directly to chlorpyrifos administration.

Gross Pathology: The gross pathological observations revealed no obvious pattern of changes in males and females of any dose group at either sacrifice interval suggestive of a treatment-related effect. All gross lesions were examined histologically. Final interpretation of these lesions was based on results of the histopathological examination.

Histopathology: There was good correlation in this study between lesions noted grossly and those examined histologically. Only 3 small lesions (in different tissues) could not be located for histopathologic examination. Significant histopathological findings that appeared to be treatment-related are summarised in the following table.

Dose (mg/kg/d)	**0**	**0.05**	**0.1**	**1.0**	**10.0**	**0**	**0.05**	**0.1**	**1.0**	**10.0**
Sex	M	M	M	M	M	F	F	F	F	F
12 month sacrifice (10sex/dose examined)										
ACFV[1] - very slight	9	10	10	10	2	0	0	0	0	0
ACFV - slight	1	0	0	0	8	0	0	0	0	0
Dose (mg/kg/d)	**0**	**0.05**	**0.1**	**1.0**	**10.0**	**0**	**0.05**	**0.1**	**1.0**	**10.0**
Sex	M	M	M	M	M	F	F	F	F	F
24 month sacrifice (50/sex/dose examined)										
ACFV - very slight	17	15	16	16	5	3	0	2	0	2
ACFV - slight	2	5	4	5	20*	2	3	0	0	3
ACFV - moderate	2	0	1	1	2	0	0	1	0	2
CPG[2] - slight	21	23	29	29	33	23	30	26	27	26
CPG - moderate	21	16	14	16	12	1	0	0	1	2

CPG - severe	7	7	4	4	1	0	0	1	0	2
MBHL[3] - slight	50	50	46	49	35*	47	44	42	46	32*

[1]ACFV = adrenal cortical fatty vacuolation
[2]CPG = chronic progressive glomerulonephropathy
[3]MBHL = multifocal biliary hyperplasia of liver
* Statistical difference from control by the Yates Chi-Square Test, alpha = 0.05.

At the 12-month sacrifice, the only remarkable histopathologic change was an exacerbation of the fatty vacuolization of the zona fasciculata in the adrenal cortex of male rats given the highest dose (10 mg/kg bw/day) of chlorpyrifos. The controls and males of the lower dose groups exhibited only a mild degree of adrenal cortical vacuolization and no incidence of vacuolization was observed in the females at any treatment level (including the controls).

At the terminal sacrifice, rats of the top dose group showed significant histopathologic changes in three organ systems that appeared to be treatment-related: an exacerbation of the fatty vacuolization of the adrenal cortex (males), a decrease in the severity of chronic progressive glomerulonephropathy (males), and a decreased incidence of mild biliary hyperplasia in the liver (males and females). As in the interim sacrifice, the higher degree of adrenal cortical vacuolization was not observed in the lower dose males nor in the females at any treatment level. This histopathologic alteration would appear to account for the noted increase in adrenal weight of the top dose males at both sacrifice intervals, and reflect chlorpyrifos-induced changes in adrenal function and/or lipid metabolism of these animals. The noted reduction in the incidence or severity of the common geriatric lesions such as chronic progressive glomerulonephropathy and mild biliary hyperplasia could be a "beneficial" result of the mild chronic stress associated with treatment in these rats, but the mechanism of action was unknown.

A number of other histopathological changes in various dose groups were identified as statistically different from their respective control group. These included: decreased "aggregates of reticuloendothelial cells" (significant at 0.5 and 1 mg/kg bw/day) and decreased "multifocal peliosis" (trend and pairwise significance at 0.1 and 10 mg/kg bw/day) in the male livers, a decreased incidence of "diffuse hyperplasia" of mammary glands in males (significant at 10 mg/kg bw/day), a decreasing linear trend for "focal cystic dilatation" of thyroid follicles in males (significant at 10 mg/kg bw/day), and an increase in "focal sinusoidal dilation" of the pituitary in females (significant at 0.1 mg/kg bw/day). These noted changes were non dose-related and interpreted as random variances not directly associated with the chlorpyrifos administration.

All other histopathological findings in the treated animals were reasonably typical and expected for aging Fischer-344 rats, and there was no evidence of exacerbation or altered incidence as a result of chlorpyrifos administration for up to 2 years.
There were no statistically identified linear trends or pairwise increases in any tumours for either males or females at any dose level. Furthermore, the total number of primary, benign or malignant tumours was comparable in all groups (Table 1).

TABLE 1

Chlorpyrifos: 2-year dietary chronic toxicity-oncogenicity study in Fischer 344 rats

Sex	**M**					**F**				
Dose	0	0.05	0.1	1	10	0	0.05	0.1	1	10

(mg/kg/d)										
rats examined	**50**					**50**				
total animals with primary tumours	50	49	50	50	50	44	39	38	38	42
total primary tumours	119	121	102	117	97	81	65	60	60	67
total animals with benign tumours	50	47	47	47	46	40	33	29	30	37
total benign tumours	98	96	81	84	83	62	49	41	42	50
total animals with malignant tumours	17	22	18	26	14	17	16	19	17	17
total primary malignant tumours	21	25	21	33	14	19	16	19	18	17

Summary of Results

Male rats administered 10 mg chlorpyrifos/kg bw/day (highest dose tested) in the diet for up to 2 years showed: (1) a consistent decrease (7-9%) in body weight gain relative to controls in the absence of reduced food consumption; (2) depression of plasma (56-87%), erythrocyte (20-40%) and brain (56-58%) cholinesterase activities; and (3) an increase in the weight of adrenal glands, characterised microscopically by an exacerbated fatty vacuolization of the zone fasciculata. Other treatment-related effects, which may be secondary, included decreases in serum cholesterol, total protein and globulin, an increase in urine specific gravity, and a reduced incidence or severity of some common geriatric conditions such as chronic renal disease and biliary hyperplasia. Altered parameters in females at 10 mg chlorpyrifos/kg bw/day were generally similar to those observed in males but were less pronounced. There were: (1) a transient decrease (<3.5%) in bodyweight gain relative to controls with no reduction in food consumption; (2) depression of plasma (82-95%), erythrocyte (generally <-20%) and brain (57-61%) cholinesterase activities; and (3) an increase in adrenal weight at the terminal sacrifice, but with no associated histopathological lesions. Other secondary effects included a mild transient depression of serum cholesterol and globulin, a slightly elevated urine specific gravity in the first 12 months of the study, and a decreased incidence of biliary hyperplasia. There was also mild perineal staining in these female rats during study months 6 - 20.

At the next lower dose tested (1 mg/kg bw/day), the only noted effect attributable to treatment was an inhibition of plasma (39-71% in males and 60-86% in females) and erythrocyte (20-40% in males and <-22% in females) cholinesterase activities. The brain cholinesterase was not affected.

No treatment-related effects were observed at the two lowest dose levels (0.05 and 0.1 mg/kg bw/day).

There was no increase in tumour incidence of any type in any organ or tissue at any of the dose levels tested (0.05, 0.1, 1.0 and 10 mg/kg bw/day).

Conclusions: Male rats were more sensitive to chronic chlorpyrifos treatment than female rats for the parameters measured. Under the conditions of this study, the NOEL was 0.1 mg/kg bw/day for both male and female rats based on plasma and erythrocyte cholinesterase depressions measured at the next higher dose level (1 mg/kg bw/day). The NOAEL for chronic toxicity of chlorpyrifos was considered to be 1 mg/kg bw/day based on brain cholinesterase inhibition at 10 mg/kg bw/day. There were no

clinical signs of cholinesterase intoxication at the highest dose tested (10 mg/kg bw/day). No tumorigenic effects of chlorpyrifos treatment were evident in this study.

[Australian conclusions: The NOEL for this study, based on toxicologically (>20%) and statistically-significant inhibition of plasma ChE activity in both sexes at 1.0 mg/kg/d was 0.1 mg/kg/d. The NOEL for erythrocyte ChE was 1.0 mg/kg/d based on toxicologically (>20%) or statistically-significant inhibition in both sexes at 10 mg/kg/d. The NOEL for brain ChE was 1.0 mg/kg/d based on toxicologically (> 10%) and statistically-significant inhibition in both sexes at 10 mg/kg/d.]

6.3 Dog

6.3.1 Dietary Administration for two years

McCollister SB, Kociba RJ, Gehring PJ & Humiston CG (1971b) Results of two-year dietary feeding studies on Dowco 179 in beagle dogs. The Dow Chemical Company, Report No.: T35.12-44793-18, dated December 10, 1971. [Dow; Submission 238, A3162/5 B40]

Beagle dogs (10 and 11 months, 7 dogs/sex/group) were dosed with chlorpyrifos (97.2%, Dowco 179, Lot No.: CP523-CD235 C) in the diet at dose levels of 0, 0.01, 0.03, 0.1, 1.0 or 3.0 mg/kg bw/d for a period of 1 year (1M & 1F/dose), 1 year + a 3-month recovery (2M & 2F/dose) or 2 years (4M & 4F/dose).

Clinical signs were recorded daily, body weights were recorded weekly for the first six months, and biweekly thereafter. Food intake was recorded weekly during months 1-3, and 1/4 weeks thereafter. Clinical pathology investigations included: haematology (PCV, Hb, RBC, WBC and prothrombin time) and urinalyses (specific gravity, occult blood, ketones, solids, pH, albumin, sugar) from the 0, 1.0 and 3.0 mg/kg/d groups; serum levels of BUN, ALP, SGOT and SGPT were measured in all dogs pretest and at various intervals thereafter. Bromsulfophthalein (BSP) retention was measured pretest and at the 1-year sacrifice in the 0, 1.0 and 3.0 mg/kg/d groups. Cholinesterase activity (pH-Stat method) was determined in most groups on blood (plasma and RBC) pretest, at various intervals during the test, and including activity in brain samples at necropsy. Necropsies of fasted dogs recorded organ weights for brain, heart, liver, kidney, spleen and testes. Portions of these organs and a standard set of tissues were preserved from the control, 1.0 (1-year only) and 3.0 mg/kg/d groups for histopathology. The animals in the 2-year feeding study portion were given complete physical examinations prior to termination; these included routine neurologic and opthalmoscopic evaluations. Statistical evaluations of data were limited to the Students "t" test.

Body weights were not significantly affected by treatment and food consumption was comparable between all same-sex groups. Consumption of the test compound was stated to be regularly monitored and to closely approximate the nominal levels in both sexes. Organ weights were not affected by treatment, except for an increase in mean liver/bw ratio in males at 3.0 mg/kg/d (3.47 g/100 g vs 2.46 for controls) in the 2-year study.

No data detailing the recorded clinical signs was presented; the study authors stated that "No clinical signs of toxicity were observed in any of the dogs in either phase of the experiment." Clinical pathology

results were presented as individual and summary data. There were no unequivocal treatment-related differences between control and treated groups recorded for the limited haematological parameters measured, nor for the urinalyses. Clinical chemistry found no effect of treatment on serum levels of BUN, ALP, SGOT and SGPT or on BSP retention..

% cholinesterase inhibition compared to concurrent controls

	Plasma	**Plasma**	**RBC**	**RBC**	**Brain**
1-year study leg[a]	Male	Female	Male	Female	M&F
3.0 mg/kg/d	72	70	79	82	8
1.0 mg/kg/d	62	62	63	61	3
0.1 mg/kg/d	39	36	18	30	0
0.03 mg/kg/d	18	17	2	33	0
0.01 mg/kg/d	1	1	16	13	0
2-year study leg[a]	Male	Female	Male	Female	Male
3.0 mg/kg/d	77	67	83	70	20
1.0 mg/kg/d	69	50	68	66	7
0.1 mg/kg/d	52	38	27	41	8
0.03 mg/kg/d	23	22	0	6	7
0.01 mg/kg/d	2	0	14	6	1

[a] average values at terminal sacrifice

Cholinesterase activity measurements were presented as individual and summary data. Cholinesterase inhibition was evident within 9 days (the first assay time) of the start of the study, and the values recorded at termination were similar to the values recorded at interim assay times. Plasma cholinesterase was decreased in a dose-related manner at both assay times, with a NOEL for this effect (< 20% inhibition) in both sexes at 0.01 mg/kg/d in the 2-year study leg. RBC cholinesterase was similarly depressed in a dose-related manner with females slightly more affected, and the NOEL (<20% inhibition) was 0.03 mg/kg/d for both sexes in the 2-year study leg. Brain cholinesterase activity was the least affected by treatment and the NOEL (<20% inhibition) was 1.0 mg/kg/d for both sexes. The recovery animals in the 1-year study leg were assayed after 2, 6 and 13 weeks on normal diet and recorded a return to control cholinesterase activity after 2 weeks for plasma cholinesterase, and after 13 weeks for RBC cholinesterase activity.

Gross pathology was reported in the form of a general summary of gross necropsy observations. There were no findings that indicated an effect of treatment. The limited (control and high-dose only) histopathology examinations were reported as a summary table with no indication of severity of lesions or abnormalities; individual data were not presented. The available histopathology data did not indicate any treatment-related effects.

Comment: This study has inadequacies in data collection and recording. The chronic effects of dietary chlorpyrifos intake could not be evaluated in the absence of complete pathology and histopathology findings; however NOELs for cholinesterase inhibition (>20%) were established. The NOEL for this study, based on plasma cholinesterase inhibition in both sexes was 0.01 mg/kg/d (LOEL 0.03 mg/kg/d). The NOEL for RBC cholinesterase for both sexes was 0.03 mg/kg/d (LOEL 0.1 mg/kg/d), and for inhibition of brain cholinesterase activity, the NOEL in both sexes was 1.0 mg/kg/d (LOEL

3.0 mg/kg/d).

Kociba RJ, McCollister SB, Keyes DG & Dittenber DA (1985) Supplement to original report entitled " Results of two-year dietary feeding studies on Dowco 179 in beagle dogs." The Dow Chemical Company, Report No.: NBT 3512-44793-18 (supplemental), dated March 26, 1985 [Dow; Submission 238, A3162/5 B40]

This supplement provides some of the data identified as lacking/inadequate in the original study report. However the "in life" individual animal observations were very limited and do not appear to consistently include basic observations. Individual body weight records, ophthalmology data, pretest and terminal physical examinations, and a tissue inventory for histopathology were included in this supplement. The conclusions of the evaluation of the original study remain unchanged by this supplement, with the additional observation that there was no effect of treatment detected by the ophthalmology examinations.

6.3.2 TCP (3,5,6-trichloro-2-pyridinol) metabolite study: One-year feeding study in dogs

Zempel JA, Rachunek BL & Szabo JR (1987) 3,5,6-trichloro-2-pyridinol: Results of a One-year dietary study in male and female beagle dogs. The Dow Chemical Company, Texas, Study No.: TXT:K-038278-009, dated September, 1987. GLP. QA. [Dow; Submission 939, volume 2 reference 4.3.5]

Beagle dogs (21 weeks, Marshall Research Animals Inc., NY) (4/sex/dose) were fed 0, 3, 12 or 48 mg/kg/d TCP (3,5,6-trichloro-2-pyridinol, batch AGR 143197, 99.7%) in their diet for one year. Prestudy and terminal physical examinations (including ophthalmology) were conducted. Clinical signs were recorded daily and body weights were recorded weekly. Food intake was recorded weekly and used to adjust TCP intake to allow for body weight changes. Blood samples were taken from fasted animals pretest and at 3, 6 and 12 months. Clinical pathology investigations on these samples included: haematology (PCV, Hb, RBC, WBC, platelets, WBC differential and prothrombin time, and serum levels of BUN, ALB, ALT, ASP, ALP, GLU, CHOL, TPRO, TBIL, GLOB, PHOS, CAL, NA, K. Urinalyses (specific gravity, occult blood, ketones, solids, pH, albumin, sugar, bilirubin and urobilinogen) were performed from samples taken at necropsy. Terminal necropsies of fasted dogs recorded organ weights for adrenals, brain, heart, liver, kidney, ovaries/testes, thyroid/parathyroid. Histopathological examinations of these organs and a standard set of tissues were performed for all animals. Appropriate statistical evaluations of data were performed.

No details of clinical observations were reported. Body weights were not statistically significantly different between groups, with 3 mg/kg/d males generally trending higher than other groups. High-dose females consistently displayed lower body weights than controls (day 364: 10.3 kg vs 13.3 kg for 48 mg/kg/d females vs controls). Statistically-significant lower body weight gains were recorded for these high-dose females (day 364: cumulative weight gain 3.1 kg vs 6.3 kg for controls). There were no changes in food consumption in treated groups, and no alterations in haematology or urinalysis parameters. Dose-related changes in clinical chemistry values attributed to TCP administration were increased serum ALP and ALT values in both males and females seen at 3, 6 and 12 months. These values were comparable to controls at 3 mg/kg/d, always elevated although generally not to a statistically-significant level at 12 mg/kg/d, and at 48 mg/kg/d exhibited an increase over controls which was generally statistically significant at 48 mg/kg/d at each assay time. There were no gross or

histopathological lesions associated with treatment and no changes in absolute or relative organ weights.

Based on the biologically significant increased levels of serum ALP and ALT values at 12 mg/kg/d, the NOEL for dietary intake for one year of TCP in male and female dogs was 3 mg/kg/d.

6.4 Chicken

6.4.1 Dietary Administration for One Year

Sherman M & Herrick RB (1973) Fly control and chronic toxicity from feeding Dursban (0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate) to laying hens. Poultry Science, 52:741-747. [Public Domain; Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Female chickens (Single Comb White Leghorns, 30 weeks old) were administered chlorpyrifos (30/dose) in the diet at 0, 25, 50 and 200 ppm (approximately 0, 2.5, 5 and 20 mg/kg bw/d) for 52 weeks. Hen mortality was unaffected by treatment; there was 13, 3, 7 and 10% mortality for the 0, 25, 50 and 200 ppm groups, respectively. Plasma cholinesterase was assayed in three hens/dose at 1, 3, 7, 14, 22 and 29 days after treatment started, and monthly thereafter, as well as 1, 2 and 3 weeks after treatment finished. The onset of cholinesterase activity inhibition was rapid and dose-related (22, 45 and 76% inhibition at week-1 for 25, 50 and 200 ppm respectively), and persisted at similar levels throughout the study but returned to control levels during the recovery period. Overall feed consumption, body weight, egg production, feed efficiency, egg weight and shell thickness were not affected by treatment. There was no NOEL for plasma cholinesterase inhibition in this study based on significant plasma cholinesterase activity inhibition at the lowest dose tested, 2.5 mg/kg/d (25 ppm).

7. REPRODUCTIVE TOXICITY

7.1.1 Three-Generation Dietary Study

Thompson DJ, Gerbig CC, Warner SD (1971) Three generation reproduction and teratology study in the rat following prolonged dietary exposure to Dursban 0,0-diethyl 0-3,5,6-trichloro-2-pyridyl phosphorothioate. The Dow Chemical Company. Study dated 20 August, 1971 [Dow; Submission 238, Part 2, vol 5: pp 2-1944-1988. A3162/5 B41]

No statements on GLP or test guidelines were issued with this report. In a three-generation, two litters per generation protocol, Sprague-Dawley rats from Laboratory Supply company, Indiana, USA, were exposed to chlorpyrifos in the diet (Dursban, Dow Batch # CD 235, purity not specified) at 0, 0.03, 0.1 and 0.3 mg/kg/d for the first generation, and 0, 0.1, 0.3 and 1.0 mg/kg/d for the second and third generations. Group sizes of rats were consistent throughout all generations, controls comprising 20 males and 40 females, low-, mid- and high-dose groups comprising l0 males and 20 females. Treatment diet started at 58 days and F0 parents were mated at 118 days of age with a 1:2 male to female ratio. Evidence of conception (vaginal plug or sperm) determined day 0 of gestation. Pairing for the second litter in each generation commenced approximately 10 days after the first litter was weaned at 21 days, and treatment diets were fed continuously to the litters from weaning. When progeny of the F1B or F2B numbered more than 10/litter, each litter was reduced to 10 after 5 days of age.

Experimental design summary

Generation	Mating at	Litters
F0	118 days of age	F1A, F1B
F1 (F1B litter)	110 days of age	F2A, F2B
F2 (F2B litter)	110 days of age	F3A, F3B

The F3B foetuses were used for teratological examination. F2B parental animals were continued on the test diet throughout the breeding period, except that females were placed on normal diet during organogenesis, at which period the test substance was administered by gavage in acetone/corn-oil. Dams were killed on day 20 gestation, foetuses removed by caesarean section and examined for external abnormalities. One third were fixed for soft-tissue examination, and two thirds stained with alizarin red for skeletal examination; however only the controls and 1.0 mg/kg/d groups were examined.

Clinical observations were stated to have been performed frequently. Body weights and food consumption were recorded weekly until breeding commenced. During gestation and lactation, the diets were adjusted based on average feed consumption of 25 and 40 g/day. Rats were re-weighed prior to breeding to allow dietary adjustment. In the teratology study, body weights of dams were recorded on days 0, 6, 15 and 20 of gestation, while food consumption was measured for the gestational intervals 0-6, 6-16 and 16-20 days.

Various indices of reproductive performance were calculated as follows: Fertility index as pregnancies/matings; Gestation index as live litters born/pregnancies; Viability index as rats alive at day 5/rats born alive; Lactation index as rats alive at day 21/rats alive at day 5.

Gross pathological examinations were made of all dying animals and of 5 pups/sex/dose from the F1A, F2A and F3A litters. An extensive set of tissues (34) were prepared and fixed from these pups, but in the absence of gross abnormalities only tissues from the F3A pups were examined histopathologically. Clinical chemistry was limited to the F2 animals, and consisted of plasma and erythrocyte cholinesterase measurements from 11 control and 5 or 6 treated dams at caesarean section, as well as 5 males/group.

Statistical analysis was limited.

Results

Very limited (7 samples) analysis of the prepared diets indicated reasonable agreement with the target dietary concentrations of Dursban. Clinical signs of toxicity were not seen in any parents or offspring. Two deaths occurred in the F1 dams, but these were unrelated to treatment. Parental male body weights were not significantly affected by treatment, although there was a trend for increased body weight in treated males with each generation. Female body weights were comparable to controls at each dose level in each generation, with a slight tendency to increased body weight in high-dose animals. Food consumption was variable but unaffected by treatment in either sex.

The fertility, gestation and lactation indices were comparable between groups and generations. The viability index was decreased at 1.0 mg/kg/d.

Viability index[a] for three generations of rats fed Dursban in the diet

Parents	F0	F0	F1	F1	F2	F2
Pups	F1A	F1B	F2A	F2B	F3A	F3B
0.00 mg/kg/d	94	90	71	87	91	na
0.03 mg/kg/d	97	96	na	na	na	na
0.10 mg/kg/d	96	92	77	92	95	na
0.30 mg/kg/d	91	94	74	91	90	na
1.00 mg/kg/d	na	na	65	73	84	na

[a] rats alive at day 5/rats born alive
na = not applicable

This effect on pup viability at the 1.0 mg/kg/d can be also seen when comparing mean litter size at day 21.

Mean litter sizes for three generations of rats fed Dursban in the diet

Generation	**Control**	**0.3 mg/kg/d**	**1.0 mg/kg/d**	**Reduction in mean litter size to day 21 (control vs treated)**
F1A - days 0, 5, 21	10.1, 9.6, 8.7	10.6, 9.7, 8.4		14% vs 21%
F1B - days 0, 5, 21	12.0, 10.9, 8.6	11.5, 10.8, 8.9		*
F2A - days 0, 5, 21	12.0, 8.5, 7.7		9.6, 6.4, 5.6	36% vs 42%
F2B - days 0, 5, 21	12.8, 11.2, 8.9		12.6, 9.2, 6.8	*
F3A - days 0, 5, 21	10.6, 9.7, 8.6		10.6, 9.1, 7.5	19% vs 29%

*litters reduced to 10 pups after day 5; insufficient data to calculate % reduction

In the dams producing the F3B pups, mean bodyweight gain showed a dose-related increase; at 0, 0.1, 0.3 and 1.0 mg/kg/d, body weight gains for gestation days 0-20 were 114, 120, 123 and 126 g respectively. In this generation, there were no treatment-related effects on fertility (% pregnant), mean number of corpora lutea or implantations, viable litter size or pup weight following caesarean section.

Teratology: External, skeletal and visceral examination was performed on pups from the control and 1.0 mg/kg/d groups of the F3B breeding. Skeletal examination of 153 pups from each group found common minor variants to be incomplete ossification of sternabrae, particularly the fifth (33 for controls vs 50 for treated), and the occurrence of extra ribs (extra right, left or bilateral summed to 41 for controls vs 21 for treated). Visceral examination of 85 control and 84 treated pups found minor variants of the urogenital system (hydronephrosis or hydroureter left, right or bilateral) to be more common in the treated group (46 vs 95). A single foetus with multiple abnormalities occurred in the 1.0 mg/kg/d group.

Cholinesterase activity: Group-average plasma and erythrocyte cholinesterase activity was decreased (>20%) at 1.0 mg/kg/d in males and females of the F2 generation.

Group-mean cholinesterase activity[a] in F2 rats

Dose	**0.1 mg/kg/d**	**0.3 mg/kg/d**	**1.0 mg/kg/d**
plasma male	112%	96%	72%
plasma female	99%	87%	39%

RBC male	108%	105%	74%
RBC female	92%	*76%*	75%

[a] expressed as percentage of control group activity

Based on the reduction in pup viability seen in each generation at 0.3 and 1.0 mg/kg/d, the decreased plasma and RBC cholinesterase activity seen at 1.0 mg/kg/d in males and females, and the decreased RBC cholinesterase activity seen in 0.3 mg/kg/d females, the NOEL for this study was 0.1 mg/kg/d.

7.1.2 Two-Generation Dietary Study

Dietz FK, Mensik DC, Hinze CA, Rachunek BK, Taylor HW (1983) Dursban insecticide: Assessment of neonatal survival in a two-generation reproduction study in rats. The Dow Chemical Company. Study No.TXT-K-44793-(45), dated July 1983. [Dow; Submission 238, A3162/5 B41]

This study was conducted to supplement the findings of a three generation dietary reproduction study of Dursban (Thompson et al, 1971), which recorded an equivocal decrease in neonatal survival at 1.0 mg/kg/d.

No statements regarding GLP or test guidelines were issued with this report. Sprague-Dawley rats (CRL, Portage, Michigan) at 6 weeks of age in groups of 30 rats/sex, were fed diets containing chlorpyrifos (Dursban F, code AGR#190183, Lot #811012-616; purity between 96.6% and 99.0%) at doses of 0, 0.5, 0.8 or 1.2 mg/kg/d for 135 days, and then bred to produce F1 litters. On day 21 of lactation, 30 pups of each sex were randomly selected for the next generation and dosed in the same manner as the F0 animals for 120 days, prior to breeding to produce the F2 litters. The F2 pups were also weaned on day 21 of lactation.

F0 and F1 matings were on a one-to-one basis, with pairing for 5 days, a 7-day rest interval, and a second pairing (different male) for a further 5 days. Litters were culled on day 4 postpartum. Litter weight and pup survival were recorded on days 1, 4, 7, 14 and 21 postpartum and individual pups were weighed on day 21. Parental animals were checked daily for signs of toxicity, body weight and food intake were recorded weekly for both sexes until pairing and for males, after pairing until sacrifice. Females were weighed on days 1, 4, 7, 14 and 21 of lactation. Litter data comprised date of parturition, litter size at birth, number of live and dead pups at birth.

Dietary concentrations were adjusted weekly during the pre-mating period according to the food consumption and body weight of the rats; for both sexes, dietary concentrations were maintained at the last adjusted pre-mating concentration from commencement of cohabitation until sacrifice. Appropriate statistical tests were applied to the data.

Results

Diets displayed satisfactory stability and homogeneity.

F0 parents displayed no significant clinical signs. Seven deaths (5 controls, being 3 males and 2 females; 1 female at 0.5 mg/kg/d; 1 male at 1.2 mg/kg/d) were reported, the deaths not being related to

chlorpyrifos exposure. Body weight and food intake were unaffected, as was female body weight during lactation. The mean fertility index (number of females delivering a litter expressed as a percentage of the total number of females placed with a male) was reduced at 0.5 and 0.8 mg/kg/d, but not at 1.2 mg/kg/d, and a similar inverted dose-response was recorded for gestation length; individual animal data were not presented. Litter sizes (10-11) were comparable in all dose levels, as were survival indices (>91% in all groups on days 1, 4, 7, 14 and 21) during lactation. Pup weights (presumably litter weight/number of pups) were also comparable.

F1 parents showed no clinical signs. Mortality was confined to 1 control female dying during cohabitation. Male body weight was reduced at 1.2 mg/kg/d on days 160-182 (post cohabitation). Female body weight showed slight sporadic increases compared to controls. Food intake was sporadically decreased in males, but not in females. There were no effects on female body weight during lactation. The fertility index in treated groups exceeded control values. Litter sizes (11-12) were comparable in all groups, as were pup weights and pup survival during lactation.

Neonatal survival during 2-generation reproduction study in rats

Dose	**0 mg/kg/d**	**0.5 mg/kg/d**	**0.8 mg/kg/d**	**1.2 mg/kg/d**
Fertility index - F1	26/30	18/28	21/30	28/30
Gestation days - F1	28 ± 6	24 ± 3	25 ± 3	26 ± 3
Live pups/litter - F1	10 ± 4	11 ± 2	10 ± 4	10 ± 4
21 day survival index -F1	179/185	145/150	141/141	200/203
Fertility index - F2	23/29	28/30	23/30	27/30
Gestation days - F2	25 ± 5	25 ± 4	26 ± 4	26 ± 4
Live pups/litter -F2	11 ± 4	11 ± 4	12 ± 3	11 ± 3
21 day survival index - F2	164/167	201/202	175/175	206/206

The adequacy of this study for regulatory purposes was limited by the dose selection. As no adverse effects were demonstrated at the highest dose, it was unclear whether the study clearly demonstrates the reproductive toxicity potential of the test material in rats. The lack of individual animal data and the inverted dose-response for some parameters does not allow for an unequivocal interpretation of the findings. However in the absence of clear adverse findings on reproductive parameters at any dose level, the NOEL for this study was considered to be 1.2 mg/kg/d.

7.1.3 Two-Generation Dietary study

James P, Stubbs A, Parker CA, Offer JM & Anderson A (1988) The effect of Pyrinex (Chlorpyrifos) on reproductive function of two generations in the rat. Huntingdon Research Centre Ltd, UK. Study completed 22 April 1988. HRC report MBS 29/881452, dated 17 August 1989. [Makteshim; Submission 11471]

This study was conducted in compliance with GLP Standards [US EPA (FIFRA) Title 40 CFR 160, 1983; OECD 1982; Japan MAFF, Notification 3850, 1984; UK Department of Health 1986], and study requirements of the US EPA 83-4.

Technical chlorpyrifos (Makteshim; stated purity 95.8%; batch 489205) was given to groups of male and female rats (CrL: COBS CD (SD) BR; Charles River UK; four weeks old upon receipt) at dietary concentrations of 0 (control), 2, 10, or 50 ppm over two generations. These dietary levels corresponded to achieved test material intakes in F0 males of 0.1-0.2, 0.5-0.9, 2.5-4.5 mg/kg/d, respectively, and 0.1-0.2, 0.6-0.9, 2.9-4.6 mg/kg/d, respectively, in F0 females. The corresponding intake ranges in the F1 generation were 0.1-0.3, 0.7-1.6, and 3.3-8.1 mg/kg/d in males, and 0.2-0.3, 0.8-1.6, 4.0-8.1 mg/kg/d in females. For each group, the test material was dissolved in a small volume of acetone, then stirred directly into an amount of sieved untreated Labsure Laboratory Diet No. 2, and placed on a rotary evaporator to remove the acetone. Further quantities of the basal diet were added to achieve the required concentrations, and homogeneity was achieved by shaking or blending for several minutes. The control diet was prepared in a similar manner using the same initial concentration of acetone. Homogeneity and stability were determined prior to treatment, and also determined during the study at the start of the F0 pre-mating period, and at the start of the mating and end of gestation/start of lactation for both the F0 and F1 generations.

In the F0 generation, animals (28/sex/dose) were approximately 7 weeks of age at the commencement of treatment, and were maintained on their respective diets for at least 56 days prior to mating. The animals were then mated on a one-to-one basis for a period of 20 days, then dams were allowed to rear their young to day 1 post-partum. During the pre-weaning period, all F1 offspring in all litters were examined for developmental indices (surface righting reflex, startle reflex, air righting reflex, pupil reflex). At day 21 post-partum 24 pups/group were selected for F1 matings. Following examination of the F1 pups, excess pups were sacrificed on day 22 post-partum and examined macroscopically. Specified organs were weighed and tissues preserved for histopathological examination from one animal/sex/litter at this time. Shortly after the F1 pups were weaned, F0 adults were killed and examined macroscopically, with the following organs and tissues preserved (* examined histopathologically) adrenals, aorta, bone, bone marrow, brain, cranial vault, caecum, colon, duodenum, eyes, heart, ilium, jejunum, kidneys, liver, lungs, lymph nodes, mammary gland, macroscopically abnormal tissues, oesophagus, ovaries*, pancreas, pituitary*, prostate* with seminal vesicle and coagulating gland, rectum, salivary gland, sciatic nerve, skeletal muscle, skin, spinal column, spleen, stomach, testes* with epididymides*, thymus, thyroids tongue, trachea, urinary bladder, uterus* with cervix* and vagina*.

In the F1 generation, the selected animals were reared on their respective diets for at least 56 days prior to mating (ie. until they were approximately 12 weeks of age). The animals were then mated on a one-to-one basis for 20 days, and dams were allowed to rear their young to day 21 post-partum. Pathological examinations were conducted as for the F0 generation.

All animals were regularly handled and examined for signs associated with treatment. All animals that died or were killed were subject to post mortem examination. The weight of each animal was determined at the start of each generation, and subsequently at weekly intervals. All pups were weighed at birth, 4, 8, 12, and 21 days post partum.

Results

Clinical signs: Clinical signs in adult animals were low in incidence, general in nature, and unrelated to dose, and were not considered to be related to treatment. At 50 ppm, one male in the F0 generation and one female in each of the two generations died during the study; one male at 2 ppm was killed when it

displayed an inability to move hindlegs; two control females also died during the study. These deaths were not considered to be related to treatment.

Body weights and food consumption: Food consumption was similar in control and treated groups. No adverse, treatment-related effects on group mean bodyweights were observed at any of the dose levels, in either of the generations. In F0 males, a slight increase in mean body weight was observed throughout the treatment period, but this effect was not considered to be toxicologically significant. No consistent effects on body weights were observed during gestation or lactation over the two generations.

Reproductive parameters: Mating performance and duration of gestation were similar in control and treated groups. Some slight intergroup variation was observed in the incidence of total prebirth loss in both the F0 and F1 generations. The percentage loss at 0, 2, 10, and 50 ppm was 6.7, 10.3, 6.6, and 7.8 respectively in F0 groups, and 5.6, 7.4, 9.4, and 4.4, respectively, in F1 groups. In the absence of any consistent relationship between dose and effect, these findings were not considered to be related to treatment.

In the F0 generation, there were statistically-significant increases in litter size and litter weight and decreases in mean pup weight at most sample intervals, at a number of dose levels. The findings were generally only slightly different from controls, and there was no consistent relationship with dose. The decrease in mean pup weights was probably related to increased litter size. In the F1 generation, litter data were similar in control and treated groups, with the exception of a single statistically-significant decrease in litter weight at 50 ppm on day 21. This isolated finding was considered to be incidental to treatment.

Summary of statistically-significant group litter data - F0 generation

	Parameter	**Control**	**2 ppm**	**10 ppm**	**50 ppm**
At birth	Litter size-total	12.0	13.4	14.3**	12.8
	Litter size-live	11.7	13.0	14.0**	12.6
	Litter weight (g)	70.1	72.2	78.7*	70.7
	Mean pup weight (g)	6.1	5.6***	5.7**	5.7*
At Day 4	Litter size-live	11.1	12.9*	13.5***	12.1
	Litter weight (g)	101.8	110.1	116.5*	106.6
	Mean pup weight (g)	9.3	8.7	8.6*	9.2
At Day 8	Litter size-live	10.9	12.8**	13.4***	11.8
	Mean pup weight (g)	15.2	14.3*	13.9*	14.9
At Day 12	Litter size-live	10.8	12.7**	13.4***	11.7
	Litter weight (g)	239.5	267.2	278.7**	253.6
	Mean pup weight (g)	22.4	21.4	20.8*	22.3
At Day 21	Litter size-live	10.7	12.6*	13.3***	11.7
	Litter weight (g)	463.8	517.2	527.3*	477.0
	Mean pup weight (g)	43.7	41.8	39.8*	42.0

*p<0.05; **p<0.01; ***p<0.001

No consistent, dose-related effect on organ weights was reported during the study. Pathological examinations did not reveal any macro-or microscopic effects that were considered to be related to treatment. Pathological findings were confined to general effects seen in laboratory rats, with a low

incidence for most findings, and no consistent relationship with dose for any findings.

Summary

Under the conditions of this study, no adverse, treatment-related effects were observed at any dose, up to and including 50 ppm (equivalent to a dose range of 2.5-8.1 mg/kg/d, depending on sex and age of animal). Slight increases in group mean body weights in high-dose F0 males were the only effect attributed to treatment. No adverse effects on reproductive performance were demonstrated.

The adequacy of this study for regulatory purposes was limited by the dose selection. As no adverse effects were demonstrated at the highest dose, it was unclear whether the study clearly demonstrates the reproductive toxicity potential of the test material in rats. The doses were selected on the basis of findings of decreased cholinesterase activity from a preliminary study, in which chlorpyrifos was given in the diet for a single generation at doses up to 250 ppm. In this preliminary study, plasma cholinesterase activity was inhibited compared with controls by 86% in adult females and by about 60% in weanling males and females at 50 ppm. At 50 ppm, erythrocyte cholinesterase activity was also inhibited in adult females (70%) and weanlings (about 50%), and brain cholinesterase was inhibited by 38% in adult females. Cholinesterase activity was not determined in the above study.

7.1.4 Two-Generation Dietary Study

Breslin WJ, Liberacki AB, Dittenber DA, Brzak KA & Quast JF (1991) Chlorpyrifos Two-Generation dietary reproduction study in Sprague-Dawley rats. The Toxicology Research Laboratory, The Dow Chemical Company. Study ID K 044793-088. Completed June 5, 1991. [Dow; Submission 11462, reference 75; Submission 11464]

This study was conducted in accordance with FIFRA Guideline 83-4, OECD Guideline 416, and GLP principles.

Four groups of rats (Sprague Dawley, CRL, MI) at 30 rats/sex/dose were utilised in a two generation, one litter per generation reproduction study at dose levels of 0, 0.1, 1.0 or 5.0 mg chlorpyrifos/kg/d administered via the diet. Chlorpyrifos (Dursban F, AGR 273801) purity was 97.8-98.5%. F0 parental animals were 6 weeks of age at study initiation and were mated after 10 weeks dietary exposure to produce FI. litters. Groups of 30 rats/sex/dose level were selected from the F1 weanlings and were treated for 12 weeks prior to breeding to produce the F2 litters.

Pairing in both generations allowed for three periods of 7 days cohabitation on a one-to-one basis. Males were changed weekly. Care was taken to avoid sibling pairings. Females were removed from pairing when vaginal lavage showed sperm, this day being day 0 of gestation. F1 and F2 litters were culled if appropriate to a total of 8 pups on day 4. Clinical observations were performed daily on all animals. Litter data were recorded, namely litter size at birth, incidence of live and dead pups on days 0, 1, 4, 7, 14 and 21 post partum, and sex and weight of pups on days 1, 4, 7, 14 and 21 of lactation. Body weights and food consumption were recorded weekly during pre- and post-breeding. Body weights of dams were recorded on days 0, 7, 14 and 21 of gestation and on days 1, 4, 7, 14 and 21 of lactation, whereas food consumption was recorded weekly, twice weekly then every 2-3 days in the first, second and third weeks of lactation respectively.

Cholinesterase (plasma, erythrocyte and brain) inhibition was measured in 10 F0 and 10 Fl parental animals/sex at necropsy (ie. after 19 and 21 weeks of exposure). A complete necropsy was conducted on all F0 and F1 adults, and included eye examinations and collection of an extensive list of tissues. Histopathological examinations were made in control and high-dose animals of adrenals, brain, gross lesions and of reproductive tissues (cervix, coagulating glands, epididymides, ovaries, oviducts, pituitary, prostate, seminal vesicles, testes, uterus and vagina). Livers of 10 F1 parental males from the control and high-dose group were also examined microscopically. Mid- and low-dose tissue examinations were limited to adrenals. Ten pups/sex/dose level from F1 and F2 litters were subject to gross autopsy. Appropriate statistical tests were applied to all data.

Results

The test diets were within 10% of target concentrations. No significant effects of treatment were seen on clinical signs, food intake or body weight. F1 males at 5 mg/kg/d were consistently slightly lighter than controls (not statistically significant). F1 females showed reduced body weight gain during lactation at 5 mg/kg/d; mean lactation body weight gains were 32.0, 33.6, 26.9 and 24.0 g for 0, 0.1, 1.0 and 5.0 mg/kg/d groups.

Plasma cholinesterase activity was inhibited in parental F0 and F1 rats in both sexes at 1 mg/kg/d (40-57%) and at 5.0 mg/kg/d (up to 72% inhibition). Plasma cholinesterase inhibition was also seen in both sexes of both generations at 0.1 mg/kg/d, as part of a dose-related trend, but generally failed to reach biological or statistically significance at this dose. Erythrocyte cholinesterase activity was strongly inhibited at 1.0 and 5.0 mg/kg/d (up to 75% inhibition). Brain cholinesterase inhibition was seen only at 5 mg/kg/d (both sexes, both generations).

Comment: An NOEL for plasma ChE inhibition was set at 0.1 mg/kg/d, on the basis that inhibition generally failed to reach biological or statistical significance at this dose.

Mean cholinesterase activity[a] in F0 and F1 parents at necropsy (percentage inhibition cf controls)

		male			female		
Generation	**Dose mg/kg/d**	**plasma**	**RBC**	**Brain**	**Plasma**	**RBC**	**Brain**
F0	0	0.54	1.08	9.33	1.95	1.02	8.98
	0.1	0.46 (15%)	1.03 (5%)	9.24 (1%)	1.55 (20%)	1.05	9.11
	1.0	0.30$ (44%)	0.33* (92%)	8.75 (6%)	0.80$ (59%)	0.36* (65%)	8.73 (3%)
	5.0	0.21$ (61%)	0.32* (92%)	4.87* (48%)	0.64$ (67%)	0.31* (70%)	4.60$ (49%)
F1	0	0.53	1.13	9.70	1.83	0.96	9.44
	0.1	0.43 (19%)	0.98$ (13%)	9.74	1.55 (15%)	0.97	9.20 (2.5%)
	1.0	0.30$ (43%)	0.37$ (67%)	9.36 (3%)	0.93$ (52%)	0.32* (67%)	9.01 (4.5%)
	5.0	0.19$ (64%)	0.33$ (70%)	4.60$ (52%)	0.52$ (72%)	0.24* (75%)	3.98* (58%)

[a]plasma and RBC activity in IU/ml, brain activity in IU/g
[$]statistically significantly different from control mean by Wilcoxon's test
[*]statistically significantly different from control mean by Dunnett's test

First breeding: Gross pathological observations of F0 parents recorded no significant observations. Significant histopathological changes in the parental animals were limited to vacuolation, consistent with fatty change in the adrenal zone fasciculata. The observations for the 30 individual adrenals of the 0, 0.1, 1.0 and 5.0 mg/kg/d F0 males were respectively: 15, 22, 17, and 6 described as "within normal limits"; 15, 8, 12 and 24 described as "very slight or slight fatty changes in the zona fasciculata". The observations for the 30 individual adrenals of the 0, 0.1, 1.0 and 5.0 mg/kg/d F0 females were respectively: 28, 30, 30 and 8 described as "within normal limits"; 0, 0, 0 and 21 described as "very slight focus of altered cells in the cortex"; 0, 0, 0 and 16 described as "very slight or slight fatty changes in the zona fasciculata".

In the F0 breeding, treatment had no effect upon fertility, length of gestation, gestation survival, time to mating, sex ratio or litter size. F1 pups showed slightly decreased body weight gain during lactation at 1.0 mg/kg/d, and body weight gain and survival were decreased (statistically significant) at 5 mg/kg/d.

No effect of treatment was seen during gross pathologic observations or daily observation of the F1 weanlings. Feed consumption and body weight gains in the F1 animals prior to breeding to produce the F2 generation were not affected by treatment, nor were they affected in F1 females during gestation and lactation.

Second breeding: Gross pathological observations of F1 parents recorded no significant observations. Remarkable histopathological changes in the parental animals were again limited to vacuolation, consistent with fatty change in the adrenal zone fasciculata. The observations for the 30 individual adrenals of the 0, 0.1, 1.0 and 5.0 mg/kg/d F1 males were respectively: 15, 12, 20, and 7 described as "within normal limits"; 15, 16, 10 and 23 described as "very slight or slight fatty changes in the zona fasciculata". The observations for the 30 individual adrenals of the 0, 0.1, 1.0 and 5.0 mg/kg/d F1 females were respectively: 26, 29, 28 and 12 described as "within normal limits"; 2, 1, 1 and 18 described as "very slight focus of altered cells in the cortex".

In the F2 breeding, treatment had no effect upon fertility, length of gestation, gestation survival, time to mating, sex ratio or litter size. F2 pups did not show dose-related decreases in body weight gain during lactation. In these F2 pups, survival was decreased during lactation in control and 5 mg/kg/d groups due to total loss of 3 and 5 litters respectively; this was stated to be due to maternal neglect since stomach of pups in these litters contained no milk. No effect of treatment was seen during gross pathological observations or daily observation of the F2 weanlings.

The NOEL for maternal toxicity based on plasma cholinesterase inhibition in adult animals was 0.1 mg/kg/d, based on significant plasma cholinesterase inhibition at 1 mg/kg/d. The NOEL for RBC cholinesterase inhibition in adults was 0.1 mg/kg/d and for brain cholinesterase inhibition was 1.0 mg/kg/d, with reduced maternal weight gain during lactation seen at this dose also. The NOEL for neonatal effects was 1.0 mg/kg/d based on decreased body weight gain and survival at 5 mg/kg/d and the NOEL for fertility and reproductive effects was 5 mg/kg/d.

8. DEVELOPMENTAL TOXICITY

8.1.1 Mouse Developmental Studies

Deacon MM, Murray JS, Pilny MK, Rao KS, Dittenber DA, Hanley TR Jr. & John JA (1980) Embryotoxicity and fetotoxicity of orally administered chlorpyrifos in mice. Toxicol Appl. Pharmacol, 54:31-40, 1980.

Deacon MM, Murray JS, Pilny MK, Dittenber DA, Hanley TR, Jr. & John JA (1979) The effect of orally administered chlorpyrifos on embryonal and foetal development in mice. Dow Chemical, USA. Study No.: HET-K-44793-32, dated July 24, 1979. [Dow; Submission 11462, reference 76. Dow; submission 238, part 2, vol 5, pp 2.1831-2.1942]

No statements on GLP or test guidelines were issued in this report. In a dose-ranging study in which pregnant mice were gavaged with up to 60 mg/kg/d chlorpyrifos, no toxic effects were noted in the dams or offspring at 3, 10 and 20 mg/kg/d, the lowest three doses tested. In the main study, pregnant mice (CF-1, Charles River, Michigan) were given chlorpyrifos (Dursban F, 96.8% chlorpyrifos, Lot No. AGR 155052) at 0, 1, 10 or 25 mg/kg/d (40 to 47/group) by gavage in cottonseed oil carrier on days 6 through 15 of gestation *(Teratology-1)*. Day 0 of gestation was the day on which a vaginal plug was observed. Due to severe maternal toxicity observed at 25 mg/kg/d, additional groups (35-41 mice/group) were similarly administered 0, 0.1, 1 or 10 mg/kg/d chlorpyrifos by gavage on days 6-15 of gestation *(Teratology-2*).

Mice were observed for signs of toxicity and were weighed daily from day 6-16 of gestation, and were sacrificed on day 18 of gestation. At caesarean section, liver weight and gravid uterine weight (with ovaries) were recorded. Uteri of non-pregnant mice were stained with sodium sulphide to ensure no implantations had occurred. Foetuses were weighed, measured (crown-rump length), sexed and examined externally. One third of the foetuses were dissected for soft tissue examinations. All foetal heads were preserved in Bouin's solution and subsequently sectioned. All foetuses were subject to alizarin staining and skeletal examination.

Additional mice were initiated into the study to assess clinical findings. Cholinesterase (erythrocyte and plasma) activity was measured in groups of 4 to 10 mice given 1, 10 or 25 mg/kg/d on day 6, days 6 through 10 or days 6 through 15 of gestation. These measurements were repeated in another group treated with 0, 0.1, 1 or 10 mg/kg/d. Blood was collected for cholinesterase assay five hours post-dosing of these groups on days 6, 10 and 15 of gestation respectively. An homogenate of foetal tissue was prepared for cholinesterase activity measurement using litters from mice sacrificed on day 15 of gestation. Data were not provided on the body weights and food and water consumption for animals in these groups.

Appropriate statistical analysis was applied to experimental data. Foetal body weights and body measurements, maternal body weights, weights of maternal livers and uteri, food and water consumption, were analysed by one-way analysis of variance and Dunnett's test. Incidences of foetal resorptions and alterations were analysed by a modified Wilcoxon test. The Fischer exact test was applied to other data.

Results

Overview of studies reported

Study	Dose (mg/kg/d)	Pregnant mice/group	Observations
Dose ranging	0, 3, 10, 20, 30, 40, 60	3-4	maternotoxicity at 30 mg/kg/d and above
Teratology-1	0, 1, 10, 25	40-47	maternotoxicity at 10 mg/kg/d and above
Study	**Dose (mg/kg/d)**	**Pregnant mice/group**	**Observations**
ChE activity-1	0, 1, 10, 25	4-10	Samples after 1, 5 and 10 days dosing
Teratology-2	0, 0.1, 1, 10	23-30	no materno- or foeto-toxicity
ChE activity-2	0, 0.1, 1, 10	4-10	Samples after 1, 5 and 10 days dosing

Dose-ranging study

The dose-ranging study found severe cholinergic signs and consequent reproductive failure at doses of 30 mg/kg/d and above.

Summary data for dose-ranging study

Dose (mg/kg/d)	**0**	**3**	**10**	**20**	**30**	**40**	**60**
Number treated	5	5	5	5	5	5	5
deaths	0	0	1[a]	0	2	4[c]	4[c]
ChE inhibition	0/5	0/5	1/5	0/5	3/5	5/5	5/5
pregnant	3/5	4/5	3/5	4/5	2/5	3/5	4/5
litters	3	4	3	4	0	1	0
implantations[b]	9±2	14±2	12±0	10±4		10	
resorptions[b]	2±0	2±1	1±1	2±0			

[a]gavage needle entered lung; cholinergic symptoms present
[b]mean±S.E
[c]significantly different from control; p<0.05

Teratology-1: Treatment induced clinical signs of cholinesterase inhibition in a dose-related manner. Frank toxicity was evident at 25 mg/kg/d; 4/47 dams from this group died, and 32/47 presented with signs of toxicity associated with cholinesterase inhibition; these symptoms were excessive salivation, tremors, urine-soaked coat, ataxia and lethargy. Dams (9/44) from the group receiving 10 mg/kg/d chlorpyrifos exhibited mild to moderate signs of cholinesterase inhibition with one death, while 2/40 dams exhibited cholinergic signs and 1 died at 1 mg/kg/d.

Body weight gain of dams at 25 mg/kg/d was depressed by 14% compared to controls, while food and water intake were only slightly affected. No effects were observed on litter size, resorption incidence or sex ratio. Foetal body weight and crown-rump length were significantly reduced at 25 mg/kg/d.

Cholinesterase activity was depressed in a dose-related manner in all tissues examined, with significant plasma activity inhibition recorded even after just one dose. Plasma cholinesterase activity, measured after 1, 5 or 10 doses, was depressed by >20% at all dose levels in adults and at 10 mg/kg/d in foetal homogenate. Erythrocyte cholinesterase depression, measured after 1, 5 or 10 doses, was depressed by >20% in adults and foetal homogenates at 10 mg/kg/d and above, and in adults at 1mg/kg/d following exposure on days 6-10 of gestation.

Significant results from Teratology-1 study including cholinesterase measurements

Dose (mg/kg/d)		**0**	**1**	**10**	**25**
No. of females		51	40	44	47
Animals with cholinergic signs		0	2	9	32
Deaths		0	1	1	4
Total pregnant[a]		40 (4)	31 (1)	32 (1)	34 (1)
Dose (mg/kg/d)		**0**	**1**	**10**	**25**
No. with litters		36	29	30	29
Maternal body weight gain (g)	days 6-17	21±5	22±3	22±4	18±4[b]
Implantation sites/dam		13±3	13±2	13±1	12±3
Foetuses/litter		11±3	12±2	12±2	11±3
Foetal body weight (g)		1.14±0.10	1.13±0.07	1.15±0.08	1.03±0.17[b]
Foetal crown - rump length (mm)		25.0±1.2	25.2±0.9	25.3±0.9	24.4±1.6[b]

Plasma cholinesterase[c]	days 6-15	100 (10)[d]	15 (7)	4 (8)	2 (9)
Erythrocyte cholinesterase[c]	days 6-15	100 (10)[d]	86 (6)	57 (9)	43 (9)
Foetal homogenate[c]		100	81	65	35

[a]Numbers in parentheses are pregnancies detected by sodium sulphide stain (not resulting in litters)
[b]Significantly different from control; $p<0.05$
[c]Values expressed as percentage of control
[d]Numbers in parentheses are number of animals

Foetal alterations: Exencephaly was reported in all groups, incidence being 1/408 (0.24%), 5/347 (1.44%), 1/359 (0.28%) and 4/326 (1.23%) at 0, 1, 10 and 25 mg/kg/d, and at these doses ablepharia (partial or complete absence of the eyelids) was noted in 1, 1, 1 and 2 foetuses, and cleft palate in 0, 2, 1 and 2 foetuses, respectively. The incidence of delayed ossification (skull and sternebra) was increased at 25 mg/kg/d. Statistically-significant increases were only seen in the case of the delayed ossification. No historical control data were provided, but in the CF-1 mouse, both exencephaly and cleft palate are known to be commonly observed malformations occurring in 1-2% of mice. Significant maternotoxicity and foetal growth retardation was seen at 25 mg/kg/d. The increased incidence of sternebral malformations at 1.0 mg/kg/d was of doubtful significance.

Teratology-1: Incidence of significant foetal alterations

Dose (mg/kg/d)	**0**	**1**	**10**	**25**
No of foetuses examined	408	347	359	326
Exencephaly*	1^{1a}	5^{5b}	1^{1}	4^{3}
Ablepharia*	1^{1}	1^{1}	1^{1}	2^{2}
Cleft palate*	0	2^{2}	1^{1}	2^{1}
Small cerebral hemisphere*	1^{1}	1^{1}	1^{1}	0
Multiple skeletal malformations*	0	0	1^{1}	0
Fused ribs*	1^{1}	0	1^{1}	0
Skull; delayed ossification	5^{5}	9^{5}	10^{6}	34^{10b}
Sternebrae; delayed ossification	37^{17}	24^{11}	19^{11}	78^{22b}
Sternebrae; unfused	2^{2}	7^{6b}	3^{3}	8^{7b}
Sternebrae; fused	24^{16}	9^{16b}	23^{12}	6^{5b}

* Considered to be a major malformations
[a] Incidence expressed as (no. foetuses [no. litters])
[b] Significantly different from control group

Teratology-2: The second study did not record any cholinergic signs in the dams at any of the dose levels of 0, 0.1, 1 or 10 mg/kg/d. Body weight, food intake, maternal liver weights, gravid uterine weight and adjusted body weight were also unaffected. Water intake was increased at 0.1 and 10 mg/kg/d, but not at 1 mg/kg/d. Cholinesterase levels were rapidly and significantly inhibited in plasma and erythrocytes at 1 and 10 mg/kg/d, but in foetal homogenates were depressed only at the high dose.

Significant results from Teratology-2 study including cholinesterase measurements

Dose (mg/kg/d)		**0**	**0.1**	**1**	**10**
No. of bred females		41	35	36	35
Total pregnant [a]		30 (0)	25 (1)	23 (0)	28 (3)
No. with litters		30	24	23	25

Maternal body weight gain (g)	days 6-17	12±6	14±5	14±5	14±5
Implantation sites/dam		10±3	10±3	10±3	9±4
Foetuses/litter		8±3	7±3	7±3	7±3
Foetal body weight (g)		1.04±1.11	1.07±0.15	1.08±0.18	9±0.12[b]
Foetal Crown-/rump length (mm)		24.4±1.1	24.8±1.6	24.9±2.1	24.8±1.2[b]
Plasma Cholinesterase [c]	days 6-15	100 (8)[d]	86 (6)	25 (7)	3 (6)
Erythrocyte Cholinesterase [c]	days 6-15	100 (8)[d]	88 (6)	75 (7)	50 (6)
Foetal homogenate[c]		100	84	95	77

[a] Numbers in parentheses are pregnancies detected by sodium sulphide stain
[b] Significantly different from control; $p<0.05$
[c] Values expressed as percentage of control
[d] Numbers in parentheses are number of animals

No effects were observed on maternal parameters or on litter size, incidence of resorptions, incidence of dead foetuses, sex ratio, foetal body weight or crown-rump measurements. Malformation incidences were 1/225, 1/177, 0/172 and 1/180 for exencephaly, 0, 0, 1 and 0 for cleft palate and 0, 0, 0 and 1 for omphalocele at 0, 0.1, 1.0 and 10.0 mg/kg/d. The incidence of delayed ossification was decreased at 1 and 10 mg/kg/d.

Teratology-2: Incidence of significant foetal alterations in mice

Dose (mg/kg/d)	**0**	**0.1**	**1**	**10**
Exencephaly*	1^{1a}	1^{1b}	0	1^{1}
Ablepharia*	1^{1}	0	0	0
Cleft palate*	0	0	1^{1}	0
Omphalocele*	0	0	0	1^{1}
Skull; delayed ossification	18^{10}	11^{6}	3^{2}	3^{3}
Sternebrae; delayed ossification	50^{20}	25^{13}	25^{13}	17^{11}
Sternebrae; unfused	3^{3}	6^{5}	0	1^{1}
Sternebrae; fused	13^{7}	9^{7}	3^{3}	12^{8}

* Considered to be a major malformations
[a] Incidence expressed as (no. foetuses $^{\text{no. litters}}$)

When comparing the two studies, the authors provided no explanation for the higher control values recorded in most dose groups of *Teratology-1* vs *Teratology-2* for the related parameters: maternal body weight gain (21g vs 12 g in controls), implantation sites/dam (13 vs 10 in controls), foetuses/litter (11 vs 8 in controls), foetal body weight (1.14 vs 1.04 g in controls) and foetal crown-rump length (25.0 vs 24.4 mm in controls). However, neither teratology study provided evidence for teratogenic activity at any dose level. Foetotoxicity was seen at 25 mg/kg/d (reduced pup weight, crown-rump length and delayed ossification), and foetal homogenate cholinesterase activity was depressed by >20% at 10 mg/kg/d in both studies. On the basis of these findings, the NOEL for foetotoxicity was 1 mg/kg/d.

Erythrocyte cholinesterase inhibition was seen at 1 mg/kg/d, and cholinergic signs were seen in one of the studies at 1 mg/kg/d; on the basis of these findings, the NOEL for maternotoxicity was 0.1 mg/kg/d.

8.1.2 Rat Developmental Studies

Ouelette JH, Dittenber DA, Kociba RJ & John JA (1983a) Chlorpyrifos : Oral teratology

probe study in rats. Dow Chemical USA. Study No.: HET K-44793-(46), dated January 4, 1983. [Dow; Submission 11462, reference 79. Dow; submission 239, part 2 vol. 5, pp 2.2019-2.2032]

A QA Statement was issued for this study. Pregnant rats (Fischer 344, Charles River, Michigan) were dosed with chlorpyrifos (Dursban F, 96.6% chlorpyrifos, Lot No. AGR 190183) by gavage in groups of 12 animals, at dose levels of 0, 3, 10 or 30 mg/kg/d on days 6 through 15 of gestation. As the purpose of this study was to establish the maximum tolerated dose level for a teratological study, further groups of pregnant rats that were given 0 or 15 mg/kg Dursban F on days 6-15 gestation (10 dams/group), using the same strain and protocol. Matings were one-to-one, and the day sperm were observed in a vaginal smear was considered day 0 of gestation.

Rats were observed daily for signs of toxicity, were weighed daily from days 6-15 of gestation, and were sacrificed on day 15 of gestation. At caesarean section, body and liver weights were recorded. The number of corpora lutea, and the number and position of live or resorbed foetuses were recorded. Uteri of non-pregnant rats were stained with sodium sulphide to determine where implantations had occurred and had not led to litters. Samples of maternal blood were collected via heart puncture for RBC and plasma cholinesterase measurements. Appropriate statistical analysis of data was reported.

Results

Maternal toxicity was observed at 15 mg/kg (slight) and 30 mg/kg (severe), with typical cholinergic signs of excessive salivation, lacrimation, urination, defecation and body tremors, and general observations of unkempt appearance, decreased body size and matting of perineal/facial hair. Four of twelve dams from the 30 mg/kg group died. Animals from this group also recorded decreased body weight gain (statistically significant), decreased food consumption (statistically significant), enlarged adrenal glands, decreased liver weight (statistically significant) and shrunken thymus glands. At the 3 and 10 mg/kg/d doses, there were no clinical signs or findings at necropsy that were unequivocally related to treatment. Both plasma and erythrocyte cholinesterase activity were depressed (dose-related) at all dose levels.

Embryo lethality was observed in the group given 30 mg/kg/d. Of the eight surviving dams at this dose, only four were pregnant, and in two of these animals, all implantations were undergoing resorption.

Summary of findings including cholinesterase measurements

Dose (mg/kg/d)		**0**	**3**	**10**	**30**
No. of females		12	12	12	12
Animals with cholinergic signs		0	0	0	12
Deaths		0	0	0	4
Total pregnant [a]		8 (0)	11 (1)	11 (0)	8 (0)
No. with litters		8	10	11	4
Maternal body weight gain (g)	days 6-15	21±4	27±6	24±6	-20±10[b]
Implantation sites/dam		10±3	10±2	10±2	10±2
Litters totally resorbed		0	0	0	2
Foetuses/litter		9±2	9±2	10±2	4±5
Plasma Cholinesterase [c]	day 15	36.6	3.5 (9.6%)	1.7 (4.6%)	0.7 (1.9%)
Erythrocyte Cholinesterase [c]	day 15	15.2	4.5 (29.6%)	4.4 (28.9%)	4.3 (28.3%)

[a] Numbers in parentheses are pregnancies detected by sodium sulphide stain

[b] Significantly different from control; p<0.05
[c]Units/ml (percentage of control)

At 15 mg/kg/d there was no evidence of severe materno- or embryotoxicity. However, at this dose there was evidence of adrenal gland enlargement in 6/10 dams, and matted integumentary haircoat in 6/10 dams. Plasma and RBC cholinesterase were decreased to 3% and 20% of control levels respectively at the end of the study.

Maternal toxicity (plasma and RBC cholinesterase inhibition) was seen in all adult animals at 3.0 mg/kg/d, and foetotoxicity (increased resorptions) was only seen at 30 mg/kg/d.

Ouelette JH, Dittenber DA, Kloes PM & John JA (1983b) Chlorpyrifos: Oral teratology study in Fischer 344 rats. Dow Chemical USA. Study No.: HET K-44793-(47), dated July 5, 1983. [Dow; Submission 11462, reference 78. Dow; submission 239, part 2 vol. 5, pp 2.2033-2.2055]

A QA statement was issued for this study. Pregnant rats (Fischer 344, CRL, NY) were dosed with chlorpyrifos (Dursban F, 96.6% chlorpyrifos, Lot No. AGR 190183) by gavage (corn oil vehicle) in groups of 31-33 animals at dose levels of 0, 0.1, 3, or 15 mg/kg/d on days 6 through 15 of gestation. Matings were one-to-one, and the day sperm were observed in a vaginal smear was considered day 0 of gestation.

Rats were observed daily for signs of toxicity, were weighed daily from days 6-16 and day 21 of gestation, and were sacrificed on day 21. At caesarean section, body and liver weights were recorded. The number of corpora lutea, and the number and position of live or resorbed foetuses were recorded at necropsy. Foetuses were sexed, examined and measured. Uteri of non-pregnant rats were stained with sodium sulphide to determine whether implantations had occurred in the absence of litters. Separate groups of 10 bred rats/dose were dosed on days 6-15 of gestation and sacrificed before samples of maternal blood were collected via heart puncture for RBC and plasma cholinesterase measurements. Appropriate statistical analysis of data was reported.

Results

There were no unscheduled deaths. Clinical signs of maternal toxicity including excessive salivation, urine staining (perineal region), porphyrin deposits about the eyes, vaginal bleeding, and tremors were observed in the 15 mg/kg/d group only (incidences not stated). Animals from this group also recorded decreased body weight gain (statistically significant on days 12 and 16); liver weights were unaffected by treatment, and no other maternal findings were reported at necropsy. Both plasma and RBC cholinesterase activity were depressed at the 3.0 and 15.0 mg/kg/d dose levels.

No adverse effects were observed on reproductive parameters, and no teratogenicity was observed. Variations and malformations were randomly distributed through the dose groups. No effect of treatment on skeletal development was seen.

This study found 0.1 mg/kg/d to be the NOEL for maternal toxicity, based on plasma and RBC cholinesterase inhibition seen in all adult animals at 3.0 mg/kg/d and above. The NOEL for foetotoxicity

was 15 mg/kg/d, the highest dose tested.

Summary of findings, including cholinesterase measurements

Dose (mg/kg/d)		**0**	**0.1**	**3.0**	**15**
No. of females		31	32	33	31
Animals with cholinergic signs		0	0	0	12
Deaths		0	0	0	0
Total pregnant[a]		30 (0)	28 (0)	26 (0)[c]	29 (1)
No. of litters		29	26	24	26
Maternal body weight gain (g)	days 6-20	65±11	64±14	71±12	58±11
Implantation sites/dam		11±2	10±2	10±2	10±3
Litters totally resorbed		0	0	0	0
Foetuses/litter		10±3	9±2	10±3	9±3
Mean foetal body weight		4.28	4.37	4.52*	4.46*
Mean foetal crown-rump length (mm)		43.91	43.93	43.96	43.64
Plasma Cholinesterase[b]	day 15	46.0	42.8 (93%)	4.9 (11%)	1.5 (3%)
Erythrocyte Cholinesterase[b]	day 15	12.1	11.9 (98%)	3.1 (26%)	2.5 (21%)

[a] Numbers in parentheses are pregnancies detected by sodium sulphide stain
[b]Mean of 6-8 dams, Units/ml (percentage of control)
[c] two dams removed during dosing for reasons not related to treatment
* significantly different from control

Rubin Y, Waner T & Nyska A (1987a) Pyrinex. Preliminary teratology study in rats. Life Science Research Israel Ltd., LSRI Report No MAK/100/PYR, dated 11 February 1987. Makteshim Chemical Works reference R-4387 [Makteshim; Submission 11471]

This study was conducted to establish appropriate dose levels for a developmental toxicology study in rats. Being a preliminary study, neither original data nor the final report were subject to Quality Assurance audit.

Female CD rats (Charles River Italia; 8-9 weeks old) were mated one-to-one with stock males (same source and strain). Pyrinex technical (chlorpyrifos; stated purity 95.5%; Makteshim Chemical Works Israel; Batch 58009/9) was formulated fresh daily as solutions in maize oil and given to mated females (10/dosage group; dosage volume 5 ml/kg/d) by oral gavage as a daily dose from day 6 to day 15, inclusive, postcoitum, at doses of 0.02, 0.10, 0.5, 2.5, 12.5, 62.5 mg/kg/d. A vehicle control group received maize oil only. The stability of chlorpyrifos in the dosing solutions was tested during the course of the study, at 4 h, 4 days, and 10 days after formulation. The active ingredient was stable in the dosage form used.

On day 14 post-coitum, half of the treated females in each group were bled for measurement of plasma cholinesterase activity, and the animals were discarded without necropsy or without a determination of pregnancy status. On day 20 of gestation the remaining females were killed, and the following recorded: macroscopic examination of the reproductive tract; number of corpora lutea in each ovary; weight of the gravid uterus; distribution of live and dead foetuses and distribution of resorption sites; individual placental weights; individual foetal weights and sexes.

Results

At 62.5 mg/kg/d, 4/10 animals died and 6/10 animals were killed *in extremis*. Deaths occurred between days 3 and 7, preceded by tremors and staining of the periorbital, nasal and/or urogenital regions. Soft faeces or diarrhoea were also seen in 6/10 animals at this dose. No mortality was reported at any other doses, but at 12.5 mg/kg/d, a single animal displayed tremor, peri-orbital and urogenital staining. These effects were confined to the period between days 11 and 16 of gestation. Group mean body weight and body weight gain were markedly and statistically significantly ($p< 0.001$) reduced during treatment at 12.5 mg/kg/d. Animals at this dose lost weight from the onset of treatment, and did not return to pre-treatment body weights until approximately day 13 of the study. After cessation of treatment, body weights improved rapidly at this dose level, but by day 20 of the study, the mean body weight in the 12.5 mg/kg/ group was still reduced by about 10% compared with controls. Body weights in other groups were unaffected by treatment. At 12.5 mg/kg/d, food consumption was reduced by about 30% during the 14-16 day period only ($p< 0.05$), and was similar to controls at other doses.

Statistically- and biologically-significant, dose-related decreases in plasma cholinesterase activity were observed at doses of 0.05, 2.5, and 12.5 mg/kg/d, with decreases in activity of 46% ($p<0.01$), 59% ($p<0.001$) and 84% ($p<0.001$), respectively, compared with controls. At doses of 0.02 and 0.1 mg/kg/d, cholinesterase activity was similar to controls.

Statistically-significant decreases in pre-implantation loss were observed in all treatment groups, suggesting that the pre-implantation loss in controls (18.9%) was higher than expected. A slight but statistically-significant ($p<0.05$) increase in post-implantation loss was seen at 12.5 mg/kg, with a mean incidence of 9.4%, compared with 6.2% in controls. This finding may be related to the maternotoxicity of the test material at this dose. Other findings were generally similar to those observed in controls.

Under the conditions of this study, no treatment-related effects were reported at doses of 0.1 mg/kg/d and below, based on the inhibition of plasma cholinesterase activity at doses of 0.5 mg/kg/d and above. No evidence of maternotoxicity was observed at doses of 2.5 mg/kg/d and below, based on clinical signs, decreased body weight and reduced food consumption at 12.5 mg/kg/d.

Rubin Y, Gal N, Waner T & Nyska A (1987) Pyrinex. Teratogenicity study in the rat. Life Science Research Israel Ltd., LSRI Report No MAK/101/PYR, dated 15 July 1987. Makteshim Chemical Works no: R-4398 [Makteshim; Submission 11471]

This study was reported to be conducted in accordance with US FDA and EPA GLP Guidelines, and testing guidelines of the US EPA (section 83-3) and OECD (no. 414).

Female CD (SD) rats (Charles River Breeding Laboratories, UK) were mated one-to-one with stock males (same source and strain). Pyrinex technical (chlorpyrifos; stated purity 96.1%; Makteshim Chemical Works Israel; Batch 58009/9) was formulated fresh daily as solutions in maize oil and given to mated females (32/dosage group; dosage volume 5 ml/kg/d) by oral gavage as a daily dose from day 6 to day 15, inclusive, postcoitum, at doses of 0.5, 2.5, and 15 mg/kg/d. A vehicle control group received maize oil only. Short-term stability of chlorpyrifos in the dosing solutions was tested during the course of the study, at 4 h and 24 h after formulation, and the active ingredient was stable in the dosage

form used.

All females were examined daily for signs associated with treatment. Animals were weighed on days 0, 3, 6-15, 17 and 20 of gestation, and food consumption was measured twice weekly. Ten mated females were bled on day 15 for measurement of plasma cholinesterase activity, with blood samples obtained from the retro-orbital sinus under ether anaesthesia. The same procedure was repeated using an additional ten animals/dose immediately prior to necropsy on day 20. Animals used for cholinesterase determinations on day 15 were discarded without necropsy after being bled. All remaining animals were killed on day 20, with the following recorded for each animal: any macroscopic abnormality of the reproductive tract; number of corpora lutea in each ovary; weight of gravid uterus; distribution of live and dead foetuses and distribution of resorption sites in each uterine horn; individual placenta weights; individual foetal weight, length and sex; external anomalies of individual foetuses. The thoracic and abdominal contents of approximately one half of each litter were dissected and examined, then these foetuses were used for skeletal staining and evaluation; the remaining foetuses were used for preparation of the free-hand sections and examination of the visceral organs.

Results

No treatment-related mortality was observed during the study, and clinical signs of intoxication were confined to tremors seen late in the study in three animals at 15 mg/kg/d. A slight (about 8% compared with controls) but statistically-significant ($p<0.01$) decrease in mean food consumption was seen in the 15 mg/kg/d group in the day 7-9 period only. At other times, and at other doses, food consumption was similar to that seen in controls.

Group mean body weight was also statistically significantly reduced ($p<0.001$) during treatment at 15 mg/kg/d. Although this decrease was clearly related to chlorpyrifos administration, the change in body weight was generally in the range of 4-5% compared with controls. Statistically-significant ($p<0.001$), dose-related decreases in plasma cholinesterase activity were observed at all test doses, with reduction in activity compared with controls of 51, 81, and 94% at 0.5, 2.5, and 15 mg/kg/d, respectively, on day 15 of gestation. Following cessation of dosing, the plasma cholinesterase inhibition was largely reversible, and on day 20 of gestation, plasma cholinesterase activity was similar to controls at 0.5 and 2.5 mg/kg/d. At 15 mg/kg/d, plasma cholinesterase activity was still reduced by about 28% on gestation day 20.

Total live litter sizes were unaffected by treatment, but at 15 mg/kg/d the mean number of live male foetuses (6/litter) was statistically significantly decreased ($p<0.05$; student T-test) compared with controls (7.6/litter). Post-implantation loss was slightly elevated at 15 mg/kg/d compared with controls (9% versus 7%); this increase was statistically significant using Freeman-Tukey arcsine transformed data ($p<0.01$), but was not significant when using rank order methods (Mann-Whitney U-test). Both control and high-dose post-implantation losses were outside the historical control incidence for this finding at the test facility (13 studies; range 4.4-6.3), but this slight increase at 15 mg/kg/d was considered to be related to the foetotoxicity of the test compound.

Statistically-significant increases in pre-implantation loss were seen at all test doses, with group mean losses of 12.2%, 11.5%, and 11.4% at 0.5, 2.5, and 15 mg/kg/d, respectively, compared with a control value of 8.6%. (In this report, pre-implantation loss also included very early post-implantation loss, up to about Day 8-9 post-coitum.) However, there was no consistent dose-response relationship

associated with this finding, the pre-implantation losses were within historical control values for this testing laboratory (13 studies; range 4.6-17%), and this finding was not considered to be related to treatment.

At 15 mg/kg/d, slight but statistically-significant ($p < 0.01$) increases were seen in mean foetal weight (3.51 g versus 3.33 g) and mean crown - rump length (36.7 mm versus 36 mm), compared with controls. The increase in foetal weight did not correlate with a decrease in litter size, and this finding was possibly associated with advanced foetal development, and was not considered to be of toxicological significance. Similarly, the statistically-significant decrease in the incidence of small foetuses (observed in all test groups) was not considered to be toxicologically significant.

Isolated instances of a number of external or internal observations were reported, but in the absence of a dose relationship, these findings were not considered to be related to treatment . These included a single incidence of right aortic arch at 15 mg/kg/d; a single incidence of ablepheron (congenital absence of eyelid) at 2.5 mg/kg; a single incidence of a major cranio-facial malformation (proboscis; anophthalmia) at 0.5 mg/kg/d; and single instances of hydroureter and hydronephrosis at 2.5 mg/kg/d.

Free-hand sectioning examination did not reveal any treatment-related malformations, and while a range of minor structural variations were reported, the incidence of such findings was very low, and/or there was no relationship with dose. As such, these findings were considered to be incidental to treatment. These findings included a single incidence of anophthalmia (0.5 mg/kg/d) and single instances of microphthalmia at 0.5 and 2.5 mg/kg/d.

Summary of litter data and foetal observations at necropsy (No. of affected foetuses)

Parameter	**0 (control)**	**0.5 mg/kg/d**	**2.5 mg/kg/d**	**15 mg/kg/d**
Pre-implantation loss %	8.6	12.2[c]	11.5[c]	11.4[c]
Post-implantation loss %	7.0	6.0	5.8	9.0[b]
Mean foetal weight (g)	3.33	3.40	3.44	3.51[b]
Mean Crown-rump length (mm)	36.0	36.2	36.3	36.7[b]
Live foetuses: male female total	 7.6 6.8 14.4	 8.0 7.0 15.0	 6.8 7.3 14.1	 6.0[a] 7.5 13.4
Small foetus[$]	48	25[b]	20[c]	13[c]
Ablepheron[$]	0	0	1	0
Major cranio - facial malformation: Proboscis; anophthalmia [$]	0	1	0	0
Hydronephrosis[@]	0	0	1	0
Parameter	**0 (control)**	**0.5 mg/kg/d**	**2.5 mg/kg/d**	**15 mg/kg/d**
Hydroureter[@]	0	0	1	0
Anophthalmia: free-hand section[&]	0	1*	0	0
Microthalmia: free-hand section[&]	0	1*	0	0

* same foetus a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

[$] number of foetuses observed 317, 315, 311, and 282, respectively

[@] number of foetuses examined 161, 158, 158, 146, respectively

[&] number of foetuses examined 156, 157, 153, 136, respectively

At skeletal examination, no adverse treatment-related findings were observed. On a number of occasions, statistically-significant differences were reported between control and high-dose groups, all of which were suggestive of more advanced skeletal development in the treated group. For example, the incidence of a small anterior fontanelle was increased, with decreases in the incidence of large anterior fontanelles; the incidence of reduced or incomplete ossification of supraoccipital, interpareital, pareital, and frontal bones was decreased in the high-dose group; and the incidence of angulated ribs and the presence of the 14th rib unilaterally was also reduced at 15 mg/kg/d. These findings were consistent with more advanced foetal development, which was also demonstrated by increased foetal size in the high-dose group.

Summary of skeletal observations at necropsy: number of affected foetuses

Parameter	0 (control)	0.5 mg/kg/d	2.5 mg/kg/d	15 mg/kg/d
Number of foetuses examined	161	157	158	146
Reduced or incomplete ossification of interparietal bone	51	38	36	28[a]
Parietal bone: incomplete or reduced unossification	16	7	6	8
Reduced or incomplete ossification of frontal bone	1	1	0	0
Anterior fontanelle small	0	1	1	5[a]
Major cranial malformation: proboscis	0	1	0	0
14th lumbar rib present bilaterally	7	4	5	1[a]
Rib(s) angulated	5	0[a]	1	0[a]
One or more of sternebrae 1-4 unossified	11	2	3[a]	0[c]
Fifth sternebra and/or xiphisternum unossified	106	99	102	77[a]
Metacarpus v unossified bilaterally	116	113	98	80[b]

a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Summary: An NOEL was not demonstrated in this study, based on the inhibition of plasma cholinesterase activity at doses of 0.5 mg/kg/d and above. The NOEL for frank maternotoxicity was 2.5 mg/kg/d, based on reduced body weights, tremors and transient reductions in food consumption at 15 mg/kg/d. The NOEL for foetotoxicity was 2.5 mg/kg/d, based on a slight increase in post-implantation loss at 15 mg/kg/d, probably associated with maternotoxicity at this dose level. No evidence of major malformations was observed at any dose.

8.1.3 Rabbit Developmental Studies

Rubin Y, Waner T & Nyska A (1987b) Pyrinex. Pyrinex preliminary teratogenicity study in rabbits. Life Science Research Israel Ltd., LSRI Report No MAK/102/PYR, dated 30 June 1987. Makteshim Chemical Works no: R-4405 [Makteshim; Submission 11471]

This study was conducted to establish appropriate dose levels for a developmental toxicology study in rabbits. Being a preliminary study, neither original data nor the final report were subject to Quality Assurance audit.

Female New Zealand White rabbits (Charles River Italia; 4-5 months old) were mated one-to-one with

stock males (same source and strain). Pyrinex technical (chlorpyrifos; stated purity 95.5%; Makteshim Chemical Works Israel; Batch 58009/9) was formulated fresh daily as solutions in maize oil and given to mated females (5/dosage group; dosage volume 2 ml/kg/d) by oral gavage as a daily dose from day 7 to day 19, inclusive, postcoitum, at doses of 0 (vehicle control), 1, 3, 10, 30, 90, 270, and 800 mg/kg/d. The original 3 mg/kg/d group was replaced after a dosing error, and the data from that group have not been included in results. The 800 mg/kg/d group was reduced to 4 animals after a single death between days 2 and 3. The stability of chlorpyrifos in the dosing solutions was tested during the course of the study, at 0 and 4 h after formulation, and the active ingredient was stable in the dosage form used.

All animals were examined daily for signs associated with treatment, and any animals found dead or killed *in extremis* were autopsied to determine the cause of death. Animals were weighed on days 0, 3, 7-19, 22, 25 and 29 of gestation, and food consumption was measured twice weekly.

Plasma cholinesterase activity was measured once before mating and again after ten days of dosing. On day 29 of gestation all remaining females were killed, and the following recorded: macroscopic examination of the reproductive tract; number of corpora lutea in each ovary; weight of the gravid uterus; distribution of live and dead foetuses and distribution of resorption sites; individual placental weights; individual foetal weights and crown-rump lengths.

Results

Treatment-related mortality was reported, with 4/5 and 3/4 animals found dead or killed *in extremis* at doses of 270 and 800 mg/kg/d, respectively. A single animal died at 1 mg/kg/d, due to an intercurrent disease, and this death was unrelated to treatment. At the two highest dose levels, the remaining animals were killed on humane grounds, and no further data were collected during the study. Diarrhoea was observed in animals at doses of 10 mg/kg/d and above. Statistically-significant reductions in group mean food consumption were observed at doses of 10, 30 and 90 mg/kg/d during treatment (up to 39% reduction compared with controls), and this decrease in food consumption persisted past the treatment period at 90 mg/kg/d.

At all doses, body weight gains were reduced during the treatment period, with animals losing weight compared with their pre-dose levels, and pre-dose body weights did not recover in treated groups until days 12-15, in a dose-dependent manner. Statistically-significant decreases in group mean body weights were seen at 90 mg/kg/d on days 19-25, with decreases of about 10% compared with controls, and this finding was considered to be related to treatment. At other dose levels, group mean body weights were also slightly lower than controls, but these differences were not dose-related, and the body weights of these animals were lower than controls pre-dosing (2-7%), so these effects were not considered to be toxicologically significant.

At day 18/19 of gestation, statistically-significant ($p<0.01$ or $p<0.001$), dose-related decreases in plasma cholinesterase activity were measured in all treatment groups compared with controls, with activity reduced by 49, 47, 60, 73, and 84% at 1, 3, 10, 30, and 90 mg/kg/d, respectively.

Slight but statistically-significant decreases in mean foetal weight was observed at 10 and 90 mg/kg/d,

but not at 30 mg/kg/d, and the toxicological significance of this finding, and its relationship to treatment, was unclear. Statistically-significant decreases in pre- and post-implantation losses were seen in almost all treatment groups compared with controls, but this effect was not considered to be toxicologically significant, and probably reflects higher than normal control values for these endpoints.

An NOEL was not established for this study, based on decreased plasma cholinesterase activity at all doses tested. The NOEL for frank maternotoxicity was 30 mg/kg/d, based on decreases in body weights at 90 mg/kg/d and above. No unequivocal foetal toxicity was demonstrated during this study, and there was no evidence of gross treatment-related malformations.

Rubin Y, Nyska A & Waner T (1987) Pyrinex. Pyrinex teratogenicity study in the rabbit. Life Science Research Israel Ltd., LSRI Report No MAK/103/PYR, dated 15 July 1987. Makteshim Chemical Works no: R-4406 [Makteshim; Submission 11471]

This study was conducted in accordance with US FDA and EPA GLP Guidelines, and testing guidelines of the US EPA (section 83-3) and OECD (no. 414). Doses were selected following a preliminary range-finding study (Report MAK/102/PYR).

Female New Zealand White rabbits (Charles River Italia; aged 4-5 months) were mated one-to-one with stock males (same source and strain). Pyrinex technical (chlorpyrifos; stated purity 96.1%; Makteshim Chemical Works Israel; Batch 58009/9) was formulated fresh daily as solutions in maize oil and given to mated females (14/dosage group) by oral gavage as a daily dose from day 7 to day 19, inclusive, postcoitum, at doses of 1, 9, 81, and 140 mg/kg/d (dosage volume 2 ml/kg/d). The daily doses for animals in the 81 and 140 mg/kg/d groups were prepared directly, and the test formulations for the 1 and 9 mg/kg/d groups were prepared by diluting the 81 mg/kg/d formulation with maize oil. A vehicle control group received maize oil only. Short-term stability of chlorpyrifos in the dosing solutions was tested at 4 h and 24 h after formulation, and the active ingredient was stable in the dosage form used.

On one occasion (day 15), three animals from the 81 mg/kg/d group were accidentally administered a dose of 140 mg/kg/d. At this time, the group was expanded to 21 animals, so that the overdosed animals might be excluded for from the study if such an exclusion was found to be warranted. Two of these three animals were pregnant, without any apparent differences between these animals and the other females treated at 81 mg/kg/d, and the maternal and foetal data for these animals were included in the report.

All animals were examined daily for clinical signs associated with treatment. Any animals found dead or killed *in extremis* were autopsied to determine the cause of death. Animals were weighed on days 0, 3, 7-19, 22, 25, and 29 of gestation, and food consumption was determined twice weekly. Plasma cholinesterase activity was measured before mating and again after at least 10 days of dosing. Females were killed on day 29 and the following recorded: any macroscopic abnormality of the reproductive tract; number of corpora lutea in each ovary; weight of gravid uterus; distribution of live and dead foetuses and distribution of resorption sites in each uterine horn; individual placental weights; individual foetal weight and crown-rump length; sex; external anomalies of individual foetuses. The thoracic and abdominal contents were dissected and examined, and the skull of each foetus was sectioned transversely through the frontal-parietal suture, and the brain examined. Each foetus was used for skeletal staining and evaluation.

Results

No treatment-related mortality was observed during the study. Four animals died during the study, with death attributed to intercurrent disease for one animal at 1 mg/kg/d and another animal at 140 mg/kg/d, and to lung dosing for two animals at 81 mg/kg/d. No treatment-related clinical signs were reported. A range of signs including diarrhoea, nasal discharge, decreased motor activity, few or no faeces, and rales were observed, but these signs were not considered to be related to treatment as the incidence was low and/or similar in treated and control groups, without a consistent dose relationship.

Food consumption and bodyweight: Maternal group mean food consumption was unaffected by treatment. On isolated occasions, statistically-significant increases in food consumption were reported in treated animals, but this finding was not related to dose and was not considered to be toxicologically significant. Group mean body weights and body weight gains were not affected by treatment at doses up to 81 mg/kg/d, with similar growth seen in control and treated animals. At 140 mg/kg/d, body weight gain was inhibited for most of the treatment period (statistically significant; $p<0.01$), though body weight gain was greater than normal ($p<0.001$) after cessation of treatment, and by day 29 the animals in this group had attained body weights similar to those in the control group. At 9 and 81 mg/kg/d, statistically-significant increases in mean body weights were seen, but this finding was not considered to be toxicologically significant.

ChE determinations: Statistically-significant ($p<0.001$), dose-related decreases in plasma cholinesterase activity were seen at all test doses, with activity reduced by 56, 68, 70, and 72% compared with controls, at 1, 9, 81, and 140 mg/kg/d, respectively, after 10 days of dosing.

Reproductive parameters: Statistically-significant decreases in pre-implantation loss were observed at most doses, but this finding was not considered to be toxicologically significant. Statistically-significant ($p<0.001$) increases in post-implantation loss were observed at 9 and 140 mg/kg/d (13.8% and 13.9%) compared with controls (6.5%), but no increase in this effect was observed at 81 mg/kg/d. In the absence of a consistent dose-response relationship, the toxicological significance of this finding was unclear, and no historical control data were supplied for this effect from the testing laboratory. A slight (about 4%) but statistically-significant ($p<0.05$) decrease in mean foetal crown-rump length, and a decrease of 10% in mean foetal weight (non-statistically significant) were observed in the 140 mg/kg/d group. These effects were considered to be related to chlorpyrifos administration.

Foetal observations: A number of foetal anomalies were observed, but these findings were not considered to be treatment-related, as the incidence was generally very low, and there was no relationship to dose. These include: a single foetus at 9 mg/kg/d with missing teeth and hydrocephalus; a single foetus at 9 mg/kg/d with an enlarged aortic arch, rudimentary pulmonary artery and agenesis of interventricular cardiac septum; abnormal flexure of the forelimb (arthrogryposis) in 2 foetuses at 9 mg/kg/d; unilateral hydronephrosis (1-2 animals at all doses including controls); discrete areas of lenticular opacity in two foetuses at 81 mg/kg/d; one foetus with an irregularly shaped thorax at 140 mg/kg/d.

Summary of litter data and foetal observations at necropsy (No. of affected foetuses)

Parameter	0 (control)	1 mg/kg/d	9 mg/kg/d	81 mg/kg/d	140 mg/kg/d
Number of foetuses examined	108	117	126	142	99
Pre-implantation loss %	15.2	13.1	8.9[c]	11.9[a]	11.5[a]
Post-implantation loss %	6.5	8.6	13.8[c]	8.7	13.9[c]
Mean foetal weight (g)	45.4	44.3	43.3	41.8	40.7
Mean Crown-rump length (mm)	97.4	95.9	95.0	94.2	93.2[a]
Teeth absent	0	0	1	0	0
Arthrogryposis, forelimb	0	0	2	0	0
Irregularly shaped thorax	0	0	0	0	1
Hydrocephalus	0	0	1	0	0
Enlarged aortic arch	0	0	1*	0	0
Pulmonary artery rudimentary	0	0	1*	0	0
Agenesis of interventricular cardiac septum	0	0	1*	0	0
Hydronephrosis (bilateral)	2	0	0	4	1
Hydronephrosis (unilateral)	2	2	1	2	1
Diffuse opacity of eye	0	0	0	2	0

* same foetus a: $p<0.05$; b: $p<0.01$; c: $p<0.001$

Skeletal observations: Skeletal examination of the foetuses revealed a number of findings with similar incidence in control and treated groups. On occasion, the incidence of some findings in treated groups was statistically significantly different to controls. In the absence of any consistent dose relationship for these findings, they were not considered to be related to treatment. These include an increased incidence of :irregular ossification of one or more sternebrae 1-4 (9 and 81 mg/kg/d only); reduced or incomplete ossification of hyoid bone (incidence within historical control incidence); and bony plaque adjacent to sternebra. A single instance of multiple malformations of the ribs and lumbar spine was reported at 140 mg/kg/d, in the same foetus described as having an irregularly shaped thorax, and this finding was considered to be an isolated incidence unrelated to treatment. An increased incidence of foetuses with fifth sternebra and/or xiphisternum unossified at 140 mg/kg/d (6/99 compared with 1/108 in controls) was considered to be related to slightly delayed development at this dose, as seen by reduced foetal weight and length in this group. However, given the variability in the incidence of other skeletal observations in treated groups, it was possible that this finding was also due to chance, and was unrelated to treatment.

Summary of skeletal observations at necropsy: number of affected foetuses

Parameter	0 (control)	1 mg/kg/d	9 mg/kg/d	81 mg/kg/d	140 mg/kg/d
Number of foetuses examined	108	117	126	142	99
Reduced or incomplete ossification of interparietal bone	3	4	3	5	2
Interparietal bone unossified	0	1	0	0	3
Reduced or incomplete ossification of hyoid bone	2	10[a]	17[c]	16[b]	8[a]
Irregular ossification of one or more of sternebrae 1-4	1	2	7[a]	8[a]	1

a: p<0.05; b: p<0.01; c: p<0.001

Parameter	0 (control)	1 mg/kg/d	9 mg/kg/d	81 mg/kg/d	140 mg/kg/d
Bony plaque adjacent to sternebra	10	6	2[b]	3[a]	1[b]
Fifth sternebra and/or xiphisternum unossified	1	1	2	3	6[a]
Multiple malformations of ribs and lumbar spine	0	0	0	0	1

a: p<0.05; b: p<0.01; c: p<0.001

Summary: Under the conditions of this study, an NOEL was not demonstrated, based on the inhibition of plasma cholinesterase activity at doses of 1 mg/kg/d and above. The NOEL for frank maternotoxicity was 81 mg/kg/d, based on decreased body weight gain at 140 mg/kg/d. The NOEL for foetotoxicity was 81 mg/kg/d, based on a slight decrease in mean foetal crown-rump length, a decrease in mean foetal weight, and an increased incidence of foetuses with fifth sternebra and/or xiphisternum unossified, at 140 mg/kg/d. No major treatment-related malformations were observed in this study.

8.2 Studies with TCP Metabolite

8.2.1 Rat Developmental Studies

Scortichini BH, Lomax LG, Wolfe EL & Hanley Jr TR (1986a) 3,5,6-trichloro-2-pyridinol: Oral teratology probe study in Fischer 344 rats. The Dow Chemical Company. Study No.HET-K-038278-006, dated March 18, 1986.[Dow: Submission 939 (1988), pp 3:699-708] done. Conducted "in the spirit" of GLP.

This was a dose-ranging study of 3,5,6-trichloro-2-pyridinol (TCP) in bred rats. Groups of 8 to 9 Fischer 344 rats (CRL, NY) were given 0, 43, 75 and 127.5 mg/kg/d TCP (99.7% pure, lot: AGR 143197) in 0.5% aqueous Methocel on days 6 through 15 of gestation, by oral gavage. Matings were one-to-one and the day sperm were observed in a vaginal smear was considered day 0 of gestation.

Rats were observed daily for signs of toxicity, were weighed daily from days 6-16 of gestation, and were sacrificed for necropsy on day 16. Body, kidney and liver weights were recorded, as were the number of corpora lutea, and the number and position of live or resorbed foetuses. Foetuses were not sexed, examined or measured. Uteri of non-pregnant rats were stained with sodium sulphide and examined for evidence of early resorption. Appropriate statistical analysis of data was reported; however individual animal data were not reported.

Results

Clinical signs (perineal soiling) were seen sporadically in all the 127.5 mg/kg/d and some of the 75 mg/kg/d dams. Food and water consumption were unaffected by treatment. Signs of maternal toxicity were limited to reduced body weight gain in the dams of the high-dose group. Relative and absolute liver and kidney weights were unaffected by treatment. There was no observable influence of treatment on reproductive parameters (see Table). The study indicates that maternal toxicity may be seen at doses exceeding 43 mg/kg/d.

Summary of reproductive parameters

Dose (mg/kg/d)		**0**	**43**	**75**	**127.5**
No. of bred females		9	8[c]	9	9
Animals with cholinergic signs		nr	nr	nr	nr
Deaths		0	0	0	0
Total pregnant[a]		9 (0)	6 (1)	6 (0)	7 (0)
No. with litters		9	6	6	7
Maternal body weight gain (g)	days 6-16	27±5	22±9	24±5	19±4[b]
Implantation sites/dam		9.4±2.7	10.7±3.4	10.8±1.7	9.1±4.0
Litters totally resorbed		0	1	0	0
Foetuses/litter		7.1±2.6	8.39±5.4	9.8±1.5	7.0±3.6
implantations resorbed		21/85	14/64	6/65	15/64

nr: not recorded

[a] Numbers in parentheses are pregnancies detected by sodium sulphide stain

[b] Significantly different from control; $p<0.05$

[c] One animal removed due to water deprivation

Hanley Jr TR, Zielke GJ & Lomax LG (1987a) 3,5,6-trichloro-2-pyridinol : Oral teratology study in Fischer 344 rats. The Dow Chemical Company. Study No. K-038278-011, dated July 23, 1987.[Dow: Submission 939 (1988), pp 3:709-923] GLP., QA.

Groups of 32-34 Fischer 344 rats (CRL, NY) were given 0, 50, 100 and 150 mg/kg/d of TCP (99.7% pure; lot: AGR 143197; 4 ml/kg) in 0.5% aqueous Methocel on days 6 through 15 of gestation, by oral gavage. Matings were one-to-one, and the day sperm were observed in a vaginal smear was considered day 0 of gestation. Rats were observed for signs of toxicity daily, were weighed on day 0, daily from days 6-16, and on d 21 of gestation and were sacrificed for caesarean section and necropsy on d 21. Body, uterine, kidney and liver weights were recorded as were the number of corpora lutea, and the number and position of live or resorbed foetuses. Foetuses were sexed, weighed, externally examined and measured. One half of each litter was dissected for evidence of internal malformations, with serial sectioning of the head also performed. Uteri of non-pregnant rats were stained with sodium sulphide and examined for evidence of early resorption. All foetuses were stained with alizarin red-s for skeletal examination. Appropriate statistical analysis of data was reported and individual animal data were presented.

Results

Clinical signs of toxicity were not seen in any group. Slight vaginal bleeding was seen in three animals at d 13 (1 at 50, and 2 at 150 mg/kg/d). Water consumption was generally unaffected by treatment, while food consumption was slightly decreased in the 100 mg/kg/d group and decreased ca. 10% in the 150 mg/kg/d group. Dose-related and significant decreases in body weight gain over the dosing period (days 6-16) were recorded at 100 and 150 mg/kg/d. Changes in the relative and absolute liver and kidney weights were restricted to a significant increase (4%) in relative liver weight at 150 mg/kg/d, consequent to a significant decrease (5%) in body weight at this dose. There was a dose-related trend (not statistically significant) in implantation sites/dam which was reflected in the number of foetuses/litter and probably in gravid uterine weight; this latter trend may also reflect the trend to lower body weight with higher dose. Foetal examination revealed only a small number of malformations in each dose group

including controls. Overall, there was no significant influence of treatment on reproductive parameters or on foetal observations.

Based on decreased body weight gain during treatment, the NOEL for maternal effects was 50 mg/kg/d. The NOEL for foetotoxicity and teratogenic effects was 150 mg/kg/d, the highest dose tested.

Summary of reproductive parameters

Dose (mg/kg/d)	**0**	**50**	**100**	**150**
No. of bred females	34	34	32	33
Animals with cholinergic signs	0	0	0	0
Deaths	0	0	0	0
Total pregnant[a]	30 (0)	27 (1)	26 (0)	26 (0)
No. with litters	30	27	26	25
Maternal body weight gain (g) days 6-16	29±5	26±6	23±6[b]	22±5[b]
Implantation sites/dam	9.7±2.1	9.0±3.4	8.7±2.7	8.4±2.6
Litters totally resorbed	0	0	0	0
Foetuses/litter	9.5±2.0	8.7±3.3	8.5±2.7	8.2±2.7
implantations resorbed	6/290	8/244	5/225	5/211
Sex Ratio (M:F; %)	46:54	43:57	51:49	55:45
Foetal body weight (g)	4.46±0.11	4.49±0.37	4.43±0.20	4.45±0.13
Gravid uterine weight (g)	57±11	52±18	50±15	49±15

[a] Numbers in parentheses are pregnancies detected by sodium sulphide staining
[b] Significantly different from control; $p<0.05$

8.2.2 Rabbit Developmental Studies

Scortichini GH, Lomax LG, Wolf EL & Hanley Jr TR (1986b) 3,5,6-trichloro-2-pyridinol:Oral teratology probe study in New Zealand White rabbits. The Dow Chemical Company, Study No:HET-K-038278-007 [Dow; Submission 939 (1988), pp3:924-935] done. Conducted "in the spirit" of GLP.

This was a dose-ranging study of 3,5,6-trichloro-2-pyridinol (TCP) in inseminated rabbits. Groups of 7 New Zealand White rabbits (Hazelton-Dutchland, Inc., Denver, PA) were given 0, 48.5, 80 and 133.5 mg/kg/d of TCP (99.7% pure, lot: AGR 143197) in 0.5% aqueous Methocel on days 6 through 18 of gestation, by oral gavage; this was designated phase 1 of the test. As there was uncertainty that the Maximum Tolerated Dose (MTD) had been reached in this phase, more dose groups were initiated. An additional 2 groups of 7 animals/group were treated with doses of 128 or 212 mg/kg/d in a similar manner to phase 1 animals; this was designated phase 2 of the test. Female animals were injected with HCG to synchronise oestrus and inseminated 3 weeks later on the presumed day 0 of gestation.

Rabbits were observed daily for signs of toxicity, were weighed daily from days 6-19 of gestation, and were sacrificed for detailed necropsy including eye examination on day 19. Body, kidney and liver weights were recorded, as were the number of corpora lutea, and the number and position of live or resorbed foetuses. Foetuses were not sexed, examined or measured. Uteri of non-pregnant rabbits were stained with sodium sulphide and examined for evidence of early resorption. Appropriate statistical

analysis of data was reported; however individual animal data were not reported.

Significant maternal findings

Phase 1

Dose (mg/kg/d)	**0**	**48.5**	**80**	**133.5**
No. of inseminated females	7	7	7	7
No. of pregnant females	4	4	3	5
Animals with cholinergic signs	nr	nr	nr	nr
Deaths	0	0	0	0
Total pregnant[a]	4 (0)	4 (0)	3 (0)	5 (0)
Maternal body weight gain (g) days 6-19 (mean±S.D.)	223±125	238±80	156±129	94±215
Implantation sites/dam	6.8±5.1	4.8±3.3	7.7±1.5	8.6±1.5
Litters totally resorbed	0	0	0	0
Foetuses/litter	6.3±5.0	4.0±3.5	7.0±1.0	8.4±1.8
implantations resorbed	2/27	3/19	2/23	1/43

[a] Numbers in parentheses are pregnancies detected by sodium sulphide staining

Phase 2

Dose (mg/kg/d)	**0**	**128**	**212**
No. of inseminated females	6[b]	7	7
No. of pregnant females	3	6	5
Animals with cholinergic signs	nr	nr	nr
Deaths	0	0	0
Total pregnant[a]	3 (0)	6 (0)	5 (0)
Maternal body weight gain (g); days 6-19 (mean±S.D.)	140±272	84±116	-212±347
Implantation sites/dam	6.0±2.6	6.0±2.8	9.6±2.5
Litters totally resorbed	0	0	0
Foetuses/litter	6.0±2.6	5.2±3.4	7.6±2.7
implantations resorbed	0/18	5/36	10/48[c]

[a] Numbers in parentheses are pregnancies detected by sodium sulphide staining
[b] One female delivered before necropsy and was removed from study
[c] Statistically different from control

Results

Maternal body weight gain during gestation was depressed. This was not statistically significant, and was highly variable at the highest dose in both phases. Gross pathology examination of all animals did not record any significant findings nor any differences between groups. All litters at 212 mg/kg/d showed resorptions; the percentage of implantations resorbed and the percentage of litters with resorptions was statistically different from the comparable phase 2 control group. There was no observable influence of treatment on reproductive parameters at doses up to 133.5 mg/kg/d. The range-finding study indicated that maternal toxicity and foetal toxicity may be seen in rabbits at doses exceeding 133.5 mg/kg/d.

Hanley Jr TR, Zielke GJ & Lomax LG (1987b) 3,5,6-trichloro-2-pyridinol : Oral teratology study in New Zealand White Rabbits. The Dow Chemical Company. Study No. K-038278-015, dated July 23, 1987.[Dow: Submission 939 (1988), pp 3:936-1083] GLP., QA.

Groups of 16 New Zealand White rabbits (Hazelton-Dutchland, Inc., Denver, PA) were given 0, 25, 100 and 250 mg/kg/d of TCP (99.7% pure, lot: AGR 143197; administered at 2mL/kg) in 0.5% aqueous Methocel on days 7 through 19 of gestation, by oral gavage. Female animals were injected with HCG to synchronise oestrus and inseminated 3 weeks later on the presumed day 0 of gestation.

Rabbits were observed daily for signs of toxicity, were weighed on day 0, daily from days 7-20, and on day 28 of gestation. Animals were sacrificed for caesarean section and detailed necropsy on day 28. Body, kidney, gravid uterus and liver weights were recorded, as were the number of corpora lutea, and the number and position of live or resorbed foetuses. Foetuses were sexed, weighed, externally examined and measured. All foetuses were dissected for evidence of internal malformations. Uteri of non-pregnant rabbits were stained with sodium sulphide and examined for evidence of early resorption. All foetuses were stained with alizarin red-s for skeletal examination. Appropriate statistical analysis of data were reported and individual animal data were presented.

Results

Clinical signs of toxicity were not seen in any group. Water and food consumption were not reported. There was a dose-related decrease in body weight gain over the dosing period (days 7-20) which reached statistically significance at 250 mg/kg/d. There were no treatment-related changes in the relative and absolute liver and kidney weights.

Summary of reproductive parameters

Dose (mg/kg/d)	**0**	**25**	**100**	**250**
No. of inseminated females	16	16	16	16
No. of pregnant females	15	16	14	14
Animals with cholinergic signs	0	0	0	0
Deaths	0	0	0	0
Litters totally resorbed	0	0	1	0
Litters aborted	0	1	0	1
Pregnancies detected by stain[a]	0	0	0	0
Litters carried to term	15	15	13	13
Maternal body weight gain (g) days 7-20 (mean±S.D.)	139±185	84±175	66±161	-67±194[b]
Number of corpora lutea/dam	10.4±1.5	11.5±2.7	11.8±3.0	11.0±2.3
Implantation sites/dam	8.7±2.0	8.5±3.1	8.7±3.2	8.6±1.9
Foetuses/litter	7.8±2.2	8.0±3.3	7.6±3.7	7.6±2.4
implantations resorbed	13/130	7/127	15/122	13/112

[a] Pregnancies detected by sodium sulphide staining

Foetal examination revealed only a small number of malformations in each dose group including controls. Overall, there was no statistically-significant influence of treatment on reproductive parameters or foetal observations. However, the increased incidence of CNS malformations at 100 and 250 (5.1%) mg/kg/d (3.7% and 5.1%, respectively) was suggestive of a possible teratogenic effect of TCP at these doses. The historical control incidence for CNS malformations was quoted as 4/2936 or 0.14%. The malformations included severe dilation of the cerebral ventricles and hydrocephaly. There was no change

in the incidence of minor alterations observed externally, viscerally or upon skeletal examination.

Selected Foetal observations after TCP administration during pregnancy in rabbits

Dose (mg/kg/d)	**0**	**25**	**100**	**250**
Number of Foetuses (litters) examined	117 (15)	120 (15)	107 (13)	99 (13)
Multiple external anomalies*	0	0	0	1 (1)
Cleft palate*	0	0	1 (1)	1 (1)
Micrognathia*	0	0	1 (1)	0
Hydrocephaly	0	0	3 (2)	3 (3)
Cerebral ventricles dilated	1 (1)	0	1 (1)	2 (2)
Total CNS anomalies*	1 (1)	0	4 (2)	5 (4)
Skull; delayed ossification	0	0	2 (2)	0
Skull; irregular ossification	3 (3)	1 (1)	0	2 (2)
Sternebrae; delayed ossification	41 (12)	37 (9)	53 (11)	32 (11)
Sternebrae; fused	0	0	0	2 (1)
Vertebrae; scoliosis*	0	0	2 (1)	1 (1)
Vertebrae; delayed ossification of dentoid process	1 (1)	4 (2)	6 (4)	1 (1)

* Considered to be a major malformations

Based on decreased body weight gain during treatment, the NOEL for maternal effects was considered to be 100 mg/kg/d. The NOEL for foetotoxicity and teratogenic effects was considered to be 25 mg/kg/d, based on increased CNS malformations at 100 mg/kg/d and above.

9. GENOTOXICITY

9.1 Gene Mutation Assays

Shirasu Y, Moriya M & Ohta T (1980) Mutagenicity test of chlorpyrifos in bacteria. Dow Chemical Pacific report GHF-R-47, dated 14 July 1980. [Dow; Submission 238, vol. 5, A3162/5, B41]

No GLP or Test Guideline statements were made regarding this study.
The mutagenic potential of chlorpyrifos technical (source not stated; stated purity 95.83%) was examined (in 2 studies) using the repair-proficient strain (H17) and recombinational repair-deficient mutant strain (M45) of *B. subtilis*, and the reversion test with *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 (Ames). The Ames test was carried out in the presence and absence of metabolic activation (S-9).

In the rec-assay tests, 0.02 ml of the test compound was dissolved in dimethylsulfoxide (DMSO), soaked into a filter paper, and placed on the culture plate to give doses of 20, 100, 200, 500, 1000, and 2000 µg/plate. Kanamycin (10 µg/plate) was the negative control, DMSO was the vehicle control, and Mitomycin C (0.1 µg/plate) was the positive control. For the reverse mutation assay, the chlorpyrifos test doses were 10, 50, 100, 500, 1000, and 5000 µg/plate, with DMSO as the vehicle control. The positive control (with and without S-9) was 2-amino-anthracene (10 µg/plate; WP2 hcr and all *Salmonella typhimurium* strains). Without S-9, the positive controls were: ß-propiolactone (50 µg/plate; TA1535), 9-aminoacridine (200 µg/plate; TA1537), 2-nitrofluorene (50 µg/plate; TA1538), and AF-2 (0.25 µg/plate, WP2 hcr; 0.05 µg/plate, TA100; 0.5 µg/plate, TA98).

Results

No increases in the inhibitory area was observed in the rec-assay at any dose of chlorpyrifos.

No statistically-significant or dose-related increases in the incidence of reversions were observed in any of the *Salmonella typhimurium* strains at any dose of chlorpyrifos. The positive control compounds gave the expected large increases in reversion rate in all strains.

Under the conditions of this study, chlorpyrifos was negative for genotoxicity, and did not induce mutations in bacteria at doses up to 5000 µg/plate.

Bruce RJ & Zempel JA (1986a) Chlorpyrifos: Evaluation in the Ames *Salmonella*/Mammalian-Microsome Mutagenicity Assay. Dow Chemical Company report TXT:K-044793-075, dated February, 1986. [Dow; Submission 11462, reference 80; submission 238, vol 5, A3162/5, B41]

A Quality Assurance Statement was issued for this study. No statement on GLP or Test Guideline compliance was noted.

The genotoxicity potential of chlorpyrifos (Dow; lot no. AGR214637 MM820905-610; stated purity 95.7%) was assessed in an Ames *Salmonella*/mammalian microsome reverse mutation assay, using tester strains TA98, TA100, TA1535, TA1537, and TA1538. The test material was dissolved in dimethyl sulfoxide (DMSO) at 10, 31.62, 100, 316.2, and 1000 µg/ml, and 0.1 ml of the test solutions were added to each culture, giving test doses of 1, 3, 3.162, 10, 31.62, and 100 µg/plate. Spontaneous reversion rates were recorded using only bacterial culture and 0.6 ml of Ames' buffer or 0.5 ml of S-9 activation mixture plus 0.1 ml Ames' buffer. The S-9 rat liver homogenate, from Aroclor 1254-induced male Sprague-Dawley rats, was obtained from Sitek Research Laboratories, USA.

The negative (vehicle control) plates were identical to the test plates, except for the absence of chlorpyrifos. Positive control chemicals were prepared in DMSO, except for quinacrine mustard, which was prepared in distilled water. The positive controls were as follows: n-methyl-n'-nitro-n-nitrosoguanisine, 2 µg/plate, TA100 and TA1535, -S9; 2-nitrofluorene, 100 µg/plate, TA98 and TA1538, -S9; quinacrine mustard, 10 µg/plate, TA1537, -S9; 2-anthramine, 5 µg/plate, TA100 and TA1535, +S9; 2-acetylaminofluorene, 100 µg/plate, TA98 and TA1538, +S9; 8-aminoquinoline, 25 µg/plate, TA1537, +S9.

The criteria for a positive finding for mutagenicity in this assay was if the mean number of revertant colonies observed was at least 3-fold higher than the mean of the negative control, and if a dose-response relationship was demonstrated over several concentrations. If a chemical produced reproducible reversion rates greater than 2x but less than 3x over controls, the results were considered to be equivocal or inconclusive. All assays, along with positive and negative controls, were conducted in triplicate.

Results

Cytotoxicity was observed at 100 µg/plate in tester strains TA100, TA1535, TA1537, and TA1538, characterised by poor lawn and/or overgrown background colonies, and at this dose the test material also formed a precipitate in the medium. In TA98, the test material also precipitated at 100 µg/plate, in the absence of toxicity.

No increases in mean revertant rates compared with negative controls were observed at any test dose in any of the strains, and the revertant rate was also similar to the spontaneous reversion rate for all strains. The positive control compounds induced the expected high reversion rates in all strains.

Under the conditions of this assay, chlorpyrifos was negative for genotoxicity, with no increases in the rate of mutations in bacterial strains at doses up to 100 µg/plate.

Anon (1996g) Reverse gene mutation study of chlorpyrifos tech (95%) in bacteria by Ames *Salmonella*/microsome test. Jai Research Foundation, Department of Microbiology, India. Report 5/JRF/MIC/96, dated 17 April 1996. [Submission 11513]

This study was conducted in accordance with OECD Guidelines 471. No statement was made regarding its GLP status.

The test material (chlorpyrifos technical; Mitsu Industries, India; Batch no. 041195; stated purity 95%) was tested for its ability to induce reverse mutations in various strains of *Salmonella typhimurium* (TA1537, TA1535, TA98, TA100) in the presence and absence of an exogenous metabolic activation system (S9). The S9 mix was obtained from Specific Pathogen Free male CD rats (Defence Research and Development Establishment, India) induced with Aroclor 1254.

In a dose-finding study, six concentrations of the test material (1, 10, 100, 500, 1000, and 10000 µg/plate) were dissolved in dimethyl sulfoxide (DMSO) and tested for mutagenicity. In the main study, test doses of 0.01, 0.1, 1, 5, 10, and 100 µg/plate were used in the presence and absence of S9. The positive control substances were as follows: sodium azide at 20 µg/plate for strains TA1535 and TA100; 2-amino-fluorine at 20 µg/plate for all strains in the presence of S9; 4-nitro 1-2-phenylene diamine at 20 µg/plate for strain TA98 in the absence of S9; and 9-aminoacridine at 20 µg/plate for strain TA1537 in the absence of S9.

Results

The test material was found to be cytotoxic at doses above 10 µg/plate. The positive control substances induced the expected increases in mutation rates, with and without S9. At plate concentrations of 0.1 µg/plate and below, the revertant frequencies, with and without S9, were similar to the solvent controls, and the revertant frequency did not increase by 2-fold, which was a criterion for a positive finding in this assay. At doses of 1 µg/plate and above, dose related increases in the revertant frequency (2-fold or greater than vehicle control frequency) were seen in all tester strains. The presence or absence of S9 made no difference to the number of revertant colonies in any of the tester strains. Under the conditions of this study, this finding was indicative of a positive genotoxicity finding. However, the number of revertant colonies induced by chlorpyrifos technical was always lower than that seen with the positive control substances (ranging from about 2-fold lower in TA1535 to 50-fold lower in TA98), and

the statistical significance of these increases (another criterion for a positive genotoxicity finding) was not provided.

Loveday KS, Findlen KMB & Yadlon S (1987) Evaluation of Pyrinex in the Ames mutagenesis assay. Arthur D Little Inc, USA. Study completed 13 April, 1987. ADL reference: 59487-00. Report dated 7 August, 1987. MCW no: R-4659 [Makteshim; Submission 11471]

This study was conducted in compliance with US EPA GLP regulations (40 CFR Part 792) and guidelines (84-2), to investigate the potential of the test material (chlorpyrifos; stated purity 96.8%, Makteshim; lot no. 489205) to induce mutations in *Salmonella typhimurium* (strains TA98, TA100, TA1538, TA1537, TA1535) using an *in vitro* mutagenesis assay. The material was tested in triplicate at 30, 100, 300, 3000, and 10,000 µg/plate. The solvent was dimethylsulfoxide (DMSO; 100 µg/plate ± S9), and positive controls were 9-aminoacridine (50 µg/plate; -S9), sodium azide (10 µg/plate; -S9), 2-nitrofluorene (10 µg/plate, -S9), 2-anthramine (5 and 10 µg/plate, +S9).

Under the conditions of this study, the test material was not mutagenic in bacteria at doses of up to 10,000 µg/plate. The test material was not toxic, nor did it precipitate at these concentrations. No statistically-significant or dose-related increases in the mean total revertants/plate were observed at any concentration, and no cytotoxicity was reported. The positive control substances induced the expected increases in revertants/plate.

Anon (1996c) Mutagenicity evaluation of chlorpyrifos technical in the point mutation - by Ames *Salmonella typhimurium* test system. Fredrick Institute of Plant Protection and Toxicology, India Project no. 05-041-96. Report no. 3049, dated 10 February, 1996. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

The genotoxicity potential of technical chlorpyrifos (Ficom Organics, India; stated purity 96.2%; batch no. C503085) was assessed in two experiments using a bacterial reverse-mutation assay in *Salmonella typhimurium* strains TA98, TA1537, TA1538, TA100, and TA1535. The test material was mixed with dimethyl sulfoxide (DMSO), with serial dilutions made to achieve test concentrations of 5, 500, and 5000 µg/plate in 0.1 ml of DMSO. These test concentrations were assayed with and without metabolic activation, provided by S9 mix obtained from an Aroclor 1254-induced Sprague-Dawley rat. Several positive control compounds were tested, namely 2-aminoanthracene (4 µg/plate) and 2-nitrofluorene (2 µg/plate) for TA98; 2-aminoanthracene (4 µg/plate) and sodium azide (0.5 µg/plate) for TA100 and TA 1535; 2-aminoanthracene (4 µg/plate) and 9-aminoacridine (50 µg/plate) for TA 1537; and 2-aminoanthracene (4 µg/plate) and 2-nitrofluorene (5 µg/plate) for TA 1538. In each instance, for each strain, the first positive control substance was used in the presence of S9, and the latter in the absence of S9.

Results

In each case, positive controls gave the expected increase in the number of revertant colonies in each of the bacterial strains. In TA1537, the presence of the test material resulted in an increase in revertants at all dose levels, in the absence of S9 only. However, this increase was small compared with positive controls, and no dose-response relationship was demonstrated, with the number of revertant colonies at 5, 500, and 5000 µg/plate being 35, 30, and 39, respectively, compared with 9 colonies in controls, and 87 colonies with the positive control compound. In all other test groups, the number of revertant colonies was similar to controls.

Under the conditions of this study, the test material was not a bacterial mutagen *in vitro* at doses up to 5000 µg/plate.

Mendrala AL (1985) Evaluation of chlorpyrifos in the Chinese hamster ovary cell-hypoxanthine (Guanine) phosphoribosyl transferase (CHO/HGPRT) forward mutation assay. The Dow Chemical Company report HET-K-044793-072, dated 3 September 1985. [Dow; Submission 238, vol 5, A3162/5, B41]

The genotoxic potential of chlorpyrifos (Dow; Lot no. MM 820905-610; stated purity 95.7%) was assessed in the Chinese hamster ovary cell-hypoxanthine phosphoribosyl transferase (CHO/HGPRT) assay, in the presence (+S9) and absence (-S9) of metabolic activation. The chlorpyrifos was dissolved in dimethyl sulfoxide (DMSO) and added to the serum-free medium to give test material concentrations of 10, 20, 25, 30, 40 and 50 µM. Chlorpyrifos was observed to form a precipitate upon introduction of the test chemical solution to the culture media at 30, 40, and 50 µM in the assay without S-9 activation. The final concentration of DMSO in all cultures was 1% v/v. The negative control cultures were treated with 1% DMSO only. In the absence of S9, the positive control was ethyl methanesulfonate (EMS; 30 µM), and in the presence of S9, the positive control compound was 3-methylcholanthrene (3-MC; 18.6 µM). For each assay the number of mutants/million cloneable cells was calculated. For a positive genotoxicity finding, compounds needed to elicit a significant concentration-dependent increase over at least 3 concentrations. Increases at a single concentration required confirmation in a repeated assay.

Results

No significant dose-related increases in mutation frequency, nor significant and reproducible increased mutation frequency at high toxicity levels were observed at any test dose in this study. The positive control compounds gave the expected large increases in mutation frequency. Under the conditions of this study, chlorpyrifos was not mutagenic in the CHO/HGPRT assay, in the presence or absence of S9 metabolic activation, *in vitro*.

Tu AS (1987) CHO/HGPRT *in vitro* mammalian cell mutation assay on Pyrinex (chlorpyrifos). Arthur D Little Inc, USA. Study completed 4 May, 1987. ADL reference: 59487. Report dated 4 August, 1987. MCW no: R-4654 [Makteshim; Submission 11471]

This study was conducted in compliance with the US EPA GLP regulations.

To assess the potential of Pyrinex (chlorpyrifos) to induce mutations in Chinese hamster ovary (CHO) cell at the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) locus, the material (technical chlorpyrifos; stated purity 96.8%; Makteshim Chemical Works; batch not stated) was tested using the CHO/HGPRT *in vitro* mutation assay. Chlorpyrifos was dissolved in dimethylsulfoxide (DMSO) at 10 or 15 mg/ml in the assay without S9 metabolic activation, and at 60 or 200 mg/ml in the assay with S9 metabolic activation. All subsequent dilutions were made using DMSO. The test sample volumes were 10 µl and 25 µl, with and without metabolic activation. The S9 fraction was prepared from the liver of Aroclor 1254 induced Sprague-Dawley rats. The negative control was DMSO, and the positive controls were ethylmethanesulfonate (EMS; 6.2 mg/ml in phosphate buffered saline (PBS)) in the nonactivated assay, and dimethylnitrosamine (DMN; 10 mg/ml in PBS) in the activated assay.

In a range-finding study, CHO cells were treated with the test material at doses ranging from 1.5 - 3748 µg/ml (without S9; 16 h exposure) and 1.5 - 5000 µg/ml (with S9; 5 h exposure). In the main study, cells were treated in two experiments without S9, at doses of 5, 10, 25, 50, or 75 µg/ml and 5, 10, 20, 30, 40, and 50 µg/ml, respectively, with a 16-h exposure period. The solvent control was 0.5% DMSO, and the positive control was EMS at 248 µg/ml. In two experiments in the presence of S9, the doses were 30, 50, 100, 300, and 1000 µg/ml. The solvent control was 0.5% DMSO and the positive control was DMN at 500 and 1000 µg/ml. The cytotoxicity of the test material was determined in a clonal assay conducted in parallel with the mutation assay.

Results

In the range-finding study, no colonies survived at doses of 150 µg/ml and above (-S9), but survival was 70% or greater at all doses in the activated assay. In the nonactivated assay, the test material precipitated in culture medium at 50 µg/ml and above.

In the assay conducted without metabolic activation, the test material was toxic at 50 µg/ml in the first experiment, resulting in a survival fraction of 6% at this dose level. In the second experiment, without S9, the surviving fraction was 79% at 50 µg/ml. No increase in the mutation frequency was observed with chlorpyrifos at any dose level, either in the presence or absence of S9. The positive controls gave the expected increases in mutation frequency, both with and without S9.

Under the conditions of this assay, the test material was not mutagenic in Chinese hamster ovary cells, with or without metabolic activation.

9.2 Chromosomal assays

Sobti RC, Krishan A & Pfaffenberger CD (1982) Mutation Research, 102 (1982) 89-102. Luxembourg Industries, Dossier file no. 5.4.1/01. [David Gray; Submission 11475] public domain

In this study, the cytokinetic and cytogenetic effects of a range of organophosphate insecticides, including chlorpyrifos, were tested on human lymphoid cells *in vitro*. Chlorpyrifos (Dursban; purity not stated) was added to 10 ml cultures of LAZ-007, a human lymphoid cell line of B-cell origin, at doses of 0.02,

0.2, 2, and 20 µg/ml, in triplicate. Control cultures were incubated with 0.1% (final concentration) of ethyl alcohol.

To test the effect of metabolic activation on the genotoxic potential of organophosphate insecticides, including chlorpyrifos, the compounds were assayed in triplicate in human lymphoblasts at 20 µg/ml, with and without S9 metabolic activation (phenobarbital-induced rat-liver S9 mix from Litton Bionetics, USA). Negative and vehicle (0.1% ethyl alcohol) were also tested, and the positive control compound (cyclophosphamide) was tested at 0.1 µg/ml.

In the absence of metabolic activation, the mean Sister Chromatid Exchange (SCE) frequency (and range) in test cultures was 8.42 (1-12), 9.07 (6-12), 10.67 (3-16), and 12.92 (4-19), respectively, at the doses listed above. At 2 and 20 µg/ml, the SCE frequency was statistically significantly different to controls (mean 6.96, range 0-12; $p<0.01$). However, for a positive genotoxicity finding, the frequency of SCEs must be at least double the control frequency, and so chlorpyrifos was not positive under the conditions of this assay.

In the study with activation, Dursban (purity not stated) did not cause an increase in the SCE frequency compared with controls, with or without S9. Cyclophosphamide increased the SCE frequency by 2-fold compared with negative controls in the presence of S9.

Loveday KS (1987) In vitro chromosomal aberration assay on Pyrinex (chlorpyrifos). Arthur D Little Inc., USA. Study completed 9 September, 1987. ADL reference: 59487. Report dated 2 October, 1987. MCW no: R-4660 [Makteshim; Submission 11471]

This study was conducted in compliance with US EPA GLP regulations (40 CFR Part 792) and guidelines (84-2), to investigate the potential of Pyrinex (chlorpyrifos; purity 96.8%; Makteshim; Batch no. 489205) to induce chromosomal breaks and aberrations in Chinese hamster ovary (CHO) cells. The test material was dissolved in dimethylsulfoxide (DMSO) at 20 and 60 mg/ml to make stock solutions, and subsequent dilutions were made with DMSO. Stock solutions of positive control substances were made, with Mitomycin C used in the assay without metabolic activation, and cyclophosphamide used for the assay in the presence of metabolic activation. The S9 microsomal fraction was prepared from the liver of Aroclor-induced Sprague-Dawley rats.

Pyrinex was tested in two non-activated (without S9) assays of 10 and 19 h, and in one 19-h and two 10-h activated assays (with S9).

The concentrations tested in the absence of S9, (19-h exposure) were 0.975, 1.47, 2.93, 4.89, 9.75, 14.7, 29.3, 48.9, 97.5, and 147 µg/ml, and 1.56, 3.12, 5.2, 10.4, 15.6, 31.2, 52, 104, and 156 µg/ml (10-h exposure).

In the presence of S9, the concentrations tested in the 19-h assay were 9.75, 14.7, 29.3, 48.9, 97.5, 147, and 293 µg/ml. For the 10-h assay, two different sets of test concentrations were used: 1, 1.5, 3, 5, 10, 15, 30, 50, and 100 µg/ml, and 2.95, 4.95, 9.85, 14.8, 29.6, 49.4, 98.5, and 296 µg/ml.

Results

Absence of S9: In the absence of S9, the highest concentrations (104 µg/ml for 10 h; 97.5 µg/ml for 19 h) resulted in an absence of cells (or an absence of metaphase cells) and these doses were considered to be cytotoxic. At 52 µg/ml (10 h) there was a statistically-significant increase in gaps only, with 31 and 36% of cells having aberrations (including gaps) in the duplicate assays at this dose level, compared with solvent control levels of 10 and 15% of cells with aberrations (including gaps). No increases in any other aberrations were seen following this incubation period at other dose levels, and there was no increase in aberrations (excluding gaps) at any test concentration. At 52 µg/ml, the incidence of cells with aberrations (excluding gaps) was 7 and 6%, which was identical to the solvent control incidence. In the 19-h assay, no increase in aberrations, including gaps, was seen at any concentration. The positive control (mitomycin C; 5 µg/ml at 10 h/0.3 µg/ml at 19 h) resulted in the expected increases in chromosomal aberrations in the absence of metabolic activation. The increase in gaps alone at the highest analysed dose at 10-h was not considered to constitute a positive response in this assay.

Presence of S9: In the presence of metabolic activation, the high-dose level for the first 10-h assay were cytotoxic (15 µg/ml), with no metaphase cells observed. There were statistically-significant increases in the percentage of cells with aberrations (including gaps) in duplicate assays at 3 (17 and 15%) and 10 µg/ml (18 and 19%) compared with solvent controls (3 and 4%). The incidence of these effects was lower than that seen with the positive controls (cyclophosphamide 50 µg/ml) with 32 and 44%. For aberrations (excluding gaps), the incidence in treated groups did not reach statistical significance, with 11 and 12% at 3 µg/ml, and 4 and 7% at 10 µg/ml. No dose-response relationship was demonstrated for these findings, and the assay was repeated. In the repeat 10-h experiment, no increases in chromosomal aberrations (including or excluding gaps) was observed. As the increase in aberrations (including gaps) in the first assay was not dose-related, and was not reproducible in a repeat experiment, this finding was not considered to constitute a positive genotoxicity outcome under the conditions of this assay.

In the 19-h assay with S9, no statistically-significant or dose-related increase in the incidence of aberrations (including or excluding gaps) was observed at doses up to 97.5 µg/ml. The test material was cytotoxic at 147 µg/ml, with few metaphase cells observed at this dose.

Under the conditions of this assay, the test material was considered to be negative for genotoxicity, at doses up to 52 µg/ml without metabolic activation, and up to 97.5 µg/ml with metabolic activation.

Linscombe VA, Mensik DC & Clem BA (1992) Evaluation of Chlorpyrifos in an *in vitro* chromosomal aberration assay utilizing rat lymphocytes. Dow Chemical Company report TXT:K 044793-092, dated 29 January, 1992. [Dow; Submission 11462, reference 81]

This study was conducted in accordance with GLP standards of the US FDA (21 CFR Part 58, 1 April 1989), US EPA (Title 40 CFR Part 160, 17 August 1989), Japan MAFF (59 Nohsan, Notification no. 3850, 10 August 1984), and OECD (Paris 1982). The study was conducted to satisfy guidelines of the OECD (1983), US EPA (1990), MAFF (1988), EEC (1984) and UK EMS.

The test material (chlorpyrifos, Dursban F; Dow; Lot no. AGR 273801 (MM-890115-616); stated purity 98.6%) was tested for its potential to include chromosomal aberrations *in vitro* in cultured rat

hepatocytes (obtained from male Sprague-Dawley rats; outbred Crl:CD BR; Charles River USA; aged approximately 12-20 weeks), with and without S-9 metabolic activation. Stock solutions of the test material were prepared in dimethyl sulfoxide (DMSO), then further diluted with the treatment medium (1:100) until the desired test concentrations were achieved. Mitomycin C was used as a positive control chemical in the absence of metabolic activation (0.5 µg/ml), and cyclophosphamide (4.2 µg/ml) was the positive control in the presence of S-9. The vehicle control was DMSO (1% v/v).

Two assays were conducted. In the first assay, cultures were treated with the test material (16.7, 50, 167, 500, 1667, and 5000 µg/ml), and cultures were harvested after 24 h. In the second assay, the test concentrations were 5, 16.7, 50, and 167 µg/ml, and cultures were harvested approximately 24 and 48 h after termination of treatment. Positive controls were harvested 24 h after treatment. One hundred metaphases/replicate (200 cells/treatment) were examined at each test material concentration, and 50 metaphases/replicate (100 metaphases/treatment) were examined for the positive controls. Those cells with 10 or more aberrations/cell were classified as severely damaged cells, and gaps were not included in calculations of total cytogenetic aberrations. The concentration of test material in the stock solutions was verified by analytical methods.

Results

In the first assay, doses of chlorpyrifos of 500-5000 µg/ml (without S-9) and 167-5000 µg/ml (with S-9) were toxic, and the mitotic index was 0 at these dose levels.

In the absence of S-9, no increase in total aberrations or in the number of cells with aberrations (excluding gaps) was observed at test doses of 16.7, 50, or 167 µg/ml, compared with vehicle controls. In the presence of S-9, no increase in total aberrations or the number of cells with aberrations (excluding gaps) was observed at test doses of 16.7 or 50 µg/ml, compared with vehicle controls. The positive control substances induced the expected increase in these effects, with and without metabolic activation.

In the second assay, in the absence of S-9 the mitotic index was decreased at 167 µg/ml at 24 h, and no mitotic indexes were measurable at 50 and 167 µg/ml at 48 h. In the presence of S-9, the mitotic index was 0 at 167 µg/ml at 24 and 48 h. No increases in the total chromosomal aberrations or in the number of cells with aberrations (excluding gaps) were observed at 5, 16.7, or 50 µg/ml in the presence or absence of S-9. The positive control compounds gave the expected results.

Under the conditions of this study, chlorpyrifos was not genotoxic at doses up to 167 µg/ml without S-9 or 50 µg/ml with S-9 in an *in vitro* chromosomal aberration assay using cultured rat lymphocytes.

Gollapudi BB, Linscombe VA & Wilkerson JE (1985) Evaluation of chlorpyrifos in the mouse bone marrow micronucleus test. The Dow Chemical Company report TXT-K-044793-067, dated August 1985. [Dow; Submission 238, vol. 5, A3162/5, B41]

A Quality Assurance Statement was issued for this study.

The genotoxic potential of chlorpyrifos technical (Dow; AGR214637, lot no. MM 820905-610; purity 95.7%) was assessed using bone marrow cells from CD-ICR BR mice (aged approximately 8 weeks; 5/sex/group; Charles River, USA). All doses of chlorpyrifos were administered in corn oil by single oral

gavage (0,7,22 and 70 mg/kg; dose volume 6 ml/kg) and bone marrow samples were taken at 24 and 48 h after dosing (positive controls 24 h only). Negative control animals received corn oil (dose volume 6 ml/kg) and positive control animals received cyclophosphamide (120 mg/kg; dose volume 12 ml/kg; distilled water vehicle). Chlorpyrifos did not induce micronucleated polychromatic erythrocytes, above control levels, at any of the dose levels tested. Positive controls produced expected results.

Under the conditions of this study, chlorpyrifos was negative for genotoxicity, and did not induce a significant increase in the frequencies of micronucleated bone marrow polychromatic erythrocytes when given as a single oral dose to male and female CD1 (ICR) BR mice.

Anon (1996d) Mutagenicity evaluation of chlorpyrifos technical in the mouse micronucleus assay. Fredrick Institute of Plant Protection and Toxicology, India Project no. 05-046-96. Report no. 3054, dated 19 February, 1996. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

The genotoxicity potential of technical chlorpyrifos (Ficom Organics, India; stated purity 96.2%; batch no. C503085) was assessed using a micronucleus test in Swiss albino mice. The test material was mixed with vegetable oil and given to male and female mice (Fredrick Institute, India; 25 g body weight) by oral gavage.

In a pilot study, conducted to determine the dose levels for the main study, mice (number not stated) were given the test material at doses of 60 or 80 mg/kg, with a dose volume of 10 ml/kg. These animals were killed 24 h later, and bone marrow smears prepared. All mice died within 24 h of administration of the 80 mg/kg dose, and 60 mg/kg was chosen as the high dose for the main study.

In the main study, the test material (dose volume 10 ml/kg) was given to groups of male and female mice on two consecutive days, at daily doses of 0 (vegetable oil control; 18/sex), 15 (6/sex), 30 (6/sex), and 60 (18/sex) mg/kg. A positive control group (6/sex) received a single dose of chlorambucil in 10% aqueous ethanol (30 mg/kg). Animals were inspected daily for signs of toxicity. Six animals/sex/dose were killed 24 h after treatment. Further lots of 6 mice/sex from control and high dose groups were killed after 48 and 72 h.

Bone marrow cells were plated, and examined for micronucleated cells.

Results

No signs related to treatment were observed during the study. No increase in the frequency of micronucleated polychromatic cells was observed in any of the test groups. After 24 h treatment, the mean incidence of micronucleated polychromatic erythrocytes per thousand scored (and standard deviation) was 0.70 $\pm$0.61 in vehicle controls, with a range of 0-1.7. The incidence (and range) was 0.67 $\pm$ 0.56 (0-1.8), 0.85 $\pm$ 0.94 (0-2.9), and 0.77 $\pm$ 0.79, (0-2.2) at 15, 30, and 60 mg/kg chlorpyrifos, respectively. In the positive control group, the mean incidence was 50.39 $\pm$ 10.27 (range

32.5-65.0). For positive controls, this value was highly statistically significant compared with vehicle controls (0.01>p>0.001). At 48 and 72 h, vehicle control and high dose values were also similar to each other.

Under the conditions of this study, the test material did not induce genotoxicity in a mouse bone marrow micronucleus assay, with doses up to 60 mg/kg/d.

Anon (1996e) Mutagenicity evaluation of chlorpyrifos technical in the mouse bone marrow cytogenetic assay. Fredrick Institute of Plant Protection and Toxicology, India Project no. 05-312-95. Report no. 3076, dated 23 February, 1996. [National Resources; Submission 11463]

A Quality Assurance Statement was issued for this study, stating that the study was conducted as per Gaitonde Committee guidelines and protocols. No information was provided on the GLP status of this study.

The genotoxicity potential of technical chlorpyrifos (Ficom Organics, India; stated purity 96.2%; batch no. C503085) was assessed using an *in vivo* bone marrow cytogenicity assay in male and female Swiss albino mice (Fredrick Institute, India; weight 25 g).

In a pilot study, mice (3/sex) received a single dose of the test material in vegetable oil at either 20 or 15 mg/kg (dose volume not stated), then were killed 24 h later. Examination of bone marrow preparations revealed marginal to severe mitotic depression at both of these dose levels.

Based on these findings, in the main study the test material was mixed in vegetable oil, and given to groups of mice (5/sex/dose) by oral gavage either as a single dose, or daily for 5 days, at doses of 0.6, 3, and 15 mg/kg/d (dose volume not stated). Similarly, a vehicle control group received vegetable oil only (10 ml/kg). A positive control group (5/sex) received a single oral gavage dose of triethylenemelamine (TEM) in distilled water at a dose of 0.5 mg/kg.

Results

In the pilot study, 1/3 males and 1/3 females given a dose of 20 mg/kg died within 1 h of administration. Surviving animals displayed restlessness, but were normal within 4-6 h. At 15 mg/kg, no overt signs of toxicity were observed.

In the main study, no mice displayed signs of toxicity. The percentage of numerical chromosomal aberrations scored in treated groups (males and females) after a single dose were 0.36 and 0.35 at 0.6 mg/kg, 0.55 and 0.54 at 3 mg/kg, and 0.55 and 0.73 at 15 mg/kg, compared with 0.35 and 0.35 in vehicle controls, and 8.18 and 6.91 in positive control animals. After repeated exposure, these figures were 0.35 and 0.52 at 0.6 mg/kg, 0.53 and 0.55 at 3 mg/kg, and 0.35 and 0.35 at 15 mg/kg, compared with 0.35 and 0.18 in vehicle controls.

Similarly, the frequency of structural aberrations was similar in all treated and vehicle control groups, while the positive control group displayed the expected increase in these findings. The mitotic index was similar in vehicle control and treated groups.

Under the conditions of this study, the test material did not cause an increase in chromosomal aberrations in mice *in vivo* at daily doses up to 15 mg/kg.

Bloom SE, Muscarella DE & Schaefer OP (1982) Toxicological evaluation of the insecticide chlorpyrifos: Assays for genetic damage and development toxicity. Department of Poultry and Avian Sciences, Cornell University, USA. Dow Chemical Company, study A1A-213, dated 2 February, 1982. [Dow; Submission 11462, reference 85; submission 238: pp 2.2088-2.2119 vol. 5, A3162/5, B41]

Muscarella DE, Keown JF & Bloom SE (1984) Evaluation of the genotoxic and embryotoxic potential of chlorpyrifos and its metabolites in vivo and in vitro. Environ Mutagen 6(1): 13-23

Chlorpyrifos (Dow; purity not stated) was tested for its genotoxicity potential in a number of assays. No statement regarding GLP status or test guidelines was made in these reports.

The genotoxicity and embryotoxicity of chlorpyrifos and two metabolites were evaluated using a chick embryo assay, Chinese hamster ovary cells, and by examining blastocysts from superovulated cows crossed to chlorpyrifos-treated bulls.

In an *in vitro* cytogenicity assay, chlorpyrifos (Dow; purity not stated) and its metabolites 3,5,6-trichloro-2-pyridinol and diethyl-3,5,6-trichloro-2-pyridyl were tested for their ability to induce Sister Chromatid Exchanges (SCEs) in Chinese Hamster Ovary (CHO) cells. The test materials were investigated at 1, 10, and 100 µg/ml, and a solvent control (acetone) and positive control (methyl methanesulfonate (MMS; 8.6 µg/ml) were also incorporated into the assay.

In an *in vivo* cytogenetics assay in chick embryos, the test material (in 10 µl of acetone) was injected into Cornell K-strain eggs (containing embryos) at doses of 1.11, 11.1, 111, 1110, and 2220 µg/embryo. The chlorpyrifos metabolites 3,5,6-trichloro-2-pyridinol and diethyl-3,5,6-trichloro-2-pyridyl were also tested. Chromosome preparations were prepared and Sister Chromatic Exchanges (SCEs) were scored in the first five pairs of macrochromosomes of each metaphase cell. Where possible, 25 metaphases were analysed from each embryo, and 8 embryos per treatment were examined. The positive control was ethyl methanesulfonate (EMS) at a dose of 240 µg/embryo.

Results

In the *in vitro* assay, the mean number of SCEs/cell was similar in control and treated cells. The positive control resulted in the expected increase in SCEs/cell. Chlorpyrifos was cytotoxic at 100 µg/ml. Under the conditions of this study, chlorpyrifos did not induce chromosomal damage *in vitro* in Chinese Hamster Ovary cells at doses up to 100 µg/ml.

In the *in vivo* assay statistically-significant increases in mortality were observed at 1110 and 2220 µg/embryo, with 8/22 and 9/13 embryos dead at these doses, respectively, compared with 2/21 in acetone controls. The pyridinol metabolite was toxic at 1110 µg/embryo, and the pyridyl metabolite at 111 and 1110 µg/embryo. Higher doses were not tested for the metabolites. No increase in the mean number of SCEs was observed at any dose of the test materials compared with acetone controls. The

positive control compound gave the expected increase in SCEs. Under the conditions of this study, chlorpyrifos and its two major metabolites did not induce chromosomal damage *in vivo* in chick embryos at doses up to 1110 µg/embryo.

Studies of bovine blastocysts obtained from superovulated cows crossed with Dursban 44-treated bulls did not reveal evidence of chromosome aberrations or developmental anomalies associated with pesticide application. The report noted, however, that the reproductive performance of breeders may have been subnormal as a result of severe poisoning.

McClintock ML & Gollapudi BB (1989a) Evaluation of chlorpyrifos in the mouse bone marrow micronucleus test. Dow Chemical Company study TXT:K 044793-067A, dated 22 September 1989. [Dow; Submission 11462, reference 82; Submission 11464, reference 5]

This study was conducted in accordance with GLP standards of the US EPA-FIFRA, Japan MAFF, and OECD, and with US EPA guidelines (84-2) to determine the potential of chlorpyrifos (Dow; lot no. AGR 214637; stated purity 97.9%) to induce micronuclei in the bone marrow polychromatic erythrocytes of mice (male and female; CD-1 (ICR) BR; Charles River USA; 10 weeks old) at doses of approximately 80% of the LD50. The test material was mixed in corn oil, and given to mice (5/sex/dose) by oral gavage at a dose of 90 mg/kg (dose volume 6 ml/kg). A positive control group received cyclophosphamide (120 mg/kg; dose volume 10 ml/kg), and negative control animals received 6 ml/kg corn oil. Test and corn oil groups were killed at 24, 48, or 72 h after dosing, and the positive control group was killed 24 h after dosing.

Bone marrow smears were prepared and 1000 polychromatic erythrocytes (PCEs) were examined from each animal, and the number of micronucleated polychromatic erythrocytes (MN-PCEs) was recorded.

Results

The frequency of micronucleated PCEs in control and treated mice were similar, and the ratios of PCE to normochromatic erythrocytes (NCE) were not affected by treatment. The positive control chemical induced a significant increase in the frequencies of MN-PCEs in the bone marrows of both male and female mice.

Under the conditions of this study, the test material was negative for genotoxicity, as the oral administration of chlorpyrifos at a dose of 90 mg/kg did not induce an increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow of CD-1 (ICR) BR mice.

9.3 Other assays

Mendrala AL & Dryzaga MD (1986) Evaluation of chlorpyrifos in the rat hepatocyte unscheduled DNA Synthesis (UDS) Assay. Dow Chemical Company report HET K 044793-073, dated 31 January, 1986. [Dow; Submission 11462, reference 83; submission 238, vol 5, A3162/5, B41]

The QA and GLP status of this study was not stated. The genotoxic potential of chlorpyrifos (Dow; reference AGR 214637 or MM 820905-610; stated purity 95.7%) was tested in the rat unscheduled

DNA synthesis (UDS) assay at targeted concentrations of 1, 3.16, 10, 31.6, and 100 µM. Rat hepatocytes were harvested from livers of male CDF Fischer 344 rats (Charles River, USA). The cultures were treated with the test material dissolved in DMSO (final concentration 0.1% v/v, except for the 10 mM concentration, which contained 1 % v/v). The positive control compound was 2-acetylaminofluorene (2-AAF) at concentrations of 0.1, 1, and 10 µM. Triplicate cultures were assayed at each concentration level. The net number of nuclear grains in treated cells were compared to the appropriate control.

Results

No increases in mean net nuclear grains were observed at any concentration of the test material, and the responses in the test cultures were similar to negative controls. The positive control compound induced to expected strong positive responses at all concentrations, with statistically-significant increases in mean net nuclear grains in these cultures.

Under the conditions of this study, chlorpyrifos was negative for genotoxicity in the *in vitro* UDS assay.

Gollapudi BB, Mendrala AL & Linscombe VA (1995) Evaluation of the genetic toxicity of the organophosphate insecticide chlorpyrifos. Mutat Res 342(1-2): 25-36

The genetic toxicity of chlorpyrifos (purity 95-7-98.6%) was examined in several test systems including the induction of gene mutations in bacteria (Ames test) and mammalian cell cultures (CHO/HGPRT assay), cytogenetic abnormalities in mammalian cells both *in vitro* (rat lymphocyte chromosomal aberration test, RLCAT) and *in vivo* (mouse bone marrow micronucleus test), and DNA damage and repair in rat hepatocytes *in vitro*.

Ames test: A modification of the standard Ames protocol (Maron & Ames, 1983) involving a 30-min preincubation of the bacteria, test chemical and metabolic activation system was used. This bacterial reverse-mutation assay used *Salmonella typhimurium* strains TA98, TA1537, TA1538, TA100, and TA1535. The test material was mixed with DMSO, with serial dilutions made to achieve test concentrations of 0, 1.0, 3.16, 10.0, 31.6 and 100 µg/plate in 0.1 ml of DMSO. The test concentrations were assayed with and without metabolic activation, provided by S9 mix (S9 mix from Aroclor 1254-induced SD rat liver). Appropriate positive control substances and solvent controls were evaluated for mutagenicity.

Positive control substances tested

Chemical	Activation	Strains	Concentration (µg/plate)
MNNG	nil	TA100, TA1535	2
2-nitrofluorene	nil	TA98, TA1538	100
quinacrine mustard	nil	TA1537	10
2-anthramine	S9	TA100, TA1535	5
2-acetylaminofluorene	S9	TA98, TA1538	100
8-aminoquinoline	S9	TA1537	25

In each case, positive controls gave the expected substantial increase in the number of revertant colonies in each of the bacterial strains. The test substance was at the limit of solubility and induced toxicity (poor background lawn and/or overgrown background colonies) at 100 µg/plate in all strains except TA98. Compared to solvent controls, there was no increase in the number of revertant colonies at any test substance concentration in any strain. Under the conditions of this study, the test material was not a bacterial mutagen *in vitro* at non-toxic doses up to 100 µg/plate.

CHO/HGPRT forward mutation assay: Chlorpyrifos in DMSO was added for 4 h to cultures of CHO-K_1-BH_4 cells at 0, 3.5, 7.0, 8.8, 10.5, 14.0 and 17.5 µg/ml in the CHO/HGPRT forward mutation assay. Cultures treated with 373 µg/ml ethyl methanesulfonate (EMS) and 5.0 µg/ml 3-methylcholanthrene (3-MCA) served as positive controls for the non-activation and activation (S9) assays, respectively. Metabolic activation was provided by S9 liver homogenates from Aroclor 1254-treated male SD rats. The protocol was not presented in detail. This protocol enabled a measure of toxicity to be estimated from cultures which were incubated for seven days post-treatment and then fixed before staining and counting. The gene mutation assay was conducted by allowing phenotypic expression time through reculture/dilution of treated cells for 8 days, at which stage the cells were plated in selective medium (6-thioguanine) and cultured for a further 7-9 days. Appropriate statistical analyses were applied to the colony count data.

Chlorpyrifos formed a precipitate at 10.5, 14.0 and 17.5 µg/ml both with and without activation. In the non-activated cultures, relative cell survival was decreased in a dose-related manner down to about 10% of control cultures at the highest dose. The EMS positive control yielded 548 TG^r mutants per 10^6 clonable cells, the solvent controls 7.3, and all chlorpyrifos levels yielded mutant counts below 17 per 10^6. Relative cell survival in the S9-activated cultures was unaffected by treatment with chlorpyrifos at any level. The 3-MCA positive control yielded 488 TG^r mutants per 10^6 clonable cells, the solvent controls 12.4 and all chlorpyrifos levels yielded mutant counts below 9 per 10^6. Under the conditions of this study, chlorpyrifos did not induce forward mutations in the CHO/HGPRT assay.

Rat lymphocyte chromosome aberration test: The protocol for this *in vitro* test was not presented in detail. Peripheral blood from male SD rats were cultured for 48 h prior to treatment (4 h) with the test and positive control chemicals. Metabolic activation was provided by S9 liver homogenates from Aroclor 1254-treated male SD rats. Cells were harvested for chromosomal analysis 24 and 48 h after treatment finished. Positive controls were 0.5 mg/ml mitomycin C without activation, and 4.2 mg/ml cyclophosphamide with activation. Chlorpyrifos (in DMSO) concentrations ranged from 16.7-5000 µg/ml.

Mitotic index scores recorded variable but generally significant toxicity at chlorpyrifos concentrations at and above 50 µg/ml both with and without activation. In repeated assays, the positive controls yielded statistically-significant elevations in the frequency of cells with aberrations, achieving >25% in all cultures. Chlorpyrifos treatment (max. 4% cells with aberrations) did not induce an increase in aberrations when compared to the control value (max. 3% cells with aberrations). The presence of the S9 activation mix had no effect on the recorded chromosome aberration frequency. Chlorpyrifos was negative in this assay.

UDS assay: The protocol for this *in vivo* test was not presented in detail. The genotoxic potential of chlorpyrifos was tested in the rat unscheduled DNA synthesis (UDS) assay at targeted concentrations

of 1, 3.16, 10, 31.6, and 100 µM. Rat hepatocytes were harvested from livers of male Fischer 344 rats, cultured with ^{3}H-thymidine, and treated for 18-20 h with the test material dissolved in DMSO. The positive control compound was 2-acetylaminofluorene (2-AAF) at concentrations of 0.22, 2.23 and 22.3 µg/ml. Triplicate cultures were assayed at each concentration level. The net number of nuclear grains in treated cells were compared to the appropriate control.

No increases in mean net nuclear grains were observed at any concentration of the test material; the responses in the test cultures were similar to negative controls. The positive control compound induced the expected strong positive responses at all concentrations, with statistically-significant increases in mean net nuclear grains in these cultures. Under the conditions of this *in vitro* UDS assay, chlorpyrifos was negative for genotoxicity.

Micronucleus assay: CD-1 mice (5/sex/dose) were given single oral doses of 0, 7, 22, 70 or 90 mg/kg chlorpyrifos (suspension in corn oil) and killed either 24 or 48 h after dosing. Positive controls received 120 mg/kg cyclophosphamide in water. Bone marrow samples were obtained from femurs and then processed, and cell smears were prepared and stained. One thousand polychromatic erythrocytes (PCEs) were evaluated from each animal and frequencies of micronucleated PCEs (MN-PCE) recorded.

At the 24-h sacrifice, the positive controls recorded >27 MN-PCEs per 1000 PCEs examined, the negative control was 2.0, and the chlorpyrifos treated mice did not exceed 1.8 MN-PCEs per 1000 PCEs examined. The results at 48 h were similar. Chlorpyrifos was negative in this mouse bone marrow micronucleus test.

9.4 TCP

Zempel JA & Bruce RJ (1986) 3,5,6-trichloro-2-pyridinol: Evaluation in the Ames *Salmonella*/mammalian-microsome mutagenicity assay. Lake Jackson Research Center, The Dow Chemical Company, Texas. Study ID: TXT:K-038278-010, dated February, 1986. [Dow; Submission 939:pp 3.1084-3.1099] QA statement provided.

The genotoxicity potential of 3,5,6-trichloro-2-pyridinol (TCP); (purity 99.7%; batch no. AGR143197) was assessed using a modification of the standard Ames protocol involving a 30-minute preincubation of the bacteria, test chemical and metabolic activation system. This bacterial reverse-mutation assay used *Salmonella typhimurium* strains TA98, TA1537, TA1538, TA100, and TA1535. The test material was dissolved with DMSO, with serial dilutions to achieve test concentrations of 3.16, 10.0, 31.6, 100, and 316 µg/plate in 0.1 ml of DMSO. The test concentrations were assayed with and without metabolic activation, provided by S9 mix (source not stated). Appropriate positive control substances and solvent controls were evaluated for mutagenicity.

Positive control substances tested

Chemical	Activation	Strains	Concentration (µg/plate)
MNNG	nil	TA100, TA1535	2

2-nitrofluorene	nil	TA98, TA1538	100
quinacrine mustard	nil	TA1537	10
2-anthramine	S9	TA100, TA1535	5
2-acetylaminofluorene	S9	TA98, TA1538	100
8-aminoquinoline	S9	TA1537	25

Results

In each case, positive controls gave the expected substantial increase in the number of revertant colonies in each of the bacterial strains. The test substance induced some mild toxicity (poor background lawn and/or overgrown background colonies) at 100 (some strains) and 316 µg/plate (all strains), especially in TA98 with and without S9, and in TA1537 without S9. Compared to solvent controls, there was no increase in the number of revertant colonies at any test substance concentration in any strain.

Under the conditions of this study, the test material was not a bacterial mutagen *in vitro* at non-toxic doses up to 100 µg/plate.

McClintock ML & Gollapudi BB (1989b) Evaluation of 3,5,6-trichloro-2-pyridinol in the mouse bone marrow micronucleus test. Dow Chemical Company USA, Study K-038278-008A, dated 15 September 1989. [Dow; Submission 11462, reference 12]

This study was performed in accordance with GLP standards of the US EPA-FIFRA, Japanese MAFF (59 Nohsan, Notification 3850, 1984), and the OECD.

It was conducted to investigate the potential of 3,5,6-trichloro-2-pyridinol (TCP) to induce micronuclei in the bone marrow polychromatic erythrocytes (PCE) of CD-1 mice when given at a dose of 1000 mg/kg (maximum tolerated dose). The test material (Dow; CAS No. 6515-38-4; stated purity 99.9%) was given to male and female CD-1 (ICR) BR mice (Charles River, USA; 10 weeks old) by single oral gavage mixed in corn oil (dose volume 6 ml/kg). The positive control compound in this study was cyclophosphamide (CP) at a dose of 120 mg/kg, and a dose volume of 10 ml/kg. Negative controls received 6 ml/kg corn oil.

In a range-finding study, groups of mice (5/sex/dose) were treated at doses of 250, 500, 750, 1000, 1500, 2000, and 3000 mg/kg, and the survival of the animals was monitored for 6 days.

In the micronucleus test, groups of mice (5/sex/dose) were given the test material (1000 mg/kg), the positive control, or the corn oil negative control. The groups given the test material or vehicle control were killed at 24, 48, or 72 h after treatment, and the positive control group was killed at 24 h after treatment. Bone marrow samples were obtained, and cell smears prepared on slides. One thousand PCEs were examined from each animal and the number of micronucleated polychromatic erythrocytes (MN-PCE) was recorded.

Results

The mortality in the range-finding study was 3/5, 3/5, and 4/5 males, and 4/5, 4/5, and 5/5 females, at 1500, 2000, and 3000 mg/kg, respectively. A single female also died at 750 mg/kg.

No significant increases in the frequency of MN-PCEs were observed between treated and vehicle control groups. The positive control resulted in a significant increase in the frequency of micronucleated erythrocytes in the bone marrow of males and females.

Under the conditions of this study, chlorpyrifos was negative for genotoxicity, and did not induce the formation of micronucleated bone marrow polychromatic erythrocytes when given to mice as a single oral dose of 1000 mg/kg.

Gollapudi BB (1987) Evaluation of 3,5,6-trichloro-2-pyridinol (TCP) in the rat hepatocyte unscheduled DNA synthesis (UDS) assay. The Dow Chemical Company report TXT-K-38278-012, dated September 1987 [Dow; Submission 939:pp 3.1108-3.1127] GLP certificate provided.

Freshly isolated hepatocytes from male Fischer CDF 344 rats were exposed to TCP (99.7% pure, lot No.: AGR143197) at final concentrations in the range 1-100 µg/ml in 0.5% DMSO, and the extent of unscheduled DNA synthesis was assessed. Cultures treated with 2.233 µg/ml 2-acetylaminofluorene (DMSO solvent) served as positive controls.

Some toxicity was observed at doses of TCP of 50 and 100 µg/ml. TCP did not increase unscheduled DNA synthesis relative to solvent control, whereas positive controls induced the expected increase in unscheduled DNA synthesis. TCP was negative in this rat hepatocyte unscheduled DNA synthesis assay.

Linscombe VA & Gollapudi B (1986) Evaluation of 3,5,6-trichloro-2-pyridinol (TCP) in the Chinese hamster ovary cell/hypoxanthine-guanine-phosphoribosyl transferase (CHO/HGPRT) forward mutation assay. Dow Chemical Company, HES, Texas, study No.: TXT:K-038278-013, dated September, 1986. [Dow submission 939: pp 3.1137-3.1147] QA statement provided

TCP (99.7% pure, Lot no.: AGR143197) in DMSO was added to cultures of CHO-K_1-BH_4 cells at 62.5, 125, 250, 500 and 750 µg/ml for 4 h in a CHO/HGPRT forward mutation assay. Cultures treated with 621 µg/ml ethyl methanesulfonate (EMS) and 4.03 µg/ml 20-methylcholanthrene (20-MCA) served as positive controls for the non-activation and activation (S9) assays respectively. Metabolic activation was provided by S9 liver homogenates from Aroclor 1254-treated male SD rats. Toxicity was estimated from cultures which were incubated for seven days post-treatment and then fixed; colonies were counted after staining the triplicate cultures. The gene mutation assay was conducted by reculture/dilution of treated cells at days 1, 3, 6 and 8, at which stage the cells were plated in selective medium (6-thioguanine) and cultured for a further 7-9 days. Appropriate statistical analyses were applied to the colony count data.

Results

Relative cell survival in the non-activated cultures was unaffected by treatment with TCP at levels up to 125 µg/ml and reduced to about 50% at 750 µg/ml. The EMS-positive control yielded 483 TG^r mutants per 10^6 clonable cells, the negative controls 10 per 10^6 (historical control range 0 - 12.9), and all TCP levels yielded mutant counts below 10 per 10^6. Relative cell survival in the S9-activated cultures was unaffected by treatment with TCP at levels up to 125 µg/ml and reduced to about 20% at 750 µg/ml. The 20-MCA positive control yielded 338 TG^r mutants per 10^6 clonable cells, the negative controls

5 per 10^6 (historical control range 1.3-13.4), and all TCP levels yielded mutant counts below 16 per 10^6 (15.2 at 250 µg/ml), which was slightly higher than the historical negative control values. Under the conditions of this study, TCP was negative in this CHO/HGPRT forward mutation assay.

Bruce RJ, Gollapudi BB & Hinze CA (1985) Evaluation of 3,5,6-trichloro-2-pyridinol in the mouse bone marrow micronucleus test. Dow Chemical Company HES, Texas, Study No.: K-038278-008, dated December, 1985. [Dow submission 939: pp 3.1148-3.1162] QA certificate provided

CD-1(ICR) BR mice (5/sex/dose) were given single oral doses of 0, 24, 76, or 240 mg/kg TCP (3,5,6-trichloro-2-pyridinol; 99.7% pure, Lot no.: AGR143197, suspension in corn oil) and killed either 24 or 48 hours after dosing. Positive controls received 120 mg/kg cyclophosphamide in water. Bone marrow samples were obtained from femurs, processed and cell smears prepared and stained. One thousand polychromatic erythrocytes (PCEs) were evaluated from each animal and frequencies of micronucleated PCEs (MN-PCE) recorded.

Results

At the 24 h sacrifice, the positive control recorded 65.4 ± 10.0 MN-PCEs per 5000 PCEs examined, the negative control was 0.2 ± 0.4, and the TCP treated mice did not exceed 2 MN-PCEs per 5000 PCEs examined. The results at 48 h were similar. There were no significant increases in the frequencies of micronucleated bone marrow PCEs in TCP-treated groups compared to negative controls, whereas positive controls showed significant increases. TCP was negative in this mouse bone marrow micronucleus test.

9.5 Studies not suitable for regulatory purposes

For the reasons given after each summary, the following reports were not considered suitable for regulatory purposes.

Domoradski JY (1981a) Sodium 3,5,6-trichloro-pyridinate [sodium salt of DOWCO 463]: Evaluation in the Ames Salmonella/mammalian-microsome mutagenicity assay. Dow Chemical USA, CRI K-65999-(6), Lab Report no.: HET-K-65999-(6), dated March 26, 1981. [Dow; submission 939: pp 3.1100-3.1107] QA statement provided.

From the study summary: The genotoxicity potential of the sodium salt of 3,5,6-trichloro-2-pyridinol (TCP); (stated purity 92%, 7% water; batch no. AGR178247) was assessed using a standard Ames protocol involving a plate assay of the bacteria, test chemical and metabolic activation system. The mutagenic potential was assessed in *S. typhimurium* (strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538). In the dose range 0.1-1000 µg/plate, sodium-TCP (92% pure) showed no mutagenic potential in the presence or absence of a metabolic activation system. Bacterial toxicity was observed at 1000 µg/plate in all strains, in both activated and nonactivated systems.

Comment: This study was not able to be evaluated because of missing report pages.

Domoradski JY (1981b) Sodium 3,5,6-trichloro-pyridinate [sodium salt of DOWCO 463]:

Evaluation in the rat hepatocyte unscheduled DNA synthesis assay. Dow Chemical USA, CRI K-65999-(7), Lab Report no.: HET-K-65999-(7), dated March 26, 1981. [Dow; submission 939: pp 3.1128-3.1136] QA statement provided.

From the study summary: Freshly isolated hepatocytes from a male Fischer 344 rat were exposed to sodium-TCP (92% pure) at concentrations of 4 nM-40 mM, and the extent of unscheduled DNA synthesis assessed. Cultures treated with 2-acetylaminofluorene at concentrations of 100 nM-10 µM served as positive controls. Sodium-TCP did not increase unscheduled DNA synthesis relative to solvent controls, whereas the positive control gave a positive response. Therefore sodium-TCP was negative in the rat hepatocyte unscheduled DNA synthesis assay.

Comment: This study was not able to be evaluated because of missing report pages.

10. SPECIAL STUDIES

10.1 Neurotoxicity

Stevenson GT (1966) A neurotoxicity study of Dursban in laying hens. Dow Chemical Company USA, Experiment no. 3-1395-2, document GH A-195; Report A1A-390, dated 29 June 1966 [Dow; Submission 238, reference 4.2.8b; Submission 11462, reference 104]

In this study, chlorpyrifos (Dow; Dursban; combination batch 2 and 3; purity not stated) was given to DeKalb Leghorn cross hens (3/group; 16 months old; source not stated) by gelatin capsule as single oral doses of 40, 75, 100, or 150 mg/kg. Daily observations were made for ataxia, morbidity and mortality for up to 27 days. 2-PAM (50 mg/kg) and atropine (1/10 grain/bird) were formulated in distilled water and administered by ip injection below the sternum 90 minutes post-dosing. Ruelene was given at 1000 mg/kg as a positive control.

Two birds died at 100 mg/kg, and one bird died at 150 mg/kg. Signs of toxicity (no details provided) were reportedly seen in all birds at 100 and 150 mg/kg. No signs of delayed ataxia or paralysis were reported in birds treated with chlorpyrifos. Delayed ataxia or paralysis were reported in positive control birds.

Capodicasa E, Scapellato ML, Moretto A, Caroldi S & Lotti M (1991) Chlorpyrifos-induced delayed polyneuropathy. Arch Toxicol 65(2):150-5. Luxembourg Industries Dossier file no. 5.7/01. [David Gray; Submission 11475]

No GLP or QA Statements were issued with this report. The acute toxicity and delayed neurotoxicity potential of chlorpyrifos and chlorpyrifos formulations were tested in adult hens (*gallus-gallus domesticus*; 1.5 - 2.5 kg body weight; source not stated). Birds were fasted for 12 h prior to dosing. Chemicals were dissolved in glycerol formal and given by oral gavage (po; dose volume <2.3 ml) or subcutaneously (sc; dose volume < 1 ml). Atropine (20 mg/kg) and physostigmine (0.1 ml/kg) dissolved in saline were given intraperitoneally (ip) 10 minutes before treatment with test materials, as a standard prophylactic treatment. In addition, treatment with atropine (20 mg/kg) and 2-PAM (100 mg/kg in saline; ip) was given when necessary. The test materials were “pure” chlorpyrifos (Dow; stated purity

99%; batch not given), and "commercial" chlorpyrifos (40% and 60% in methylene chloride; local suppliers). The positive control was DFP (di-isopropyl phosphorodiaminofluoridate; Fluka).

In the acute toxicity studies, groups of five birds were given "pure" chlorpyrifos at doses of 4, 8, 16 or 32 mg/kg po, or "commercial" chlorpyrifos at 4, 8, or 16 mg/kg po, and then observed for cholinergic signs and mortality.

In the delayed neurotoxicity studies, the experimental deign was as follows:

- Birds (21 in total) were dosed with 60 or 120 mg/kg po with "pure" chlorpyrifos after prophylaxis with atropine/physostigmine. Additional treatment with atropine and oximes was given twice-daily for 6 days. Groups of birds (3-4) were killed daily to measure neuropathy target esterase (NTE) and brain cholinesterase (ChE) activity.

- Birds (31 in total) were dosed with 90 mg/kg po of "pure" chlorpyrifos after prophylaxis with atropine/physostigmine. Additional treatment with atropine and oximes was given twice-daily for 6 days and then once a day for 5 days. Birds were observed for 2 weeks for signs of organophosphate-induced delayed neuropathy (OPIDN). Groups of birds (3-4) were killed daily to measure neuropathy target esterase (NTE) and brain cholinesterase (ChE) activity.

- Birds (26 in total) were dosed with 150 mg/kg po of "commercial" chlorpyrifos after prophylaxis with atropine/physostigmine. Additional treatment with atropine and oximes was given twice-daily for 5 days and then once a day for 3 days. Two birds were killed daily for NTE and brain, spinal cord and peripheral nerve ChE determinations. Surviving animals were observed daily for a further two weeks for signs of OPIDN.

- Positive control birds (14 in total) were dosed with DFP (1,5 mg/kg sc) after prophylaxis with atropine/physostigmine. Two birds were killed at daily intervals to measure NTE and brain, spinal cord and peripheral nerve ChE activity.

Assays were also conducted on the effect of route of administration on ChE inhibition, kinetics of NTE and ChE inhibition by chlorpyrifos-oxon (hen and human brain tissues), and reactivation by oximes of ChE inhibited by chlorpyrifos-oxon.

Results

The design and general reporting of this study reduced the usefulness of the results, as no two treatment groups and/or controls were treated and/or assayed in an identical manner. However, there were some general findings from this study that were useful.

In hens given a single oral dose of chlorpyrifos, birds dosed at 90 or 150 mg/kg returned positive mean ataxia scores (on a scale of 0-4). At 90 mg/kg, the mean ataxia score was 1.1 $\pm$ 0.6 (4/7 birds with positive findings), and at 150 mg/kg, the ataxia score was 2.5 $\pm$ 0.6 (6/6 birds). At 4, 8, 16, and 32 mg/kg, the ataxia scores were 0. Little difference was seen in acute toxicity findings between "pure" and "commercial" chlorpyrifos preparations, and deaths occurred in a dose-dependent manner within 48 h of administration.

Time-dependent decreases in NTE activity were seen at all doses of chlorpyrifos, but the extent of inhibition was similar at all doses between 60 and 150 mg/kg, reaching 60-80% inhibition after up to 6 days of administration. Significant brain ChE inhibition was observed at all chlorpyrifos doses, with 60-100% inhibition observed after 1 day of dosing, regardless of the dose. Positive control birds showed maximal NTE inhibition 4 h after dosing, which was much faster than the inhibition seen in birds treated with chlorpyrifos. After a five-day recovery period, birds treated with chlorpyrifos displayed extensive NTE inhibition (44-55% compared with controls) in brain, spinal cord and peripheral nerve, along with continued ChE inhibition in the same tissues. The extent of inhibition in positive control birds was similar to that seen in birds treated at 150 mg/kg/d chlorpyrifos after about 4 days of treatment, and through the recovery period.

Under the conditions of this study, doses of chlorpyrifos at about 5 times the oral LD50 in hens resulted in significant inhibition of NTE and ChE activity, suggesting that chlorpyrifos had the potential to induce OPIDN, but at doses that required extensive and aggressive antidote treatment to keep the birds alive, both prior to treatment and throughout the treatment and recovery periods.

Rowe LD, Warner SD & Johnston RV (1978) Acute delayed neurotoxicologic evaluation of chlorpyrifos in white Leghorn hens. Dow Chemical Company, USA report A1A-715, dated 22 May 1978 [Dow; Submission 11462, reference 105; Submission 11464, reference 8]

No GLP or test guideline statements were provided for this study.

Chlorpyrifos (Dow; AGR 155052; stated purity 96.9%) was given to adult White Leghorn hens (local source) to assess the acute neurotoxicity potential of the test material. In a dose-ranging study, birds were given a single oral dose of chlorpyrifos in a gelatin capsule at doses of 0, 25, 50, 100, 200, or 400 mg/kg (6 birds/group; 14.5 month old; mean body weight 1630 g). No atropine was administered to these birds. The mortality was recorded for 8 days.

Based on the acute toxicity findings, a neurotoxicity evaluation was conducted on 17-month old hens (10/group; mean body weight 1661 g), with chlorpyrifos given in gelatin capsules at doses of 50 and 100 mg/kg. All birds were dosed without prior fasting. A negative control group received empty gelatin capsules, and a positive control group received tri-o-tolyl phosphate (TOCP) by intubation at a dose of 232 mg/kg. Atropine sulphate (30 mg/kg) was given by crop intubation approximately 30 minutes prior to administration of TOCP or chlorpyrifos. A control group received empty gelatin capsules in addition to the atropine sulphate.

Each bird was examined for 21 or 24 days for signs of toxicity or behavioural abnormalities. Bodyweights were determined at the beginning of the study, then every 7 days and immediately prior to the terminal sacrifice. Immediately after death, sciatic nerve and cervical, thoracic and lumbar spinal cord were taken from each bird and fixed. Representative samples of these tissues were used for light microscopic examination. All fixed tissues were used for histopathological examination.

Results

In the dose-ranging study, mortality was 0/6, 1/6, 4/6, 4/6, 6/6, and 6/6 at 0, 25, 50, 100, 200, and 400 mg/kg, respectively. The LD50 was estimated to be 50 mg/kg, with 95% confidence limits of 30 and 83.4 mg/kg.

In the neurotoxicity study, no death or moribundity was observed in any birds. Signs of neurotoxicity were scored on a scale of 0 (normal in appearance in behaviour) through 9 (complete paralysis; the bird exhibits loss of all or nearly all motor control in its legs and cannot move itself). A score of 4 indicated slight ataxia (the bird can walk and run upright, but exhibits constant or intermittent incoordination that was slight in degree and may be evident only following exercise).

No signs of neurotoxicity were observed in control birds. In the positive control group, signs of neurotoxicity began to develop in some birds from day 10 onwards, and by day 21, all birds had neurotoxicity scores of between 4 and 7.

At 50 mg/kg chlorpyrifos, scores of up to 7 (partial paralysis) were seen in some birds after treatment, but these effects were transient, and no adverse effects were observed by day 2 of the study. Similar effects were seen at 100 mg/kg, but these findings were more severe, with scores of up to 9 seen in most birds by day 1 after treatment, and some signs persisting in a single bird on day 4. No adverse effects were observed at or after day 5.

Some slight reductions in body weights of birds treated with chlorpyrifos or TOCP were observed, but none of these effects were statistically significantly different to control group bodyweights.

Microscopic assessment of the spinal cord and sciatic nerves from control animals revealed no spinal cord abnormalities in control birds, but 1/10 control birds had focal lymphocytic infiltration of the sciatic nerve. Positive control birds all had fragmented, necrotic, swollen axons and demyelination of the spinal cord, and 3/10 had vacuolated, swollen sciatic nerve neurolemmal sheaths. Birds treated with chlorpyrifos at 50 or 100 mg had a low incidence of focal lymphocytic infiltrate in the spinal cord and/or the sciatic nerve, but no other adverse neuropathological findings were observed in these groups.

Under the conditions of this study, chlorpyrifos did not cause acute delayed neurotoxicity in hens after single doses up to 100 mg/kg.

Roberts NL, Phillips CNK, Gopinath C, Begg S, Anderson A, & Dawe IS (1987) Acute delayed neurotoxicity study with chlorpyrifos in the domestic hen. Huntingdon Research Centre Ltd, UK. Study completed 14 May 1987. HRC report no. MBS 16/87764. MCW reference no. R-4661. [Makteshim; Submission 11471; reference 39]

This study was conducted in compliance with GLP regulations of the US EPA (40 CFR Part 160), OECD, and Japan MAFF, and according to US EPA Guideline 81-7.

Pyrinex (technical chlorpyrifos; stated purity 96.8%; Makteshim; batch no. 489205) was tested for its acute toxicity (LD50) and delayed neurotoxicity potential in adult female domestic hens (approximately 12 months old; L & M Barnes, UK). For the acute toxicity determination, a range-finding study was conducted where the test material was given orally in a corn oil vehicle (dose volume not stated) to ten groups of two birds, at single doses of 20, 40, 50, 60, 80, 100, 120, 150, and 200 mg/kg. Surviving

birds were killed 14 days after dosing, but were not subject to post-mortem examination. One bird died in each of the 50, 80, and 120 mg/kg groups, and two birds died in each of the 100, 150, and 200 mg/kg levels.

Doses for the LD50 study were selected based on the results of the range-finding study, and groups of 10 birds were given the test material by oral gavage at doses of 0 (control), 18, 27, 40, 60, 90, and 135 mg/kg, in a corn oil vehicle, using a dose volume of 2 ml/kg. Dosing was followed by a 14-day observation period, at the end of which all birds were killed. Birds were not subject to post-mortem examination. The health of birds and signs of toxicity were recorded daily, and the bodyweights were measured weekly.

In the neurotoxicity assessment, groups of 10 birds were tested with:
- corn oil (vehicle control; dose volume 2 ml/kg);
- tri-ortho-cresyl phosphate (positive control; TOCP; 20% in corn oil; 500 mg/kg; dose volume 2.5 ml/kg);
- chlorpyrifos (110 mg/kg; 5.5% in corn oil; dose volume 2 ml/kg) plus atropine sulphate (intramuscular injection; 10 mg/kg; 1% aqueous solution);
- chlorpyrifos (110 mg/kg; 5.5% in corn oil; dose volume 2 ml/kg) plus 2-PAM (intramuscular injection; 50 mg/kg; 5% aqueous solution); or
- not dosed (maintained as a source of additional birds if necessary).

The intramuscular injections were given immediately prior to dosing with the test material.

A 21-day observation period followed dosing, and all vehicle control birds were re-dosed with corn oil, and all test birds were re-dosed with chlorpyrifos at the same dose level at the end of day 21, followed by anther 21-day observation period. Positive control birds were killed at the end of the first 21-day observation period. Birds were examined daily for mortality or clinical signs of toxicity, body weights and food consumption were recorded weekly and birds were examined daily for signs of delayed locomotor ataxia. Macroscopic examinations were conducted on all positive control birds after day 21 and all birds surviving until the end of the study on day 42, as well as all birds which died more than 7 days after dosing.

Tissues were taken from a number of birds from all groups for histopathological examination of the brain (medulla/pons, cerebellar cortex and cerebral cortex), spinal cord (multiple longitudinal and transverse sections of the cervical, thoracic and lumbar-sacral regions), and peripheral nerve (proximal and distal sciatic nerve and tibial nerve (distal branches).

The stability and test material concentration in the dosage formulation was tested and found to be acceptable.

Results

In the LD50 determination, four birds in the 27 mg/kg group died or were killed within three days of dosing as a result of injuries sustained from other birds. This group was discarded and replaced by another group of birds. A high incidence of this 'bullying' behaviour was also seen in other test groups,

including controls, and this behaviour was observed both before and during chlorpyrifos administration. Mortality was observed at 60 mg/kg (3/10 birds), 90 mg/kg (4/10), and 135 mg/kg (6/10), with a calculated LD50 of 106 mg/kg. Treatment-related clinical signs were seen in all treatment groups, and included: unsteadiness, trembling, subdued appearance, inability to stand, and gasping, with the severity of these signs related to dose. Surviving birds recovered after 5-6 days.

In the neurotoxicity assessment, no clinical signs of toxicity were observed in vehicle control birds. In the TOCP treated group, no signs of toxicity were observed immediately following dosing, but all birds developed signs of delayed locomotor ataxia, as early as day 11 after dosing. The birds dosed with chlorpyrifos displayed signs of toxicity after dosing (or re-dosing), including subdued appearance, unsteadiness, inability to stand and weakness. Mortality was observed in both chlorpyrifos treated groups, with 4/10 birds dying after the first dose of chlorpyrifos plus atropine sulfate (no deaths after the second dose), and 3/10 after the first dose of chlorpyrifos plus 2-PAM (one death after the second dose). Surviving birds recovered completely within 5-8 days of dosing. Additional doses of atropine sulfate were given to several birds in both of the chlorpyrifos groups due to the severity of clinical signs observed.

Histopathological examination revealed a low incidence of minor neurological changes (Grade II on a scale of grade I being normal to Grade V being most severe) in several birds from the vehicle control group, and this finding was considered to demonstrate a normal background incidence of axonal degeneration. The majority of positive control birds displayed grade III changes in at least one level of spinal cord and Grade III or IV changes in at lest one level of peripheral nerve, indicating a significant treatment-related axonal degeneration. Three birds treated with chlorpyrifos showed Grade II changes at one level of peripheral nerve only, and these findings were considered to be similar to the incidence and severity of changes seen in vehicle control animals.

Incidence of neuropathological gradings

	Fore-brain	Mid- and hind-brain	Upper cervical spinal cord	Lower cervical spinal cord	Thoracic spinal cord	Lumbar spinal cord	Proximal sciatic nerve	Distal sciatic nerve	Tibial nerve
Corn oil control	10/10 (I)	10/10 (I)	2/10 (I) 8/10 (II)	8/10 (I) 2/10 (II)	9/10 (I) 1/10 (II)	7/10 (I) 3/10 (II)	6/10 (I) 4/10 (II)	9/10 (I) 1/10 (II)	10/10 (I)
Positive control TOCP 500 mg/kg	10/10 (I)	9/10 (I) 1/10 (II)	1/10 (I) 1/10 (II) 8/10 (III)	5/10 (II) 5/10 (III)	1/10 (I) 7/10 (II) 2/10 (III)	2/10 (I) 7/10 (II) 1/10 (III)	4/10 (I) 4/10 (II) 2/10 (III)	2/10 (I) 1/10 (II) 4/10 (III) 3/10 (IV)	3/10 (I) 3/10 (II) 3/10 (III) 1/10 (IV)
Chlorpyrifos 110 mg/kg	12/12 (I)	12/12 (I)	5/12 (I) 7/12 (II)	4/12 (I) 8/12 (II)	9/12 (I) 3/12 (II)	9/12 (I) 3/12 (II)	9/12 (I) 3/12 (II)	12/12(I)	12/12 (I)

(I) = Grade I; (II) = Grade II; etc.

Under the conditions of this study, the oral administration of a single dose of chlorpyrifos (110 mg/kg) to hens followed by a repeat dose after 21 days did not produce any clinical signs of delayed neurotoxicity, and this result was confirmed by histopathological examination, which showed no treatment-related change in nerve tissues as a result of chlorpyrifos administration.

Barna-Lloyd T, Szabo JR & Young JT (1986) Chlorpyrifos : Subchronic organophosphate-induced delayed-neurotoxicity (OPIDN) study in laying chickens hens. The Dow Chemical Company, report no. TXT:K-044793-064, dated April 1986. [Dow; Submission 238]

The following study assessment report was obtained from the Pest Management Regulatory Agency of Canada (PMRA) under the auspices of the OECD Ad Hoc Exchange Program. The Canadian review was completed 14/1/93, and the PMRA evaluation was incorporated with minimal changes. An independent assessment of the original data has not been conducted by Australian regulatory authorities. **Australian regulatory conclusions and comments are enclosed in square brackets [ ].**

This study was conducted in accordance with the U.S. EPA Good Laboratory Practice Regulations and Standards, and the Standard Operating Procedures of Health & Environmental Science Texas, Dow Chemical Inc., Lake Jackson Research Centre.

Test Material: Chlorpyrifos technical, Lot #MM820905-610 (DURSBAN F, AGR-214637) with a purity of 95.7-97.0% w/w. Supplied as white granular crystals by the Agricultural Products Department, Dow Chemical U.S.A., Midland, MI.

Test System: Mature laying domestic Babcock White Leghorn chicken hens (*Gallus gallus domesticus*) were purchased from M`M Farm Supply, Franklin, TX. The hens were between the ages of 8 and 14 months at the start of the study.

Experimental hens were housed in enclosed study pens (8 X 4 ft. each, with an epoxy-painted, nonslip concrete floor covered by hardwood chips; 5 hens/pen) in an air-conditioned room with an automatic 12-h light/dark photocycle. Lab Cage Layer Feed #5070 (Lot No. JAN04852, Ralston Purina Co., Richmond, IN mill) and tap water were provided *ad libitum*, l feeder and 1 automatic waterer per study pen.

Dose Levels: Dose levels of 0 (negative control), 0 (positive control), 1, 5 and 10 mg/kg low/day of chlorpyrifos were selected on the basis of a 4-week dose-setting dietary probe study in which a higher dose of 15 mg/kg low/day resulted in high mortality and excessive loss of body weight (Barna-Lloyd et al., 1985).

Chlorpyrifos was suspended in Mazola corn oil (Best Foods, Union, NJ) and a dosage volume of approximately 3 ml/bird for a bird of average weight was used. The negative control group received the vehicle (corn oil) only. The positive control group was given 10 mg/kg bw/day of tri-ortho-cresyl phosphate (TOCP) (Lot #C10A, Kodak Lab. & Specialty Chemicals, Eastman Kodak Co., Rochester, NY), a known inducer of delayed-neurotoxicity in mature chicken hens. No atropine or any other agent was given for protection against possible acute cholinergic intoxication.

The dosing solutions were prepared weekly from more concentrated premix solutions. Both chlorpyrifos and TOCP suspensions in corn oil were shown to be homogeneous and stable for at least 14 days. All dosing solutions were prepared within $\pm$10% of the target concentrations, with the exception of two 1 mg chlorpyrifos/kg bw/day batches being at $\pm$15%.

Study Design: Prior to the start of the study, the birds were ranked by body weights and assigned by random numbers to the treatment and control groups. Five groups of 10 hens/group were administered chlorpyrifos (one dose/day, 7 days/week) in corn oil via gavage at dose levels of 0 (negative controls), 0 (positive controls), 1, 5 or 10 mg/kg bw/ day for 91 days (13 weeks).

All birds were weighed weekly. As part of the assessment of general health status, the number of eggs found daily in each pen was recorded. The birds were observed at least once daily for clinical signs of delayed-neurotoxicity. Once a week starting the 3rd week of the study, the birds of all groups were induced to walk about on a bare concrete floor and to climb a short, inclined (30°) ramp. The gait/stance of each bird was observed and recorded on a graded scale of 0 - 5 (0-normal gait; 1-slight ataxia; 2-severe ataxia; 3-slight paralysis; 4-moderate paralysis and 5-severe paralysis).

At the end of 91 days of treatment, all surviving birds were sacrificed, necropsied and subjected to gross examination. Each bird was perfused (open-heart) with a heparinised saline solution followed by 200 - 300 ml neutral phosphate-buffered 10% formalin. The cranium and spinal column were removed *in toto* and fixed in formalin. The sciatic nerves (2) and tibial nerves (2) were also dissected free from the musculature and fixed in formalin for subsequent histopathological evaluation.

Histological sections of the formalin-fixed nervous tissues from birds of the negative control (corn oil only), the positive control (10 mg TOCP/kg bw/day) and the highest dose chlorpyrifos treatment (10 mg/kg bw/day) groups were prepared, stained with haematoxylin and eosin (also luxol fast blue-periodic acid-Schiff for myelin and Sevier-Munger silver stain for axons), and examined microscopically for lesions indicative of delayed neurotoxicity. All sections were examined blind to treatment and scored

according to a classification scheme modified from that of Bickford and Sprague (1982) and Prentice and Roberts (1983).

Only body weight data were evaluated for differences of statistical significance.

Results

Clinical Signs and Mortality: All experimental animals survived till scheduled sacrifice on Day 92 of the study. None of the hens of the negative control or chlorpyrifos-treatment groups (1, 5 or 10 mg/kg bw/day) exhibited any clinical signs characteristic of OPIDN during the 13 weeks of the study. Five of the 10 birds in the high dose chlorpyrifos group showed occasional signs of transient (lasting fewer than 24 hours) gait disturbance (temporary ataxia and failure to maintain balance) indicative of acute neurotoxicity. All 10 birds of the positive control group (10 mg TOCP/kg low/day) displayed sustained clinical signs characteristic of OPIDN (abnormal gait, ataxia and impaired motor co-ordination) beginning in Week 6 of the study. Slight paralysis (inability to rise off the hocks) was observed in one TOCP bird during Weeks 11-13.

Body Weights: Group mean body weights were comparable among the negative control, the low- and mid-dose chlorpyrifos treatment groups throughout the study. Birds of the high dose chlorpyrifos group (10 mg/kg bw/day) showed a significant ($p<0.05$) decrease in mean body weight within two weeks of commencing treatment; the weekly group average stabilised at 10-12% below that of the negative controls from Week 5 onward. The weight loss at high dose was judged to indicate acute toxicity.

Birds of the positive control (TOCP-treated) group lost weight starting in Week 4 of treatment, but the group mean body weights did not show significant differences ($p<0.05$) from the negative controls until Week 6 of the study. The decline in body weight progressed over the remaining course of the study, with a loss of 27% (relative to negative control) after 12 weeks of treatment. Reduction in body weight of the TOCP group coincided with the onset of clinical signs of delayed neurotoxicity and was judged to be a sign of OPIDN caused by administration of TOCP.

Egg Counts: The weekly average egg counts/hen/day were comparable among the negative control, the low- and mid-dose chlorpyrifos treatment groups during the 13 weeks of the study. Birds of the high dose chlorpyrifos group (10 mg/kg bw/day) showed a decrease in their egg production starting the first week of the study. Reduced egg production was not evident in the birds of the positive control (TOCP) group until Week 4 of treatment. The difference in the times of onset was judged to be due to an acute cholinergic intoxication in the high dose chlorpyrifos group and the onset of OPIDN in the TOCP group.

Necropsy Findings: No gross abnormalities were observed in the negative controls. No treatment-related gross lesions were found in birds of the chlorpyrifos groups. One of the birds in the high dose group had a pale, shrunken comb and shrunken, opaque left eye. Microfocal hepatic mineralisation was seen in one hen of the mid-dose group. These lesions, not accompanied by any clinical signs of toxicity, were considered incidental and not ascribed to OPIDN.

Many birds of the positive control (TOCP) group had shrunken, atrophic, pale combs (5/10 birds) and generalised muscle wasting (4/10 birds), the pectoral muscles being most visibly affected. Skeletal

muscle atrophy was likely to be the result of damage to innervating fibres characteristic of OPIDN.

Histopathological Findings: The type and incidence of histopathological lesions recorded in birds of the positive control (TOCP), the negative control (corn oil) and the high dose chlorpyrifos treatment (10 mg/kg bw/day) groups were summarised in Table 1.

The central and peripheral nervous tissues from the birds treated with TOCP consistently displayed microscopic lesions (of varying degree) characteristic of OPIDN. All TOCP-birds exhibited mild to moderate axonal degeneration in the dorsolateral aspects of the brain stem with a minimal to moderate glial response. Mild to extensive axonal degeneration confined primarily to the dorsal columns was observed in all cervical spinal cord tissue sections with mild to extensive focal gliosis. In the thoracic cord of TOCP-birds axonal degeneration was primarily localised in the lateral columns, whereas in the lumbosacral cord it was found in the ventral columns. Examination of peripheral nerve tissue from TOCP-treated birds revealed lesions in both the tibial and sciatic nerves: randomly distributed, mild to extensive nerve fibre degeneration, and corresponding Schwann cell hyperplasia and swelling of the axis cylinder.

Similar histopathological lesions (in terms of type, incidence and/or degree of severity) were not found in comparable nervous tissues from the negative controls or from the birds given chlorpyrifos at 10 mg/kg bw/day. The incidence and severity of lesions observed among the negative control and chlorpyrifos-treated birds were essentially the same. Randomly distributed axonal degeneration was commonly seen in all nervous tissues examined. These changes appeared to correlate positively with the incidence of lymphocytic perivascular cuffing and were judged to be the result of mild inflammation and normal ageing.

Conclusions: Under the conditions of this study, no compound-related clinical signs and increase in histopathological lesions of the nerve tissues characteristic of organophosphate-induced delayed-neurotoxicity were evident up to and including the highest dose level of 10 mg chlorpyrifos/kg bw/day. Concurrent positive TOCP-treated controls exhibited both toxic signs and histopathological lesions of the nerve tissues typical of a delayed neurotoxicant.

Table 1. Chlorpyrifos: Subchronic OPIDN study

Histopathological observations

Test substance		**TOCP**	**Control**	**Chlorpyrifos**
Treatment (mg/kg bw/day)		10	0	10
Number of hens examined		10	10	10
Brain No abnormality noted:		0	4	5
Axonal degeneration:	-minimal -mild -moderate	0 7 3	1 0 0	2 0 0
Lymphocytic perivascular cuffing:	-minimal -mild	0 2	1 1	1 0
Focal gliosis:	-minimal	2	2	3

Test substance		TOCP	Control	Chlorpyrifos
	-mild	7	0	0
	-moderate	1	0	0
Neuronal swelling/chromatolysis:	-minimal	0	1	0
Cervical spinal cord				
No abnormality noted:		0	3	0
Axonal degeneration (dorsal and lateral funiculi):	-minimal	0	1	1
	-mild	1	0	0
	-moderate	3	0	0
	-extensive	6	0	0
Axonal degeneration (random):	-minimal	7	3	6
	-mild	0	0	1
Lymphocytic perivascular cuffing:	-minimal	4	5	6
	-mild	3	1	2
	-moderate	2	0	1
Focal gliosis:	-minimal	0	6	5
	-mild	1	0	1
	-moderate	5	0	0
	-extensive	4	0	0
Thoracic spinal cord:				
No abnormality noted:		0	3	4
Axonal degeneration (ventral and lateral funiculi):	-minimal	0	1	1
	-mild	2	0	0
	-moderate	2	0	0
	-extensive	6	0	0
Axonal degeneration (random):	-minimal	6	4	4
	-mild	1	0	1
Lymphocytic perivascular cuffing:	-minimal	6	2	4
	-mild	2	2	0

Test substance		TOCP	Control	Chlorpyrifos
Focal gliosis:	-minimal	0	3	1
	-mild	0	0	1
	-moderate	4	0	0
	-extensive	6	0	0
Lumbo-sacral spinal cord				
No abnormality noted:		0	6	3
Axonal degeneration (ventral funiculi):	-mild	2	0	0
	-moderate	2	0	0
	-extensive	6	0	0
Axonal degeneration (random):	-minimal	0	0	2
Lymphocytic perivascular cuffing:	-minimal	0	3	4
	-mild	1	0	1
Focal gliosis:	-minimal	1	3	2
	-mild	2	0	1
	-moderate	3	0	0
	-extensive	4	0	0
Sciatic nerve:				
No abnormality noted (right/left):		0/0	5/3	6/2

Test substance		TOCP	Control	Chlorpyrifos
Swelling of axis cylinder (right/left):	-minimal	2/1	1/0	0/2
	-mild	4/5	0/0	0/0
	-moderate	4/1	0/0	0/0
	-extensive	0/1	0/0	0/0
Nerve fibre degeneration (right/left):	-minimal	0/0	2/3	3/4
	-mild	3/4	0/0	0/1
	-moderate	3/2	0/0	0/0
	-extensive	4/4	0/0	0/0
Lymphocytic foci (right/left):	-minimal	3/3	2/5	2/3
	-mild	0/1	1/0	1/1
	-moderate	0/0	1/1	0/0
Schwann cell hyperplasia (right/left):	-minimal	2/3	½	2/2
	-mild	3/2	0/0	0/0
	-moderate	3/2	0/0	0/0
	-extensive	2/3	0/0	0/0
Tibial nerve				
No abnormality noted (right/left):		0/0	1/4	4/2
Swelling of axis cylinder (right/left):	-minimal	0/6	1/0	0/0
	-mild	4/3	0/0	0/0
	-moderate	2/0	0/0	0/0
	-extensive	3/1	0/0	0/0
Nerve fibre degeneration (right/left):	-minimal	1/0	4/4	2/5
	-mild	0/1	0/0	1/0
	-moderate	2/5	0/0	0/0
	-extensive	7/4	0/0	0/0
Lymphocytic foci (right/left):	-minimal	6/4	1/3	1/4
	-mild	1/1	3/0	4/2
	-moderate	0/1	1/0	0/0
	-extensive	0/0	0/1	0/0
Schwann cell hyperplasia (right/left):	-minimal	0/1	2/3	½
	-mild	1/4	0/0	0/0
	-moderate	2/2	0/0	0/0
	-extensive	6/3	0/0	0/0

Wilmer JW (1992) Acute neurotoxicity study in Fischer 344 rats. Dow Chemical Company study K-044793-093B, dated 11 September 1992. (Summary only) [Dow; Submission 11462, reference 87]

The following text was submitted by the sponsors as a summary for the above study. No data were submitted for this study. An independent assessment of this study has not been conducted as part of this review. This summary was not adequate for regulatory purposes.

"Neurotoxicological effects of chlorpyrifos were evaluated in male and female Fischer 344 rats following a single-dose exposure by oral gavage to 0, 10, 50 or 100 mg/kg. Parameters examined during the two-week observation period included body weights, a Functional Observation Battery (FOB), motor activity and neuropathology. The FOB consisted of hand-held and open-field observations, grip performance and landing foot splay testing. Ten animals/sex/group were evaluated by FOB and motor activity assay; once prior to exposure, on day 1 at the time of the peak effect (approximately 6 hours after dosing), and on days 8 and 15 of the study period. Body weights were determined on days -1, 1, 2, 8, and 15. After completion of 2 weeks of study, neuropathology of central and peripheral nervous

tissues was conducted on 5 animals/sex/group.

Prominent FOB changes occurred in high-dose rats on day 1 only, females were more affected than males. Only females had FOB changes at the middle dose. Female mid- and high-dose rats had a mild decrease in grip performance, and high-dose females had a severe effect on hindlimb splay. No FOB effects were noted at the high dose. The day 1 effects were consistent with those expected of acute organophosphate overexposure. It should be noted that, on day 1, the FOB was conducted at the time of peak effects as determined by preliminary dose range-finding study. Hand-held clinical examinations on days 2, 3, and 4 indicated diminishing clinical signs of treatment to near normal by day 4. The FOB conducted on day 8 indicated the rats at all dose levels appeared clinically normal by this time. Body weights of both high- and mid-dose males and females were significantly reduced on day 2 of the test period. Body weights were not significantly altered on days 8 or 15. No significant changes in any of the FOB or clinical parameters were observed on day 15 of the study period. A statistically-significant decrease in motor activity was observed at 50 and 100 mg/kg on day 1, approximately 5 to 6 hours after dosing. On day 8, motor activity was significantly reduced at 100 mg/kg in females only. No statistically-significant changes in motor activity were observed on day 15 of the study. Histologic evaluation of the central and peripheral nervous systems did not reveal any treatment-related effects.

The results of this study indicated a No-Observed Effect Level (NOEL) of 10 mg/kg."

Mattsson JL, Wilmer JW, Shankar MR, Berdasco NM, Crissman JW, Maurissen JP & Bond DM (1996) Single-dose and 13-week repeated-dose neurotoxicity screening studies of chlorpyrifos insecticide. Food Chem Toxicol 34(4): 393-405. [Dow; Submission 11634, reference 129]

The studies in this published paper were conducted in accordance with US EPA guidelines for neurotoxicity testing. In this report, details were provided on both acute and 13-week neurotoxicity tests conducted in rats by Dow Chemical Company, USA. The citation details of the unpublished Dow reports were not provided, but it appeared as if the studies described in this report were those referred to as Wilmer, 1992 and Shankar et al., 1993, respectively. The 13-week study (Shankar et al., 1993) has been assessed later in this Chapter. The following text relates to the acute neurotoxicity study only (Wilmer, 1992).

The test material (technical chlorpyrifos; Dow; Dursban F; AGR 273801; lot no. MM-890115-616; purity 98.1%) was given to fasted male and female Fischer 344 rats (Charles River USA; 9 weeks old at start of study) as a single oral gavage dose in corn oil. In a range-finding study to determine the highest non-lethal dose and the time of peak effect, rats (2/sex/dose) were treated at 50, 100, 150, or 200 mg/kg. The rats were examined several times on the day of exposure, and then daily for 14 days.

In the main study, animals were fasted overnight before treatment, and given the test material in corn oil (dose volume 2.5 ml/kg) at doses of 10, 50, and 100 mg/kg (10 animals/sex/dose). All animals were assessed in a Functional Observational Battery (FOB) and motor activity test (prior to treatment, then on the day of dosing at 1 and 6 h, then on days 8 and 15), and tissues from 5 rats/sex/dose were fixed for neuropathological examination of central and peripheral nervous tissues. Cage-side observations were conducted twice-daily (once daily on days when FOB was conducted). The FOB consisted of

measurements (body weight, hindlimb grip performance, forelimb grip performance, landing foot splay), hand-held observations (general, palpebral closure, pupil size, lacrimation, salivation, skin or haircoat abnormalities, perineal staining, abnormal movements and/or respiration, reactivity to handling), and open-field observations (level of activity, responsiveness to sharp noise/ touch/tail pinch, abnormal behaviour, gait abnormalities, urine quantity voided, number of faecal pellets).

The 5 rats/sex/dose that were randomly preselected for neuropathology were fasted overnight, and examined for gross pathological alterations. The brain, head, spinal column with spinal cord, fore- and hind-limbs, and tail were prepared for immersion fixation. The muscles of the hindlimbs were reflected to expose the nerves, and abdominal and thoracic viscera were also saved in the same fixative. The remaining 5 rats/sex/dose were killed and a standard set of tissues was saved.

Tissues for histopathologic evaluation were prepared from rats of the control and high-dose groups only. Nine transverse sections of the brain included: olfactory bulb, cerebral cortex (frontal, parietal, temporal and occipital lobes), thalamus/hypothalamus, midbrain, pons, cerebellum, medulla oblongata, and nucleus gracilis/cuneatus. Other tissues examined were: trigeminal ganglion and nerve, pituitary, eyes (retina and optic nerve), spinal cord (cervical and lumbar), olfactory epithelium, and skeletal muscles (gastrocnemius and anterior tibial). These tissues were embedded in paraffin, sectioned approximately six microns thick, and stained with hematoxylin and eosin. Spinal nerve roots (cervical and lumbar), dorsal root ganglia (cervical and lumbar), and peripheral nerves (sciatic, tibial and sural) were osmicated, embedded in plastic, cut to 2-3 microns thick, and stained with toluidine blue.

Results

In the range-finding study, no mortalities occurred, but clinical signs of intoxication were observed at all dose levels, and increased in incidence in a dose-related manner. At 50 mg/kg, signs consisted of decreased activity (2 males/2 females), tremor (1/1), lacrimation (0/1), and faecal (1/0) and urinary (1/1) perineal staining. Additional signs were seen at higher doses, and these included salivation, incoordination, hyper reactivity to stimuli, laboured and/or rapid respiration, chromodacryorrhea, chromorhinorrhea, and reduced body weight.

In the main study, no treatment-related effects were observed at 10 mg/kg in the FOB or motor activity test. A range of effects were observed at 50 and/or 100 mg/kg, with the incidence and/or magnitude of these findings increasing with dose, and these included gait abnormalities, landing foot splay, decreased body weight, decreased grip performance, decreased motor activity, tremor, incoordination, decreased muscle tone, increased salivation, increased lacrimation, chromodacryorrhea, chromorhinorrhea, perineal staining, and hyper reactivity to stimuli. Females appeared to be more sensitive to the effect of treatment. For example, tremors were seen in a single male at 100 mg/kg, in a single female at 50 mg/kg, and in 3 females at 100 mg/kg, and increased incoordination (5/10 animals), decreased muscle tone (5/10), pronounced gait abnormalities (7/10), exaggerated lacrimation (7/10), and pronounced (4/10) to exaggerated (4/10) salivation were only seen in females (100 mg/kg).

Cage-side or clinical observations commonly revealed excessive chromodacryorrhea (high dose females only), decreased activity and perineal staining. These effects were generally confined to high dose animals, though 1/10 males and 1/10 females at 10 mg/kg were reported with decreased activity, and 1/10 control males and 2/10 low dose males reportedly had perineal staining. Clinical signs were

confined to days 2-3 after treatment.

Neuropathological examination did not reveal any gross or histopathological lesions related to treatment in animals treated at 100 mg/kg. Animals from other groups were not examined.

Under the conditions of this study, single oral doses of chlorpyrifos at 50 to 100 mg/kg resulted in treatment-related effects and clinical signs for several days after dosing. No mortality occurred at any dose. At 10 mg/kg effects were confined to isolated observations of perineal staining and/or decreased activity. No neuropathological lesions were reported in animals receiving chlorpyrifos at up to 100 mg/kg.

Maurissen JP, Shankar MR & Mattsson JL (1996) Chlorpyrifos: Cognitive study in adult Long-Evans rats. Dow Chemical Company, Study no. K-044793-096, report dated 29 April 1996. [Dow; Submission 11634; reference 130]

This study was conducted in accordance with GLP standards of the US EPA-FIFRA (Title 40 CFR Part 160), OECD, EEC and Japanese MAFF.

The test material (chlorpyrifos technical; Dow; Dursban F Insecticide; lot #MM-890115-6-6; stated purity 98.1%) was given in corn oil to female Long-Evans rats (Charles River USA; approximately 4 months old at beginning of training) by oral gavage for 4 weeks, 5 days/week at doses of 0, 1, 3, and 10 mg/kg/d, with a dose volume of 1 ml/kg. Dosing was carried out after the daily cognitive test, where applicable.

This study consisted of two components: the main behavioural study, using 10 animals/dose, and a satellite study to determine clinical chemistry parameters only, using 6 animals/dose.

In the main study, 50 animals were trained on a cognitive task (delayed matching to position or DMTP), and the first 40 animals to reach a preset stability criterion were retained, and assigned to control and treatment groups so that the group differences in overall percent correct choice were minimised. A one-week baseline was established for the cognitive function task, after which time the dosing commenced for the 4-week test period. The testing was conducted 6 days/week during the treatment phase of the study, and then for 4 weeks post-dosing.

Clinical observations were conducted twice-daily for 5 weeks (5 times/week), then daily during the recovery period. During the treatment phase of the study, animals were observed about 3 h and 21 h after each dose. The clinical observations consisted of hand-held examination of pupil size, lacrimation, salivation, perineal staining, tremors, general appearance, palpebral closure, skin/haircoat abnormalities, abnormal movements, abnormal respiration, and reactivity to handling. Rectal temperature was measured during baseline, dosing and recovery phases of this study at approximately 3 and 21 h post-dosing.

The delayed matching-to-position (DMTP; a delayed-response task) sessions were each terminated after 100 completed trials, or after approximately 150 minutes, whichever came first. An internal Dow report had previously addressed the validation issue of the DMTP testing in male and female Fischer 344 rats, with some slight variations from this study design.

Plasma, erythrocyte (RBC) and brain cholinesterase (ChE) activity assays were performed on the satellite group on the morning after the last day of the 4-week treatment phase, and on 24 rats from the DMTP groups 4 weeks after the end of dosing. Brain neuropathy target esterase (NTE) was assayed in the satellite group (6 rats from each of the control and high dose groups) on the morning after the 4-week dosing period.

Results

Clinical examinations: A dose-related decrease in plasma and brain ChE activity was observed on the first day after dosing, and RBC ChE activity was decreased by similar amounts at all doses. The decreases in ChE activity at 1, 3, and 10 mg/kg/d (percentage inhibition compared with controls) were 69, 82, and 93% for plasma, 56, 65, and 65% for RBC, and 8, 63, and 86% for brain, respectively. Recovery of ChE activity was observed by 4 weeks after cessation of dosing, and the ChE activity was generally 80% of the control values by this time. NTE activity in the high dose group was 93% of control activity, and a decrease of this magnitude was not considered to be toxicologically significant.

Rectal temperature was generally similar in control and treated groups. At 10 mg/kg/d, the rectal temperature was approximately 1^{o}C lower than control for the first week of dosing only.

A number of clinical signs were observed, including salivation, tremor and myosis. Salivation was seen in high dose animals throughout the dosing period. The incidence of this finding was generally greatest 3 h after dosing, but was also noted at the 21-h inspections. At doses below 10 mg/kg/d, the incidence of salivation was similar to controls. An increased incidence and severity of miosis was noted at 10 mg/kg/d throughout the study, and this finding was observed at both the 3-h and 21-h inspections at about the same incidence. At 3 mg/kg/d, there was also a suggestion of an increase in the incidence of miosis, though the relevance of treatment to this effect was complicated by a high background incidence of miosis in control animals, and at 1 and 3 mg/kg/d, the incidence and severity of miosis was generally comparable with that seen in controls. No animals had miosis pre-treatment, but the control incidence increased to 50-60% by the end of the study. The reason for this effect was not clear. Tremor was observed at 10 mg/kg/d in all 4 weeks of treatment, almost exclusively at the 3-h inspections. A single observation of tremor was also noted in animals treated at 1 and 3 mg/kg/d in the first week of treatment. The isolated nature of these last findings made it difficult to attribute them to treatment, but no control animals displayed tremors during the study, and no tremors were seen prior to initiation of treatment. A range of other minor signs were observed in all groups, including controls, and were not considered to be treatment-related.

Non-cognitive measures: A number of performance (non-cognitive) measures were examined, but not statistically analysed. The results of these investigations were outlined below.

Actual total delay: the actual total delays (time spent between pressing the sample lever and the choice lever) increased during dosing weeks 1 and 2 at 10 mg/kg/d for all delays (0-, 5-, 10-, 15-, and forced 15-second delays). These delays then stabilised or decreased slightly in weeks 3-4 of dosing, but generally remained higher than the total delays seen in control or other treated groups. The actual total delay increase was seen at all intervals (1-4 weeks). The actual total delays recovered in the 10 mg/kg/d group by the first week post-exposure. These delays could possibly be attributed to decreased motor

function in rats at the high dose level.

Total void trials: the total void trials (trials that were declared void if the appropriate response was not given within any one of the limited holds) increased during the first two weeks of treatment at 10 mg/kg/d, then decreased to baseline levels by week 1 post-exposure. The number of void trials at this dose level was higher than controls at each interval during treatment. Along with the increased means came large increases in variability, and these increases in variability were also seen at 3 mg/kg/d (weeks 2-8) and 1 mg/kg/d (weeks 6-8). The increase in void trials at 10 mg/kg/d was considered to be related to treatment, but at lower doses the total number of void trial was generally similar to controls, and/or was not dose-related.

Retracted sample lever presses: data variability for this parameter tended to increase with time, especially in the 1 mg/kg/d group, making the interpretation of this results more difficult, but there was an apparent increase in the number of lever presses per trial in treated groups compared with controls, though there was generally no apparent dose-response relationship for this finding. The exception was in the forced 15-second delay trials, where there was a dose-related increase in all nominal delay times, though these mean increases were within the large variability seen at all dose levels.

Nose-pokes: The number of nose-pokes increased compared with baseline levels as a function of the increased delay, in all groups, indicating that the rats were spending proportionally more time with their nose in the food cup as the delay increased. In weeks 1 and 2, the number of nose-pokes was lower at 10 mg/kg/d than in other groups, but this effect had largely disappeared in weeks 3-4.

Cognitive measures: The slopes of the retention gradient (retention of information) did not indicate a relationship between chlorpyrifos and a measure of mnesic retention. The different dose groups generally retained the position they held when tested under baseline conditions. Repeated-measure analysis of variance of the data provided a $p = 0.37$ for the time-by-dose interaction (Pillai trace statistic).

When the intercept of the retention gradient during baseline and dosing was plotted, chlorpyrifos did not apparently affect the intercept (used as a measure of the attention/encoding processes) at any dose over time. The data suggested that groups generally stayed within the baseline range of variation for this parameter. The exception was for the 10 mg/kg/d group, where there was an increase in the intercept at week 2 and a decrease in the intercept at week 3, and this resulted in a finding of a statistically-significant time-by-dose interaction ($p = 0.013$). However, the data from weeks 1 and 4 for the 10 mg/kg/d group were similar to controls, and so there was no consistent relationship with time for this effect at the high dose level.

Summary

Under the conditions of this study, the oral administration of chlorpyrifos to rats at doses up to 10 mg/kg/d resulted in dose-related inhibition of cholinesterase activity (1 mg/kg/d and above for plasma and RBC; 3 mg/kg/d and above for brain), accompanied by clinical signs of intoxication at 10 mg/kg/d. A cognitive behavioural study indicated some non-cognitive changes associated with impaired motor activity at 10 mg/kg/d, but no clear treatment-related effects on cognitive function were observed.

Anon (1996f) Neurotoxicity study of chlorpyrifos technical to hen. Department of Toxicology, Jai Research Foundation, India. Report 1539/JRF/TOX/96, dated 10 February 1996. [Submission 11513]

This study was conducted in accordance with the Guidelines of Gaitonde Sub-Committee report to the Central Insecticides Board, Govt. of India. No statement was made on the GLP status of this study. This study was performed between 6 October 1995 and 26 October 1995.

To assess the acute delayed neurotoxicity potential of chlorpyrifos technical (Mitsu Industries, India; stated purity 95%; batch no. 041195), the test material was given to white leghorn hens (*Gallus gallus domesticus;* Vikram farm, India; 10 months old; 1.2-1.6 kg; 6/group) as a daily oral dose by gavage at 0 (vehicle control), 2, 5, and 12 mg/kg/d in a peanut oil vehicle (dose volume 5 ml/kg for 21 days). All birds were observed for signs of toxicity and behavioural changes, with ataxia scored daily prior to dosing. The body weight of all birds was measured daily. Blood was collected on day 22 and used for the evaluation of differential leucocyte count and blood, plasma, and RBC cholinesterase activity. At the terminal kill, brain, and spinal cord (from three different levels), sciatic nerve and tibial nerve were collected and fixed for microscopic pathological examination.

Results

No mortalities were observed during the study. A dose- and time-related increase in the incidence of ataxia was observed. No ataxia was seen in control birds. At 2 mg/kg/d, ataxia was observed in all birds, and the severity of this finding increased from severity scores of 0-1 (on a scale of 0-8) in the first half of the dosing period, to scores of 1-2 by the end of the dosing period, and similar ataxia results were observed at 5 mg/kg/d. At 12 mg/kg/d, scores of 2 (slight incoordination: occasional stumbling or wing dropping especially after exertion) were seen in 3/6 birds; a scores of 3 (frequent incoordination or stumbling especially on alighting or after exertion), 4 (staggering gait, tail and leg reflexes may be affected and bird lands awkwardly), and 6 (bird stands for short periods only, normally moves by shuffling on hocks, tail and leg reflexes usually noticeably affected) were seen in single birds.

Body weights were not affected by treatment, and egg yield and weight, and differential leucocyte counts were similar in control and treated groups. Plasma cholinesterase activity was decreased by 46 and 53% at 5 and 12 mg/kg/d, respectively, and whole blood cholinesterase activity was reduced by 22 and 20%, respectively, at the same dose levels. RBC cholinesterase activity was not affected by treatment, and no reductions in plasma or whole blood ChE activity were seen at 2 mg/kg/d.

Necropsy examination did not reveal any findings associated with treatment. Histopathological examination revealed a number of findings at a low incidence, without any relationship with dose. These include: mild focal haemorrhage of the cerebellum (1/6 birds at 2 and 5 mg/kg); mild gliosis of the cerebrum (2/6 in controls and 1/6 in other groups); satelitosis of the cerebrum (1/6 at all doses including controls); satelitosis (1/6 control birds only) and mild vacuolation (1/6 high dose birds only) of the spinal cord at mid-thoracic region; and a single control bird with mild vacuolation of the spinal cord at the lumbosacral region. These findings were considered to be incidental to treatment.

The ongoing administration of chlorpyrifos resulted in ataxia in test groups during the study, which would have made it difficult to observe any delayed neurological effects in treated birds, had any such effects

been manifest. The histopathological examination suggested that the administration of chlorpyrifos to hens did not cause any neuropathological effects at any dose in this study. No inhibition of plasma cholinesterase activity was observed at 2 mg/kg/d.

Dittenber DA (1997) Chlorpyrifos: Evaluation of single oral doses on cholinesterase and neurotoxic esterase inhibition in F344 rats. Dow Chemical Company study no. 960036, dated 13 March 1997. [Dow; Submission 11634, reference 127]

This study was reportedly conducted in accordance with Good Laboratory Practice Standards of the US EPA-FIFRA GLPS, Title 40 CFR Part 160.

This study was conducted to establish the dose response for inhibition of cholinesterase (ChE) and neurotoxic esterase (NTE) in female Fischer 344 rats (Charles River USA; approximately 7 weeks old at start of study; 6/dose) given a single oral dose of technical chlorpyrifos (Dow; Dursban F; Lot #MM-890115-616; stated purity 98.1%). The test material was delivered in a corn oil vehicle (dose volume 10 ml/kg), at doses of 0 (control), 0.5, 1, 5, 10, 50, and 100 mg/kg. At 0.5 mg/kg, only ChE activity was measured.

Analysis of test material/vehicle to verify the test material concentration was conducted at the start of the study. The test material concentration was found to be 90-98% of the targeted doses.

Cageside observations were conducted twice-daily for the incidence of mortality, morbidity, moribundity, treatment-related signs, and the availability of feed and water. Body weights were measured prior to randomisation and once prior to dosing. Heparinised blood samples were obtained by orbital sinus puncture from anaesthetised animals, and the brain and hearts were removed from all animals. The brains were divided in half, with one half used for NTE determinations. The remaining brain portions, hearts, plasma and erythrocytes were used for ChE determinations 24 h post-exposure.

Results

The report stated that no treatment-related signs were noted at any of the cageside observation periods. These data were not provided in this report.

Mean body weights in treated groups were similar to control values.

Under the conditions of this study, no treatment-related effects were observed on NTE activity at any dose. Plasma, RBC, and heart ChE activities were statistically significantly decreased compared with controls at doses of 5 mg/kg and above, and brain ChE was decreased at 50 mg/kg and above. The percentage decreases in ChE activity, compared with controls, at 5, 10, 50, and 100 mg/kg were: 45, 61, 87, and 90%, respectively, for plasma; 17, 29, 52, and 51%, respectively, for RBC; 2, 7, 53, and 70%, respectively, for brain; and 20, 40, 73, and 91%, respectively, for heart. No significant ChE inhibition was observed at 0.5 or 1 mg/kg/d.

Nostrandt AC, Padilla S & Moser VC (1997) The relationship of oral chlorpyrifos effects on behaviour, cholinesterase inhibition, and muscarinic receptor density in rat. Pharmacology

Biochemistry and Behaviour, Vol 58, No. 1, pp. 15-23, 1997. [Dow; Submission 11634, reference 128]

No GLP or Test Guideline statements were made in this report.

The neurotoxicity potential of chlorpyrifos (99.5% purity; Chem Serve Co., USA) was assessed in male Long-Evans Hooded rats (20/group; approximately 70 days old; Charles River USA; mean body weight 350 g). The test material was dissolved in corn oil, and given to the rats by oral gavage (dose volume 1 ml/kg) at doses of 0, 10, 30, 60, and 100 mg/kg. All rats were evaluated for behavioural signs at 3.5 h post-dosing, and half the animals in each group were killed at this time. The remaining 10 animals/group were again examined at 24 h post-dosing, and killed at this time. Immediately following sacrifice, the following tissues were collected from all animals: brain, retina, liver, heart, diaphragm, and *quadriceps femoris* muscle. The brain was sectioned longitudinally (mid-sagitally), and the remaining half-brain was further sectioned to give frontal cortex, hippocampus, striatum, hypothalamus, cerebellum, and pons/medulla. Trunk blood was collected.

All animals were examined using a Functional Observational Battery (FOB), consisting of home cage, open-field and manipulative neurobehavioural evaluations. The test measures were: autonomic (lacrimation, salivation, miosis, defecation), activity (motor activity, rearing, home cage posture), convulsive (tremors), neuromuscular (gait score, landing foot splay, righting reaction), general reactivity (arousal, removal reactivity), sensorimotor (tail-pinch response, click response, approach response), and physiological (body temperature, body weight). Motor activity data were collected shortly after FOB testing, using a maze, and activity was recorded during a 1-h session. Tissue samples were obtained after the motor activity tests, so sample times for tissue collection were 4.5 and 25 h post-dosing.

Muscarinic receptor density was determined in all brain regions as well as heart and retina. Cholinesterase (ChE) determinations were conducted on brain tissues, muscle tissues, retina, plasma, erythrocytes (RBC), whole blood, and liver.

Results

One rat died within 24 h of receiving a dose of 100 mg/kg, and some weight loss was observed in animals at doses of 30 mg/kg and above, after the 24 h treatment.

Behavioural effects: No behavioural changes were observed in animals treated at 10 mg/kg/d. At the 3.5 h observation, effects that were statistically significantly different to controls were: decreased defecation, decreased motor activity, decreased rearing response, smacking, increased gait score, decreased click response, and decreased body temperatures (all at 30, 60, and 100 mg/kg); increased landing foot splay, decreased removal reactivity, decreased tail pinch response, and decreased body weight (all at 60 and 100 mg/kg); and lacrimation, salivation, miosis, flattened home cage posture, and righting reaction (all at 100 mg/kg). At the 24-h assessment, the effects were: decreased motor activity, decreased rearing, and increased gait score (all at 60 and 100 mg/kg); and flattened home cage posture and decreased body temperature at 100 mg/kg. Decreased arousal and increased approach response were observed at 60 mg/kg only. Statistically-significant decreases in motor activity at the 3.5 h assessment improved considerably by 24 h, but there were still reductions compared with controls.

Biochemical changes: Marked decreases in plasma, RBC, and whole-blood cholinesterase activity were observed at all doses tested, at both 4.5 and 25 h. The data were represented as graphs only, and so the percentage inhibition figures that follow were approximates only. RBC ChE was slightly more sensitive than plasma ChE at the doses used in this study. At 4.5 h, the inhibition of RBC ChE was approximately 90, 95, 100 and 100% at 10, 30, 60, and 100 mg/kg, respectively, compared with 80, 85, 85, and 90% inhibition, respectively, for plasma ChE. Plasma ChE activity also recovered more quickly than RBC activity, and after 25 h, the RBC activity was inhibited by approximately 75, 90, 100 and 100%, compared with inhibition of plasma ChE of 35, 75, 95, and 95%, respectively. Whole-blood cholinesterase inhibition was somewhere between the plasma and RBC figures, both at 4.5 and 25 h.

At 10 mg/kg, brain ChE activity was inhibited by approximately 40% at 4.5 h, and by 30% at 25 h. At 30, 60, and 100 mg/kg, these figures were 70, 85 and 90% at 4.5 h, and 60, 80, and 85% at 25 h, respectively. Whole-brain cholinesterase activity could be considered roughly representative of that in the various brain regions, although a number of brain regions demonstrated less ChE inhibition than was measured in the half-brain in this study.

In the peripheral tissues, the ChE inhibition was much lower in the quadriceps than in other tissues at 4.5 h, and inhibition of heart, liver, and retina ChE was similar at 25 h.

Percentage inhibition of cholinesterase activity compared with controls* (Approximate values)

Time	Dose mg/kg	Plasma	RBC	Blood	Half-Brain	Retina	Heart	Diaphragm	Quadriceps	Liver
4.5 h	10	80	90	85	40	40	55	40	5	70
	30	85	95	90	70	65	65	55	20	70
	60	85	100	90	85	80	70	70	70	80
	100	90	100	95	90	90	75	85	85	85
25 h	10	35	75	55	30	30	40	5	+10	25
	30	75	90	85	60	60	75	35	20	70
	60	95	100	95	80	75	90	65	35	85
	100	95	100	95	85	85	90	75	55	85

* In all instances where inhibition was 20% or greater compared with corresponding controls, the change in ChE activity was statistically significant ($p<0.05$)

At 100 mg/kg, apparent down-regulation of muscarinic receptor density was noted in the pons/medulla at 4.5 h (decreased by 23%), and in the striatum at 25 h (decreased by 27%).

Correlation between behavioural and biochemical changes: The study was conducted with the aim of investigating any relationships between specific behavioural measures and ChE inhibition. At 10 mg/kg, there was significant inhibition of ChE in most tissues, but no effects were observed in the FOB at this dose level. Correlation coefficients were calculated for the analysis of behavioural measures and ChE inhibition, and at 4.5 h, for example, the r-values suggested that some behavioural measures (eg. activity, smacking, and temperature) were more closely related to brain ChE inhibition, whereas other findings (eg. lacrimation and tremors) were better related to peripheral ChE inhibition. However, r-values were often comparable for both brain and peripheral tissue ChE inhibition, and so the results of this portion of the study were not definitive. The authors noted that the correlations between behavioural and biochemical effects were generally poor, because behavioural signs were not observed

at the low dose (and ChE inhibition was), and behavioural signs showed recovery at 24 h, whereas ChE activity did not. This was generally true. However plasma, whole blood, liver, and diaphragm ChE activity did recover markedly between 4.5 and 25 h after treatment at 10 mg/kg.

To examine the relationship between clinical and behavioural findings and ChE inhibition, the study authors also grouped rats according to the extent of their ChE inhibition (instead of by dose), and indicated the incidence of cholinergic signs in relationship to the individual ChE inhibition. The 10 'cardinal' cholinergic signs that were used for this analysis were stated to be 'temperature, gait, motor activity, smacking, foot splay, tail pinch, tremors, lacrimation, pupil response, and salivation'. In animals that had <60% inhibition of brain ChE activity (n=18), none of these signs were observed. There was an increase in incidence of these findings related to the increase in brain ChE inhibition. In animals that had <80% whole blood ChE inhibition, no signs were observed, while all signs were seen, with varying incidences, in animals with 90-100% whole blood ChE inhibition. The correlation between findings and other tissue ChE inhibition (RBC or plasma, for example) was not provided in this report. These results were interpreted by the report authors as being indicative of a threshold-effect, with no clinical signs observed until ChE was inhibited by a certain percentage in various tissues after a single dose of chlorpyrifos. The study authors cautioned against extrapolating these findings to other pesticides, other behavioural endpoints, or other species.

Incidence (%) of each cardinal cholinergic sign for rats, grouped according to ChE inhibition

	Brain ChE inhibition					**Blood ChE inhibition**		
End Point (no. of rats)	90-100% (10)	80-90% (8)	70-80% (7)	60-70% (7)	<60% (18)	90-100% (25)	80-90% (14)	<80% (11)
Temperature	100 %	100 %	86 %	57 %	0 %	92 %	36 %	0 %
Gait	100	100	57	71	0	84	43	0
Motor activity	90	88	86	57	0	84	36	0
Smacking	90	63	57	0	0	64	14	0
Foot splay	40	38	29	0	0	32	14	0
Tail Pinch	70	38	0	0	0	40	0	0
Tremors	90	25	0	0	0	44	0	0
Lacrimation	80	13	0	0	0	32	7	0
Pupil response	60	0	0	0	0	24	0	0
Salivation	30	0	0	0	0	12	0	0

Summary

Under the conditions of this study, cholinesterase activity was significantly inhibited in a range of tissues and tissue components, including brain, plasma and erythrocytes, at all doses of 10 mg/kg and above. No clinical or behavioural signs were observed at 10 mg/kg, but were observed at 30 mg/kg and above at the 4.5 h sample interval.

The selection of doses in this study resulted in a skew of some of these correlation data (acknowledged by the study authors), especially in the correlations for ChE inhibition in blood and blood components with behavioural and clinical findings, as marked ChE inhibition was observed at the lowest dose in this

study in the absence of clinical findings. The use of a single acute dose in this study also limited the usefulness of the findings. Although no clinical signs were associated with inhibition of brain ChE activity of less than 60% compared with controls, or whole blood ChE inhibition of less than 80% compared with control following a single dose of chlorpyrifos, there were no data to suggest that these figures could be extrapolated to repeat-dose situations. It is quite likely that the apparent threshold ChE inhibition that is needed before clinical and/or behavioural signs were observed after repeated doses would be lower than the 60% (in brain) or 80% (in blood) that were observed after a single dose in this study.

Shankar MR, Bond DM & Crissman JW (1993) Chlorpyrifos: 13-week Neurotoxicity Study in Fischer 344 rats. Dow Chemical Company Study no. K-044793-094, dated 16 September 1993 [Dow; Submission 11462, reference 88; Submission 11464, reference 12]

This study was conducted in accordance with the following requirements for GLP: US EPA 40-CFR Part 160, 1990; Japan MAFF, 59 NohSan, Notification 3850, 1984; and OECD, 1982, and to fill US EPA-FIFRA Guideline no. 82-7.

The test material (chlorpyrifos technical; 98.1% purity; Dursban F; Dow USA; AGR 273801; lot # MM-890115-616) was given to groups of male and female Fischer 344 rats (Charles River USA; 7-weeks old at start of study; 10/sex/dose) in the diet (Certified Purina Rat Chow #5002) for 13 weeks at doses of 0 (control), 0.1, 1, 5, or 15 mg/kg/d. The dietary concentrations used to achieve these doses were not stated. The dose levels in this study were selected based on the results of a previously conducted subchronic study (Szabo et al., 1988).

Homogeneity of the test material/vehicle (not stated) mixture was determined at the start of the study. Samples were taken from five different locations in the low dose diet, and the test mixture was determined to be homogenous. Concentration analyses were conducted at the beginning, middle and end of the study, and mean concentrations were stated to range from 95 to 103% of target concentrations. Reference samples (1/dose/sex/mix) were retained and stored at ambient temperature.

Body weights and feed consumption data were measured during the pre-study period, and weekly during the dosing period. Chlorpyrifos had previously been determined to be stable in basal rodent feed for at least 56 days. Premixes were prepared every 3-4 weeks. Ophthalmic examinations were conducted pre-study and at necropsy.

Cageside observations were conducted twice-daily, including evaluation of morbidity, mortality, skin, mucous membranes, respiration, central nervous system function, and behaviour. On weekends and holidays, evaluation was confined to assessment of morbidity and mortality. Clinical examinations were conducted weekly, and included observations of general appearance, palpebral closure, lacrimation, salivation, perineal staining, abnormal movements, abnormal respiration, and reactivity to handling.

A Functional Observation Battery (FOB) was conducted on all rats pre-study and during weeks 4, 8, and 13 of treatment. The FOB was conducted as a blind study, and consisted of measurements (body weight, hindlimb grip performance, forelimb grip performance, landing foot splay), hand-held observations (general, palpebral closure, pupil size, lacrimation, salivation, skin or haircoat abnormalities, perineal staining, abnormal movements and/or respiration, reactivity to handling), and open-field

observations (level of activity, responsiveness to sharp noise/touch/tail pinch, abnormal behaviour, gait abnormalities, urine quantity voided, number of faecal pellets).

Each animal was tested for motor activity, consisting of six 8-minute periods in a motor activity cage utilising an infra-red beam to measure activity. The experimental design was referred to as a split-plot factorial design with two between-block (sex and dose) treatments and two within-block (epoch and week) treatments.

After 13 weeks of treatment, 5 rats/sex/dose that were randomly preselected for neuropathology were fasted overnight, and examined for gross pathological alterations. The brain, head, spinal column with spinal cord, fore- and hind-limbs, and tail were prepared for immersion fixation. The muscles of the hindlimbs were reflected to expose the nerves, and abdominal and thoracic viscera were also saved in the same fixative. The remaining 5 rats/sex/dose were killed and a standard set of tissues was saved.

Tissues for histopathological evaluation were prepared from rats from the control and high-dose groups only. Nine transverse sections of the brain included the olfactory bulb, cerebral cortex (frontal, parietal, temporal and occipital lobes), thalamus/hypothalamus, midbrain, pons, cerebellum, medulla oblongata, and nucleus gracilis/cuneatus. Other tissues examined were the trigeminal ganglion and nerve, pituitary, eyes (retina and optic nerve), spinal cord (cervical and lumbar), olfactory epithelium, and skeletal muscles (gastrocnemius and anterior tibial). These tissues were embedded in paraffin, sectioned approximately six microns thick, and stained with hematoxylin and eosin. Spinal nerve roots (cervical and lumbar), dorsal root ganglia (cervical and lumbar), and peripheral nerves (sciatic, tibial and sural) were osmicated, embedded in plastic, cut to 2-3 microns thick, and stained with toluidine blue.

Results

Cageside and clinical observations: A low incidence of perineal soiling was observed in females (one female at weeks 12 and 13 at 5 mg/kg/d, and one female at week one at 15 mg/kg/d). No other findings related to treatment were observed.

FOB observations: A low incidence of slight perineal staining was observed in females at 5 mg/kg/d (2/10 at week 8 and 1/10 at week 13) and 15 mg/kg/d (2/10 at weeks 4, 3/10 at week 8 and 1/10 at week 13). No perineal staining was observed in control animals, or at 0.1 or 1 mg/kg/d. The FOB did not identify any other findings that were considered to be related to treatment.

Group mean body weights were similar in control and treated animals. In the high-dose males, a reduction in body weight of approximately 3% compared with controls was observed, but this effect was not statistically or toxicologically significant. Slight decreases in hindlimb and/or forelimb grip strength compared with controls were observed in high-dose males in the second half of the study, but these changes were not statistically significant, and appear to be within normal variability for such effects in this study. Hindlimb landing foot splay was similar in control and treated groups.

Motor activity: The report described a statistically-significant decrease in the overall treatment-by-week interaction (ie. the groups significantly differed overall with respect to time). Following this analysis, a step-down analysis compared the control group versus each treatment group in a repeated-measure design, and indicated that only the high-dose group was significantly different from the control group

(decreased activity). However, the results from week 4 appear to have contributed substantially to this finding, and if the week-4 results were removed from the assessment, the statistical significance of this finding is lost. As there was no apparent treatment-related effect on motor activity at the 8 and 13 week assessments, there does not appear to be a strong relationship between any effects on motor activity and time.

It is possible that the decrease in motor activity at 4 weeks was related to treatment, and that, through adaptation or accommodation, the animals were less sensitive to this effect at the 8 and 13-week intervals. The doses used in this study were based on a study by Szabo et al., 1988. In this earlier study, at the high dose of 15 mg/kg/d, significant inhibition of erythrocyte cholinesterase activity (43 and 90 days) and brain cholinesterase activity (measured at 90 days only) were observed. Erythrocyte cholinesterase activity was similar at 43 days (47%) and 90 days (45%), compared with controls, though these sample times were different to those in the current study. If similar cholinesterase inhibition occurred in this current study, then the decrease in motor activity at 4 weeks, if considered to be treatment-related, was reversible and possibly transient, and was likely to have been accompanied by significant inhibition of brain cholinesterase activity also (62% at 90 days). However, the absence of this effect at other doses and at other intervals makes interpretation of the toxicological significance of the motor activity finding at week 4 difficult, especially as observations were not made prior to the 4-week interval.

Pathology: Gross pathological examination did not reveal any treatment-related effects. A single female in the 5 mg/kg/d group had a diaphragmatic hernia nodule on the cranial aspect of the left middle lobe of the liver, and the stomach of this female was adhered to the liver. Two control females and one female at 5 mg/kg/d had unilaterally distended ovarian bursas, and one of these control females had a dark ovary. A single male at 5 mg/kg/d had unilaterally decreased testes size.

Histopathological examination revealed a small number of isolated findings that were similar in control and treated groups, and which were not considered to be related to treatment. These included very slight degeneration of individual nerve fibres in the trapezoid body of the medulla oblongata (4/5 control males and 3/5 control females versus 1/5 high dose males and 2/5 high dose females); very slight degeneration of individual nerve fibres in the cervical spinal cord in a single high-dose male; and single myelin ellipsoids in the lumbar spinal cord of one high-dose male and one control female.

Under the conditions of this study, the dietary administration of chlorpyrifos to rats at doses up to 15 mg/kg/d for 13 weeks resulted in a low incidence of perineal staining in females at 5 and 15 mg/kg/d. No decrease in body weights was recorded at any dose. A slight reduction in motor activity was observed at 15 mg/kg/d at week 4 only, but the toxicological significance of this finding was unclear. Cholinesterase activity was not measured in this study. No neuropathological findings related to treatment were observed at any dose. The NOEL for this neurotoxicological study, based on the clinical signs at 5 and 15 mg/kg/d, was 1 mg/kg/d.

Moser VC & Padilla S (1998) Age- and gender-related differences in the time course of behavioral and biochemical effects produced by oral chlorpyrifos in rats. Toxicol Appl Pharmacol 149(1): 107-119

This study compared the behavioral and biochemical toxicity of chlorpyrifos in young (postnatal Day 17; PND17) and adult (about 70 days old) rats.

Dose ranging studies were carried out to establish the magnitude of the differences in effective doses and the time course of chlorpyrifos effects in rats of various ages. The observed Maximum Tolerated Dose (MTD, single oral dose) for each age group induced less than 10% mortality but did induce clear signs of toxicity at 30 and 60 minutes post-dose. These observations (Table I) indicate that the magnitude of age-related differences in chlorpyrifos toxicity decreased as the rat matures.

Table I

Age (days)	MTD (mg/kg)	Increased Sensitivity (adult MTD/age-specific MTD)
10	15	6.7
17	20	5.0
27	50	2.0
Ca. 70	100	

The main study evaluated the time course after a single oral dose of chlorpyrifos in adult and PND17 male and female rats. Behavioral changes were assessed using age-appropriate functional observational batteries and an evaluation of motor activity. Cholinesterase (ChE) activity was measured in brain and peripheral tissues and muscarinic receptor binding assays were conducted on selected tissues. Rats received either vehicle (corn oil) or chlorpyrifos (adult dose: 80 mg/kg; PND17 dose: 15 mg/kg); these doses were equally effective in inhibiting ChE. The rats were tested, and tissues were then taken at 1, 2, 3.5, 6.5, 24, 72, 168, or 336 h after dosing.

In adult rats, peak behavioral changes and ChE inhibition occurred in males at 3.5 h after dosing, while in females the onset of functional changes was sooner, the time course was more protracted and recovery was slower. In PND17 rats, maximal behavioural effects and ChE inhibition occurred at 6.5 h after dosing, and there were no gender-related differences. Behavioral changes showed partial to full recovery at 24 to 72 h, whereas ChE inhibition recovered markedly slower. Blood and brain ChE activity in young rats had nearly recovered by 1 week after dosing, whereas brain ChE in adults had not recovered at 2 weeks. Muscarinic-receptor binding assays revealed apparent down-regulation in some brain areas, mostly at 24 and 72 h. PND17 rats generally showed more receptor down-regulation than adults, whereas only adult female rats showed receptor changes in striatal tissue that persisted for 2 weeks. Thus, compared to adults (1) PND17 rats showed similar behavioural changes and ChE inhibition although at a five-fold lower dose; (2) the onset of maximal effects was somewhat delayed in the young rats; (3) ChE activity tended to recover more quickly in the young rats; (4) young rats appeared to have more extensive muscarinic receptor down-regulation, and (5) young rats showed no gender-related differences in functional changes.

Campbell CG, Seidler FJ & Slotkin TA (1997) Chlorpyrifos interferes with cell development in rat brain regions. Brain Res Bull 43(2): 179-189

Chlorpyrifos was administered (SC in DMSO vehicle) to neonatal rats (strain not stated, numbers/group not clear) daily at doses of 1 or 5 mg/kg/d from postnatal days 1 through 4 (PND1-4) or daily at 5 or 25 mg/kg/d to neonates from PND11-14. These two groups of pups were examined at postnatal days

5 and 10 or 15 and 20 respectively. Investigations examined the developing brain regions viz. brainstem (including midbrain, colliculi, pons, medulla oblongata and thalamus), forebrain (basal ganglia, hippocampal formation, neocortex) and cerebellum for signs of interference with cell development, using markers for cell packing density and cell number (DNA concentration and content) and cell size (protein/DNA ratio). The authors reasoned that the brainstem undergoes its primary phase of neurogenesis prenatally and develops prominent cholinergic innervation during the first week postpartum; the forebrain, which also becomes a prominent cholinergic target region, develops somewhat later; and the cerebellum, which undergoes a postnatal peak of neurogenesis, remains poor in cholinergic innervation.

Results

Control animals (vehicle only) displayed the expected pattern of brain weight increases. From postnatal day 5-20 when body weight increased 4-fold, increases in weight of brain regions were: brainstem 2-fold, forebrain 3-fold, and cerebellum more than 10-fold. Other data indicated that the brainstem and forebrain grew mainly through cell enlargement, while the cerebellum grew through cell replication.

Animals treated with chlorpyrifos from postnatal days 1-4 showed significant mortality at 5 mg/kg/d (>50%) but not at 1 mg/kg/d. The body weights of these 5 mg/kg/d pups were decreased by 10-15% (PND 5), and the brain as a whole was significantly smaller compared to controls. The survivors exhibited severe cell loss in the brainstem; brainstem growth was maintained by enlargement of the remaining cells. This effect was not seen at 1 mg/kg, a dose that did not compromise survival or growth, nor was there any adverse effect at either dose in the forebrain, despite the fact that both brainstem and forebrain possess comparable cholinergic projections.

Observations of animals treated from postnatal days 11-14 were made on PND15. There was no mortality at 5 mg/kg/d but significant mortality (>80%) at 25 mg/kg/d. On PND15 the body weights of the pups at 25 mg/kg/d were significantly decreased (>40%) and the major target for cell loss shifted from the brainstem to the forebrain. The pups treated with 5 mg/kg/d recorded a small transient loss in body weight and no significant effects on whole brain weight. However, these pups displayed a loss of forebrain cell number between 15 and 20 days of age. The total amount of DNA in the forebrain declined in the 5 mg/kg/d group (from 0.77 ± 0.01 mg to 0.70 ± 0.02 mg) when compared to the rise seen in the control pup forebrains (from 0.75 ± 0.01 mg to 0.89 ± 0.02 mg). The brainstem also showed a smaller but not significant cell loss during this period. The cerebellum differed from the other regions in that it showed short-term elevations of DNA after chlorpyrifos exposure in either early or late postnatal periods; nevertheless, values then regressed to subnormal, in parallel with the loss of cells in other regions.

The authors concluded that chlorpyrifos likely causes delayed cell death. Although regions rich in cholinergic projections, such as brainstem and forebrain, may be more affected than noncholinergic regions (cerebellum), the maturational timetable of each region (brainstem earliest, forebrain intermediate, cerebellum last) appears to be more important in setting the window of vulnerability. Brain region weights remained within normal limits despite severe reductions in cell number, probably because reactive hypertrophy of the remaining cells masked the cell loss. These results suggested that even when growth or survival were unaffected, chlorpyrifos produced cellular deficits in the developing brain that could

contribute to behavioural abnormalities. However, no information on clinical effects was provided and no assessment of behaviour or cognitive function of the pups was made, thus it is not possible to draw a conclusion between the effects on DNA and any behavioural effects. The conclusions regarding the nature of the cellular defecits (ie. regarding cellular hypertrophy) appear to have been based solely on quantitative measurements of DNA and protein concentrations, and not on histopathological examination of the tissues. The usefulness of this study for regulatory purposes was limited by the route of administration (subcutaneous injection), which may have had a significant effect on the toxicokinetics of the test material, and the lack of information on the study design, including the number of animals per group and the strain of rat used.

Song X, Seidler FJ, Saleh JL, Zhang J, Padilla S & Slotkin TA (1997) Cellular mechanisms for developmental toxicity of chlorpyrifos: targeting the adenylyl cyclase signalling cascade. Toxicol Appl Pharmacol Jul;145(1): 158-174

Chlorpyrifos was administered (SC in DMSO vehicle) to neonatal rats (strain not stated, numbers/group not clear) at doses of 1 or 5 mg/kg/d from postnatal days 1-4 (PND1-4) or at 5 mg/kg/d from PND11-14. These two groups of pups were examined at postnatal days 5 and 10 or 15 and 20 respectively. Investigations for signs of interference with the adenyl cyclase signalling cascade examined the heart and developing brain regions viz. brainstem (including midbrain, colliculi, pons, medulla oblongata and thalamus), forebrain (basal ganglia, hippocampal formation, neocortex) and cerebellum (including flocculi).

Results

Animals treated with chlorpyrifos from postnatal days 1-4 at 1 mg/kg/d showed no signs of toxicity, while there was significant mortality at 5 mg/kg/d (>50%). Animals treated from postnatal days 11-14 at 5 mg/kg/d showed no mortality or clinical signs. There were no significant changes in brain region weights at the apparently non-toxic doses. Cholinesterase activity in the brainstem of the 1 mg/kg/d pups was inhibited by 25% when measured 24 h after the last dose and recovery was substantial (<10% inhibition) by PND10. Cholinesterase activity in the brainstem of the pups administered 5 mg/kg/d from PND11-14 was inhibited by >60% when measured 24 h after the last dose and recovery was again substantial (<30% inhibition) by PND20.

Separate examinations of the forebrain, cerebellum and heart found that chlorpyrifos evoked deficits in multiple components of the adenylyl cyclase cascade: expression and activity of adenylyl cyclase itself, functioning of G-proteins that link neurotransmitter and hormone receptors to cyclase activity, and expression of neurotransmitter receptors that act through this cascade. Disruption of signalling function was not restricted to transduction of cholinergic signals but rather extended to adrenergic signals as well. In most cases, the adverse effects were not evident during the immediate period of chlorpyrifos administration, but appeared after a delay of several days.
Comment: results from this study were generally presented in statistical form only.

Moser VC (1995) Comparisons of the acute effects of cholinesterase inhibitors using a neurobehavioural screening battery in rats. Neurotoxicol Teratol 17(6): 617-625

This report was a comparative study of the acute effects of 7 cholinesterase inhibitors, as determined

by a functional observational battery (FOB) and motor activity.

Test animals were adult male Long-Evans hooded rats (from CRL) of ca. 10 weeks of age. The acute effects of two carbamates (carbaryl, aldicarb) and five organophosphates (OP) (chlorpyrifos, diazinon, parathion, fenthion, and diisopropyl fluorophosphate, or DFP) were evaluated on the day of dosing at the time of peak effect, at 1 and 3 days, and at 1 week after dosing (oral gavage, in corn oil). All doses were administered at dose volumes of 1 ml/kg, except carbaryl (2 ml/kg). A high dose was selected that produced clear cholinergic signs, and lower doses were chosen to produce a range of effects. After some preliminary studies, the doses used in this study (10 animals per group) and the time of peak effect (TOPE) were chosen as shown in the table below. Statistical analysis of all data was reported.

Experimental design for each test substance

Substance	Dose (mg/kg/d	TOPE (hours)
Aldicarb	0. 0.2, 0.4, 0.7	1.5
Carbaryl	0, 20, 75, 150	1
Parathion	0, 2, 4, 7	2
DFP	0, 4, 8, 14	2
Chlorpyrifos	0, 20, 50, 100	3.5
Fenthion	0, 20, 75, 150	1.5
Diazinon	0, 100, 200, 400	4

Results

The selected doses produced few deaths; one high dose parathion and two high dose aldicarb animals died. Weight loss (ca. 10%) was recorded for each substance at the high dose only. Generally all cholinesterase inhibitors produced autonomic signs of cholinergic overstimulation (salivation, lacrimation, and miosis), hypothermia, mild tremors and mouth-smacking (chewing motions), lowered motor activity, decreased tail-pinch response, and altered neuromuscular function (gait changes and increased foot splay). The measures generally found to be most sensitive on the day of dosing were body temperature, motor activity, gait, and the presence of mouth-smacking and fine tremors. However, no single measure was the most sensitive across all compounds; for example, the lowest dose of fenthion decreased motor activity by 86% but did not alter the tail-pinch response, whereas the lowest dose of parathion did not lower activity but did decrease the tail-pinch response.

For some measures, differences in the slopes of the dose-response curves were evident. Many effects were still observed at 24 h, but recovery was apparent for all compounds. Interestingly, residual effects at 72 h were obtained with the carbamates (carbaryl, aldicarb) as well as with the OP fenthion, but not with the other compounds; this observation may be explicable, at least in the case of the carbamates, by slow absorption from the GI tract. Thus, in summary: the overall clinical picture of toxicity was similar for these cholinesterase inhibitors, but compound-specific differences emerged in terms of the individual measures, dose-response, and time course; behavioural effects produced by these compounds may be dependent on both cholinergic and noncholinergic mechanisms.

Comment: Only summary data was available for evaluation.

Whitney KD, Seidler FJ & Slotkin TA (1995) Developmental neurotoxicity of chlorpyrifos: cellular mechanisms Toxicol Appl Pharmacol 134(1): 53-62

Chlorpyrifos was administered (SC, in DMSO vehicle) to neonatal rats (strain not stated, numbers/group not clear) at doses of 2 mg/kg on postnatal day 1 (PND1) or at 11 mg/kg on PND 6-9. These two groups of pups were examined within 5 h of dosing ie. on postnatal days 1 and 6-9 respectively. Investigations examined the developing brain regions viz. brainstem (including midbrain, colliculi, pons, medulla oblongata and thalamus), forebrain (basal ganglia, hippocampal formation, neocortex) and cerebellum, for signs of interference with cell development using markers for cell division. A 4-h pulse with tritiated leucine or thymidine was used to assess protein and DNA synthesis respectively. Ornithine decarboxylase was also assayed in these brain segments.

Results

One-day-old rats given 2 mg/kg SC of chlorpyrifos showed significant inhibition (ca. 15%) of DNA synthesis in all brain regions within 4-h of treatment. Equivalent results were obtained when a small dose (0.6 µg or 2 µg/g brain) was introduced directly into the brain via intracisternal injection, suggesting that the actions may not have been secondary to systemic toxicity. Comparable inhibition (>10%) of DNA synthesis was also seen at 8 days of age; however, at this point, there was regional selectivity, with sparing of the cerebellum (ca. 1% inhibition).

Another phase of this study was designed to test whether the effect of chlorpyrifos was mediated through cholinergic actions on nicotinic receptors known to mediate inhibition of DNA synthesis. In this phase, 6-8 day-old animals were pretreated with mecamylamine, a nicotinic receptor antagonist (SC 2 mg/kg), followed by chlorpyrifos (11 mg/kg). The pretreatment caused a decline in DNA synthesis by itself, and also prevented any further decrement in DNA synthesis due to chlorpyrifos. Chlorpyrifos administration at 1 day of age caused a large (>30%) inhibition of protein synthesis throughout the brain; the effect was distinct from that on DNA synthesis, as it diminished substantially by 8 days of age (<10%) and did not develop any regional selectivity. Assays of ornithine decarboxylase activity 4 and 48 h after chlorpyrifos treatment of 1 and 8-9 old rat pups found no significant alteration of activity in any brain region when compared to appropriate controls. The authors concluded that the effects of chlorpyrifos on DNA and protein synthesis were not secondary to generalised cell damage or suppression of cell metabolism, as evidenced by the maintenance of normal ornithine decarboxylase activities. However, the data do not necessarily support such a conclusion, as effects on DNA synthesis might arise following systemic effects (such as inhibition of acetylcholinesterase) leading to secondary effects such as regulation of receptor number and changes in secondary messenger pathways. In the absence of cholinesterase inhibition data, it is not possible to determine whether any effects on DNA synthesis, if such effects did occur, would occur at doses that do not cause other measurable findings. The use of subcutaneous and intracisternal routes of administration in this study make the results limited for regulatory purposes. Results from this study were presented in summary form only.

Hoberman AM (1998) Developmental neurotoxicity study of chlorpyrifos administered orally via gavage to Crl:CD.BR VAF/Plus presumed pregnant rats. Sponsor: Dow Agrosciences, protocol No.: K-044793109. Performing laboratory: Argus Research Laboratories,

Pennsylvania. Study ID: 304001, dated May 1, 1998. [Dow submission No.: 11709] GLP

In this developmental neurotoxicity study, pregnant rats were orally dosed with chlorpyrifos commencing on day 6 of gestation and continuing until day 11 of lactation (GD6-LD11), so that the pups were potentially exposed *in utero* and through part of lactation. The pups were divided into several groups and reared for varying periods before evaluation of neurobehavioural performance and collection of neuropathological findings. All data was extensively analysed using appropriate statistical methods.

Presumed pregnant Sprague-Dawley (Crl:CD.BR VAF/Plus) rats (25/group) were given chlorpyrifos (Lot MM930503-17 TSN 100227) in corn oil vehicle once a day from day 6 of gestation through to day 11 of lactation (GD6-LD11) orally via gavage at doses of 0, 0.3, 1 or 5 mg/kg/d in a volume of 1 ml/kg. Rats were supplied by Charles River Laboratories, Inc., Portage, Michigan, and bred, dosed and delivered in the test facility. Dosages were adjusted daily for body weight changes; food consumption was also monitored daily. An additional satellite group of 20 mated females rats (5/dose group) were similarly dosed every day from GD6 through to GD20, and used for blood and brain sample collection and cholinesterase analysis.

The dams were evaluated for duration of gestation, litter sizes and pup viability at birth. Litters were examined twice daily during the 22-day lactation period, and dead pups were necropsied. Litter observations included recording of physical signs (daily), pup body weights, sexes and nursing behaviour (days 1, 5, 12, 18 and 22 postpartum).

The day of birth was regarded as day one of lactation (LD1) or day one postpartum (DPP1). On LD5, litters were culled to 10 pups/litter, and pups were randomly assigned to one of four sets (80 M and 80 F/set) for continued observation. On LD12, litters were reduced to eight pups/litter (4M and 4F). Dams that delivered a litter were sacrificed on LD22; those with no surviving pups were sacrificed after the last pup died. Those not delivering a litter were sacrificed on LD25; having been dosed daily from GD6-GD24. All dams were subjected to gross necropsy of the thoracic, abdominal and pelvic viscera.

Pup viability was assessed twice daily and clinical observations were made daily during the pre-weaning period and on weigh-days during the post-weaning periods in subset 4 only. One or more of the subsets of pups were evaluated for the following: bodyweights (various times); feed consumption; pup development in subset 4 (pinna unfolding – DPP2 onwards, and eye opening – DPP12 onwards); sexual maturation in subset 4 (preputial separation – DPP39 onwards; vaginal patency -DPP28 onwards) and body temperature in subset 3 (DPP 22 and 61±2).

Motor activity was monitored by passive infrared sensor mounted outside the cage. The test sessions were 1 h with the number of movements and time spent in movement tabulated at each 5-minute interval.

Auditory startle habituation was conducted by placing the test rat in a cage sited on an accelerometer, and after a 5 minute adaptation period exposing the rat to 50 trials of 20 msec 120 dBA bursts of noise.

Experimental protocol for pups

Test	Days performed	Subset 1	Subset 2	Subset 3	Subset 4

		80M & 80 F	80M & 80 F	80M & 80 F	80M & 80 F
Brain weights	DPP 12	40M, 40F			
Neuropath exam	DPP 12	24M, 24F*			
Brain weights	DPP 66-71				40M, 40F
Neuropath exam	DPP 66-71				24M, 24F*[a]
Learning & memory	Between DPP 22-25 & DPP 62-92		32M & 32F		
Motor activity	DPP 14, 18, 22, 61			80M & 80F	
Auditory startle habituation	DPP 23 & 62			80M & 80F	
Necropsy for gross lesions	DPP 22	40M, 40F	48M, 48F		40M, 40F

* selected from within the animals assigned to brain weight determination
[a] data not provided

Neuropathological examinations were conducted to GLP standards. Brain weights were recorded on fixed material with olfactory bulbs intact. Brain weight and morphometry were recorded for 48 pups on DPP12, and microscopic neuropathology on 24 of these pups (0 and 5 mg/kg/d only). A selection of these brains were measured and divided into multiple coronal sections and prepared for histopathology examination including quantitative assessments and statistical analysis. Measurements included: anterior to posterior length of the cerebellum and cerebrum; thickness of the cerebral cortex (2 locations) and of the hippocampal gyrus; the height of the cerebellum at the level of the deep cerebellar nuclei; the thickness of the corpus callosum; the thickness of the external germinal layer of the cerebellum (in juveniles only).

Brains from the adult (DPP66) rats were were perfusion fixed (10% buffered formalin) intact within the cranial vault. Neuropathological examinations were conducted to GLP standards. Meaurements of cerebrum length (exculsive of olfactory bulbs) and cerebellum were recorded from the intact brains. Brain weights were recorded on fixed material with olfactory bulbs intact. Brain weight and morphometry were recorded for 30 rats (6 each from control and high-dose males and females, and 6 mid-dose females). Microscopic neuropathology was performed on 24 adults (6 M and 6 F at 0 and 5 mg/kg/d). These brains were divided into multiple coronal sections and prepared for histopathology examination including quantitative assessments and statistical analysis (paraffin-embedded). Vertebral columns from these same rats were processed for examination of the dorsal and ventral nerve roots and multiple sections of the spinal column (paraffin-embedded). Hind legs from these animals were processed for cross and longitudinal sections of the sciatic and tibial nerves, and longitudinal sections of the tibial, fibular and sural nerves (glycol methacrylate-embedded). Morphometric measurements included: anterior to posterior length of the cerebellum and cerebrum; thickness of the parietal cortex; thickness of the frontal cortex; thickness of the hippocampal gyrus; the height of the cerebellum at the level of the deep cerebellar nuclei; the thickness of the corpus callosum.

Learning and memory were assessed by training rodent pups (22 to 25 days of age) in a T-maze, using a Spatial Delayed Alternation (discrete trials) methodology. Training was conducted on food and water deprived rodents. Each trial consisted of a pair of runs, the first being a forced run, in which the animal is presented with only one of the maze arms and is rewarded for entering it. The second run is a choice run, in which both arms of the maze are available and the animal is rewarded only for entering the maze arm it did not enter on the forced run. Animals were trained using a series of trials involving 0 second,

10-second and 20-second delay periods between the forced run and the choice run. This procedure is considered to evaluate learning and working memory because the only cue that can guide performance on the choice run is the memory of the immediately precedinq forced run. The equipment utilised in this test was a Plexiglas T-maze scaled to the size of the pup. The maze was fitted with a start box, a runway leading from the start box to the choice-point and left and right runways extending from the choice-point to the left and right goal (supplied with liquid dippers). Data from a methodology study which was conducted to provide background and validation data for the spatial delayed alternation trials was presented for pups and adults. These data demonstrated an increase in the learning curve during the acquisition phase of training for the rats. They also demonstrated that an increase in the delay between the forced run and the choice run resulted in a decrease in the retention curve.

Cholinesterase measurements were performed by an external laboratory (the USEPA) on plasma, RBC and brain samples from the satellite group. The animals were sacrificed 4-5 h after dosage administration. Blood was collected from the inferior vena cava and the brain was removed. No methodological details of the assays were presented, and the raw data was not directly interpretable. Summary data were provided by the sponsor.

Results

Satisfactory quality assurance analyses for food composition, water quality, bedding quality and general environmental conditions were presented.

Dosage: The solutions of the test substance administered to the rats were within ±4 % of the target values. Stability of the test substance was acceptable; there was no change in chlorpyrifos concentration over 42 days.

General observations: During observation of the F0 generation females twice daily between days 6-21 of gestation there were no mortalities or clinical signs other than a significant increase in fasciculations at the high dose (6 observations in 6 animals on GD 21 only vs nil observations at all other doses). Twice daily observations during lactation recorded no mortalities but did record a significant increase in: fasciculations at the high dose (30 observations in 16 animals vs nil observations at all other doses, LDs 1-5 mainly); hyperpnea (0 in 0, 0 in 0, 1 in 1 and 10 observations in 8 animals for 0, 0.3, 1 and 5 mg/kg/d respectively) and hyperreactivity (while being handled) at the high dose (3 in 2, 7 in 7, 3 in 2 and 27 observations in 17 animals for 0, 0.3, 1 and 5 mg/kg/d respectively).

Body weights were unaffected by treatment during gestation. Body weight gain was slightly reduced (11% cf. controls) at the high dose towards the end of gestation (GDs16-20). Body weights during lactation were unaffected by treatment in the 0, 0.3 and 1.0 mg/kg/d groups. The high dose group did not gain weight during the early days of lactation (LDs1-4) compared to the other groups which gained 4.6-6.8 g. Food consumption (absolute or relative to body weight) was decreased (up to 14%) by treatment at the high dose only.

Gestational observations

Dosage (mg/kg/d)	0	0.3	1.0	5.0

Pregnant dams	25	24	24	24
[a]Gestation index	25/25	24/24	24/24	23/24
Gestation days	23.1 ± 0.5	23.2 ± 0.4	23.0 ± 0.5	23.0 ± 0.2
Implantation sites/delivered litter	13.6.1 ± 3.2	14.5 ± 2.0	14.2 ± 2.4	14.2 ± 1.6
Dams with stillborn pups	1	1	4	1
Dams with no live-born pups	0	0	0	1
Dams with all pups dying during DPP 1-5	0	0	0	3 (31 pups total)
Total pups delivered	309	320	318	298
Live pups/litter F1	12.3 ± 3.1	13.3 ± 1.9	13.0 ± 2.4	12.7 ± 2.4
Pups found dead or presumed cannibalised DPP 2-5	4/308	4/319	6/311	88/292
[b]Viability index	98.7%	98.7%	98.1%	69.9%

[a] Number of dams with live offspring/number of pregnant dams

[b] Number of live pups on day 5 postpartum/number of live-born pups on day 1 postpartum

Between LD 1-5, a total of 3 dams in the 5 mg/kg/d dosage group had total litter loss and an additional 57 pups died or were cannibalised; these losses led to a decreased viability index. There were no similar effects of treatment in the other dose groups.

Post-partum observations

Dosage (mg/kg/d)	0	0.3	1.0	5.0
[a]Surviving pups/litter Day 5 preculling	12.2 ± 3.1	13.1 ± 1.7	12.7 ± 2.5	10.2 ± 3.7
Surviving pups/litter Day 5 postculling	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	8.7 ± 2.5
Pup weight/litter (g) Day 1 - males	6.6 ± 0.6	6.7 ± 0.6	6.4 ± 0.5	6.1 ± 0.7
Pup weight/litter (g) Day 1 - females	6.3 ± 0.6	6.2 ± 0.5	6.1 ± 0.5	5.6 ± 0.6
Pup weight/litter (g) Day 5 postculling – males	9.8 ± 0.8	10.2 ± 1.1	10.1 ± 1.2	8.8 ± 1.7
Pup weight/litter (g) Day 5 postculling – females	9.4 ± 0.9	9.6 ± 1.0	9.5 ± 1.2	8.2 ± 1.6

[a]Pups were culled on postpartum day 5. One control litter had no females, and one 5 mg/kg/d litter had no males.

Male and female pup weight in the high dose group was significantly lower than the other dose groups on post partum days 1 and 5 pre- and post-cull. These findings are consistent with the decreased food consumption and lack of body weight gain in high dose dams.

There were no statistically significant group differences in transient or persistent clinical observations in the pups between LDs 1-22. There was a slight delay in pinna detachment in the high dose group (4.0 days vs. 3.5 in controls) which is considered treatment-related and consistent with lower body weight and body weight gain in this group; however mean number of days to eye-opening did not differ between groups (mean range 14.0 – 14.9 days). There were no statistically significant differences in necropsy findings between groups of pups whether found dead or sacrificed on LDs 5, 6, 8, 12 or 22. The necropsy of the pups found dead did indicate treatment-related lack of maternal care; no milk was found in the stomach of 14 pups (12 from the same litter) from the high dose group vs. the same finding in only one other pup from the 0.3 mg/kg/d group.

Cholinesterase measurements: Both plasma and erythrocyte cholinesterase activity were significantly inhibited at all three dose levels, while brain cholinesterase was significantly inhibited at the two higher

doses. No information was provided for the cholinesterase levels in pups at any stage, and no information was provided on chlorpyrifos levels in milk during lactation, thus restricting evaluation of this data to the observations of NOELs for cholinesterase inhibition in the dams.

Cholinesterase measurements (% of controls); satellite group females (GD20, 5/group)

Dosage (mg/kg/d)	**0.3**	**1.0**	**5**
Plasma	56.7	31.1	8.5
Erythrocyte	58.7	15.6	0.13
Brain	99.7	82.1	10.2

Subset 1 pups: Sacrifice of subset 1 pups on DPP12 revealed a significantly lower body weight and slightly lower brain weight in the high dose males and females when compared to controls. This led to a significantly higher relative brain weight in both sexes at the high dose compared to controls. Necropsy observations for all subset 1 pups were listed as normal (20pups/sex/dose except only 15 M and 16 F at 5 mg/kg/d).

Brain weights (mean ± S.D.) – Male pups in subset 1 (DPP 12)

Dosage (mg/kg/d)	**0**	**0.3**	**1.0**	**5.0**
Pups tested	10	10	10	10
Terminal body weight (g)	23.5 ± 1.6	27.4 ± 2.4	25.9 ± 2.4	19.4 ± 4.3
Brain weight (g)	1.284 ± 0.042	1.409 ± 0.068	1.356 ± 0.075	1.173 ± 0.163
[a]Brain (%TBW)	5.48 ± 0.36	5.16 ± 0.25	5.26 ± 0.36	6.21 ± 0.87

Brain weights (mean ± S.D.) – Female pups in subset 1 (DPP 12)

Dosage (mg/kg/d)	**0**	**0.3**	**1.0**	**5.0**
Pups tested	10	10	10	10
Terminal body weight (g)	23.1 ± 2.3	23.2 ± 1.8	23.1 ± 2.8	18.8 ± 3.6
Brain weight (g)	1.284 ± 0.079	1.279 ± 0.043	1.273 ± 0.106	1.171 ± 0.127
[a]Brain (%TBW)	5.59 ± 0.37	5.54 ± 0.36	5.54 ± 0.35	6.36 ± 0.87

[a]Relative brain weight = (organ weight/total body weight) x100

Neuropathological examination of 38 tissues was performed on sections from 6 control and 6 high dose pups for each sex (24 total); no abnormalities were reported. Morphometric measurements of the brain and brain sections of 48 pups (6/sex/dose group) were reported. When compared to the controls, the male pups displayed a pattern of increase in morphometric measurements at the low dose and a decrease at the high dose, paralleling the brain weight differences between the groups. In the female pups, this trend was present but weaker. In the female pups, decreased brain weight exhibited a weak dose response, but most of the morphometric measurements do not. Brains of both male and female pups were consistently smaller at the high dose when compared to controls but there did not appear to be a particular area of the brain that was selectively targeted. These results suggest a delay in brain

development due to undernutrition secondary to maternal toxicity.

Morphometric data – mean values from 6 male pups in subset 1 (DPP 12)

Dosage (mg/kg/d)	**0**	**0.3**	**1.0**	**5.0**
Brain weight (g)	1.291 ± 0.047	1.428 ± 0.077	1.362 ± 0.091	1.142 ± 0.203
Ant/Post cerebrum (mm)	12.53 ± 0.34	13.42 ± 0.50	13.12 ± 0.49	11.75 ± 0.95
Ant/Post cerebellum (mm)	3.27 ± 0.31	3.45 ± 0.35	3.33 ± 0.19	2.47 ± 0.55
[a]Frontal Cortex	1348 ± 54	1360 ± 100	1352 ± 47	1272 ± 153
Parietal Cortex	1336 ± 56	1448 ± 58	1448 ± 33	1256 ± 138
Caudate Putamen	2240 ± 84	2240 ± 109	2312 ± 93	2224 ± 148
Corpus Callosum	293 ± 25	303 ± 25	290 ± 36	293 ± 56
Hippocampal Gyrus	904 ± 93	1004 ± 114	972 ± 54	824 ± 66
Cerebellum	3504 ± 129	3456 ± 173	3416 ± 200	3008 ± 504
Ext. Germinal Layer	37.17 ± 2.04	38.33 ± 3.62	40.00 ± 7.35	37.67 ± 2.58

[a] measurements in micrometers unless otherwise stated

Morphometric data – mean values from 6 female pups in subset 1 (DPP 12)

Dosage (mg/kg/d)	0	0.3	1.0	5.0
Brain weight (g)	1.292 ± 0.055	1.278 ± 0.054	1.261 ± 0.128	1.179 ± 0.114
Ant/Post cerebrum (mm)	12.40 ± 0.26	12.70 ± 0.28	12.80 ± 0.63	12.15 ± 0.58
Ant/Post cerebellum (mm)	3.18 ± 0.22	3.03 ± 0.32	3.30 ± 0.17	3.00 ± 0.31
[a]Frontal Cortex	1376 ± 92	1388 ± 79	1356 ± 54	1368 ± 86
Parietal Cortex	1380 ± 54	1376 ± 20	1368 ± 80	1304 ± 72
Caudate Putamen	2384 ± 131	2224 ± 116	2288 ± 108	2152 ± 134
Corpus Callosum	307 ± 38	288 ± 27	304 ± 36	274 ± 40
Hippocampal Gyrus	936 ± 82	912 ± 50	932 ± 96	828 ± 79
Cerebellum	3512 ± 200	3176 ± 130	3120 ± 328	3208 ± 226
Ext. Germinal Layer	38.67 ± 3.44	36.33 ± 5.89	41.17 ± 6.43	40.83 ± 5.67

[a] measurements in micrometers unless otherwise stated

Subset 2 pups: These pups were tested for learning and memory on DPP23-25 and DPP62-92, and the data presented were the results from blocks of trials in the Delayed Spatial Alternation apparatus. Learning was measured by Average Acquisition Training (a plot of percent correct guesses in the apparatus, versus number of trial blocks). There were no statistically significant differences between the dose groups at either test period in either sex when either the final total or rate of acquisition were compared. Memory was assessed using the same apparatus and the data were recorded as response time and percent correct responses as a function of variable time delay. The slope of the regression line fitted to the raw data represented how quickly the rats forgot the information just learned. There were no statistically significant differences in the calculated slope of the delayed response between the dose groups at either test period in either sex. Necropsy observations (DPP22 or DPP97-100) of all subset 2 animals (Males: 20, 20, 20, 14; females 19, 20, 20, 15 for 0, 0.3, 1.0 and 5.0 mg/kg/d respectively) did not record any treatment related abnormalities.

Subset 3 pups: These pups were tested for motor activity and auditory startle habituation on several occasions. The statistical analysis showed no overall differences in motor activity between the treatment groups in either sex. While the high dose group showed decreased motor activity compared to controls

on DPP14, it appeared to be increased on DPP61 and increased, decreased or unchanged on the other assay days, depending on the sex. The auditory startle evaluation found an overall statistically significant difference in the latency of the startle response among the dose groups in both sexes; however, this effect was limited to the high dose group and was present on DPP23 but not DPP62. Similarly the data for the peak response (grams) showed that the high dose group on DPP23 only had a lower peak response than other groups in both sexes, but this difference was not statistically significant. There was no effect of treatment on body temperatures which were measured in all rats in this subset on DPP22 and around DPP61. Necropsy observations (DPP5-64) of all subset 2 animals (Males: 20, 20, 20, 19; females 20, 20, 20, 19 for 0, 0.3, 1.0 and 5.0 mg/kg/d respectively) did not record any treatment related abnormalities.

Subset 4 pups: There were no treatment related clinical signs observed in subset 4 pups post-weaning. Bodyweights were lower in both sexes in the high dosage group compared to the other groups, and this difference was generally statistically significant, although female bodyweights approached control values by DPP66. Sexual maturity was delayed in both sexes at the high dose (statistically significant in females); this may be related to lower body weight. Necropsy of subset 4 pups on DPP66-71 did not reveal any statistically significant differences in the treatment groups.

Body weights (mean ± S.D.) and maturity – Male pups in subset 4 (DPP 66)

Dosage (mg/kg/d)	**0**	**0.3**	**1.0**	**5.0**
Pups tested	20	20	20	17
Terminal body weight (g)	388.9 ± 24.8	385.4 ± 35.6	389.8 ± 31.8	348.0 ± 36.8
Preputial separation (d)	44.2 ± 1.9	43.4 ± 1.9	45.2 ± 3.2	47.0 ± 5.9
Pups tested	10	10	10	10
Terminal body weight (g)	410.7 ± 24.6	384.8 ± 25.4	408.2 ± 44.4	368.7 ± 30.8
Brain weight (g)	2.27 ± 0.08	2.26 ± 0.08	2.29 ± 0.11	2.24 ± 0.10
[a]Brain (%TBW)	0.50 ± 0.04	0.59 ± 0.06	0.57 ± 0.04	0.61 ± 0.04

Body weights (mean ± S.D.) and maturity – Female pups in subset 4 (DPP 66)

Dosage (mg/kg/d)	**0**	**0.3**	**1.0**	**5.0**
Pups tested	20	20	20	18
Terminal body weight (g)	228.7 ± 15.4	238.1 ± 27.5	228.8 ± 20.6	220.3 ± 14.0
Vaginal patency	32.4 ± 1.0	31.5 ± 1.5	32.1 ± 2.3	33.4 ± 2.2
Pups tested	10	10	10	10
Terminal body weight (g)	234.5 ± 17.4	254.2 ± 28.4	233.1 ± 28.9	230.4 ± 18.1
Brain weight (g)	2.09 ± 0.07	2.18 ± 0.09	2.12 ± 0.08	2.09 ± 0.10
[a]Brain (%TBW)	0.89 ± 0.07	0.86 ± 0.09	0.92 ± 0.09	0.91 ± 0.05

[a]Relative brain weight = (organ weight/total body weight)x100

Morphometric data – mean values (±SD) from pups in subset 4 (DPP 66)

	males		females		
Dosage (mg/kg/d)	**0**	**5.0**	**0**	**1.0**	**5.0**
Pups tested	6	6	6	6	6
Brain weight (g)	2.300 ± 0.069	2.299 ± 0.021	2.103 ± 0.071	2.127 ± 0.079	2.048 ± 0.050
Ant/Post cerebrum (mm)	15.900 ± 0.400	16.183 ± 0.264	15.617 ± 0.306	15.633 ± 0.344	15.517 ± 0.248
Ant/Post cerebellum (mm)	5.683 ± 0.232	5.667 ± 0.216	5.517 ± 0.232	5.500 ± 0.261	5.383 ± 0.098
[a]Frontal Cortex	1792 ± 105	1768 ± 75.3	1744 ± 56	1748 ± 75	1724 ± 79.5
Parietal Cortex	1756 ± 79	1792 ± 58.1	1792 ± 36	1716 ± 36	1700 ± 55.6
Caudate Putamen	2800 ± 176	2744 ± 98	2576 ± 131	2552 ± 178	2704 ± 112
Corpus Callosum	265.7 ± 29.0	246.5 ± 17.9	244.8 ± 24.8	257.5 ± 17.6	233.7 ± 18.9
Hippocampal Gyrus	1640 ± 91.9	1612 ± 95.3	1708 ± 57.6	1644 ± 129	1592 ± 86.8
Cerebellum	5152 ± 218	5104 ± 351	5016 ± 120	4888 ± 150	4968 ± 207

[a] measurements in micrometers unless otherwise stated

The morphometric measurements reveal minor variations (ca. 5%) which might be expected for such a small sample (6 animals). The neuropathological microscopical examinations (generally 48 sites/tissues reported) were restricted to the control and high dose animals and no effects of treatment were evident. While data comprising the morphometric measurements were provided for mid-dose DPP 66 females (1 mg/kg/d), no neuropathological examinations were reported for this group. These results suggest that the animals had generally recovered from the delayed development that was evident at DPP 12.

Attachments to this study: Limited results from a study which evaluated positive control substances (acrylamide, d-amphetamine, trimethyltin chloride and MK-801) using tests of motor activity, auditory startle habituation and neurohistological examination were presented. These tests were restricted to 5 adult rats/sex/substance. These data were provided to demonstrate that the motor activity test system was capable of detecting increases or decreases in activity. The only results presented were clinical observations and motor activity trials for rats treated with vehicle (controls), acrylamide and amphetamine. Evaluation indicated that this technique was capable of detecting increases or decreases in motor activity.

A summary of a developmental neurotoxicity study of lead nitrate exposure (gavage; 0, 5 and 50 mg/kg/d) in the same rat strain, conducted according to USEPA guidelines, was also provided. This study recorded a very similar pattern of findings to the present study viz. maternotoxicity and increased early mortality and delayed development of pups at the high dose. Functional evaluation included motor activity, auditory startle habituation, performance in a passive avoidance task and adult performance in a watermaze task. Additionally, assessment of absolute and relative brain weights and neurohistopathology examinations (data not presented) were performed. No effect of treatment was observed for these parameters, however the relevance of this study is limited by the differing toxicological mechanism of lead acetate to chlorpyrifos, the lack of raw data and the different functional evaluation battery used in the main study.

Data from a morphometric measurement validation study were also provided. When comparing brains of DPP10 and DPP12 pups this study found statistically significant differences for 5/9 of the two gross and seven microscopic measurements of rat pup brains. The authors concluded that this methodology can function as a screening technique for detecting developmental neurotoxicity when neuropathological lesions are not present. However, while the data support the hypothesis that the technique may allow discrimination of a gross delay in rat-pup brain development, they do not appear to be sufficiently sensitive to address more subtle aberrations in normal pup-brain development.

Summary

This study found that treatment of pregnant dams with oral doses of chlorpyrifos at 0, 0.3, 1.0 and 5.0 mg/kg/d during pregnancy and early lactation resulted in no deaths and few clinical signs (fasciculations, hyperpnea, hyperreactivity and decreased body weight gain) in the dams at the high dose (NOEL 1.0 mg/kg/d). Plasma and RBC cholinesterase activity were significantly inhibited at all three doses (LOAEL 0.3 mg/kg/d), while brain AChE activity was significantly inhibited at the two highest doses (NOEL 0.3 mg/kg/d).

Pups which were exposed to chlorpyrifos *in vivo* and for a period post partum, displayed signs of toxicity (decreased: viability index; relative brain weight and delayed sexual maturity) at the high dose only (NOEL 1.0 mg/kg/d). Cognitive functions in the pups (learning, memory and habituation) were not affected by treatment (NOEL 5.0 mg/kg/d). The lack of a functional observational battery and analysis of reflex ontogeny, and the fact that cholinesterase activity was not measured in pups, means that this study was of limited regulatory value in determining the relative sensitivity of young and adult animals to the effects of chlorpyrifos.

Mattsson JL, Maurissen JP, Spencer PJ, Brzak BS & Zablotny CL (1998) Effetcs of chlorpyrifos administered via gavage to CD rats during gestation and lactation on plasma, erythrocyte, heart and brain cholinesterase, and analytical determination of chlorpyrifos and metabolites. Sponsor: Dow Agrosciences. Performing laboratory: Dow - Health & Environmental Research Laboratories, Michigan. Study ID: 971162, dated August 31, 1998. [Dow submission No.:] GLP

This study was performed to measure the induction of cholinesterase inhibition and analyse the metabolic transformation of chlorpyrifos in dams and developing pups following repeated oral chlorpyrifos administration to dams; pups were potentially exposed *in utero* and through part of lactation. The dams and pups were divided into two main groups (for chlorpyrifos/metabolites or cholinesterase measurement; 80 & 120 dams respectively) and the pups were reared for varying periods before sacrifice. The strain of rat, dosage levels and timing of samples were designed to match important time points in the developmental neurotoxicity study of Hoberman (1998).

Presumed pregnant Sprague-Dawley (Crl:CD.BR) rats (25/group) were given chlorpyrifos (Lot MM930503-17 TSN 100227) in corn oil vehicle once a day from day 6 of gestation through to day 10 of lactation (GD6-LD10) orally via gavage at doses of 0, 0.3, 1 or 5 mg/kg/d in a volume of 1 ml/kg. Rats that not deliver a litter were dosed through to the equivalent of GD23. Rats were supplied and bred by Charles River Breeding Laboratories, Portage, Michigan and delivered to the test facility on GD1 or 2. Dosages were adjusted daily for body weight changes; food consumption was also monitored daily.

Twice daily all animals were visually evaluated at cageside for general appearance, morbidity, moribundity, mortality and availability of feed and water. Detailed clinical signs were recorded weekly. Visible physical abnormalities or behavioural changes in the neonates were recorded daily during the lactation period.

The day of birth was regarded as day one of lactation (LD1) or postnatal day 1 (PND1). On PND4 litters were culled to 10 pups/litter (5/sex); if necessary pups from other litters were used to supplement the numbers, but these pups were not used for subsequent testing. Bodyweights of pregnant dams were recorded from GD6 through to PND10. On PND11, litters were reduced to eight pups/litter (4M and 4F). All pups were weaned on PND21.

Chlorpyrifos and metabolite measurements
Chlorpyrifos and chlorpyrifos-oxon were determined in milk, and chlorpyrifos, chlorpyrifos-oxon and 3,5,6-trichloro-2-pyridinol (TCP) were determined in blood samples from fetuses/pups and dams. Five dams from the chlorpyrifos group,as well as 5 pups/sex/dose (total of 40/day; half from cholinesterase group and half from chlorpyrifos group) were sacrificed on gestation day 20 (GD20) and postnatal days 1, 5, and 11. The pups were separated from the dams at 2 h post-dose and sacrificed after blood (2 x 0.1 mL from the abdominal aorta) was taken. Milk (01-0.3 mL) and blood (0.5 mL & 0.1 mL by cardiac puncture) were collected from the dams at 4 h post-dose (28 h post-dose on PND11), immediately before sacrifice. At GD20 blood (abdominal aorta) was taken from fetuses at the 4 h sacrifice. Blood samples of littermates were pooled if necessary to achieve a minimum of 0.2 mL of blood.

Cholinesterase measurements
Cholinesterase was determined in plasma, RBC, heart and brain samples from dams and pups. Five dams from the cholinesterase group were sacrificed on gestation day 20 (GD20) and postnatal days 1, 5, 11and 22, while 5 pups/sex/dose (total of 40/day; half from cholinesterase group and half from chlorpyrifos group) were sacrificed on GD20 & PND's 1, 5, 11, 22 & 65. The pups were separated from the dams at 2 h post-dose and sacrificed after blood (0.25 mL from the abdominal aorta) was taken; dams were bled (0.5 mL) via cardiac puncture and dams and fetuses were bled at 4 h post-dose. Cholinesterase measurements were made at the US EPA using an autoanalyser method.

Data analysis
All data was extensively analysed using appropriate statistical methods. The authors state that part of the results from 5 dams were eliminated from the data sets prior to analysis due to extremely aberrant nature of the values. Three dams (1 low-, 1 mid-, and 1 high-dose group) had aberrant values for plasma/RBC cholinesterase activity on GD20 and two dams (1 control & 1 high-dose group) had aberrant values for for hindbrain cholinesterase activity on PND5. Chlorpyrifos, chlorpyrifos-oxon and TCP had many samples below the limit of detection; in these cases means were calculated if at least three samples were quantifiable, using a value of 1/2 the limit of detection value for the remaining one or two samples. For chlorpyrifos and chlorpyrifos-oxon the limit of detection was 0.7 ng/g and for TCP the limit of detection was 10 ng/g.

Results
The dose solutions were within 6% of the target concentrations and recorded satisfactory homogeneity with a <6% top-to-bottom variation. Treatment had no effect on litter size or viability. There was no effect of treatment on clinical observations of dams or pups. Bodyweight and bodyweight gain were unaffected by treatment.

Chlorpyrifos was quantifiable in the blood of dams of the 5.0 mg/kg/d dose group at PNDs 1 and 5, in the blood of the 1.0 mg/kg/d group at GD20 only, and never at 0.30 mg/kg/d. TCP concentration in

dams' blood showed a clear dose- and time relationship, reaching a peak concentration on PND5 in the 0.3 and 1.0 mg/kg/d groups, but peaking at PND1 in the 5.00 mg/kg/d. The concentration of chlorpyrifos in dams' milk also showed a clear dose- and time relationship, first appearing at PND1 in all dose groups and only present in the high-dose group by PND11. Chlorpyrifos-oxon was not detected in blood or milk at any dose level at any time point in dams.

Summary chlorpyrifos concentrations (ng/g): Dams - blood

	Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NQ[c]	NQ	2.55 (0.88)[b]	109 (59)
PND1	NQ	NQ	NQ	15 (6)
PND5	NQ	NQ	NQ	15 (11)
PND11	NQ	NQ	NQ	NQ

[a] 5 dams/point. [b] numbers in parentheses are Standard Deviations.
[c] NQ = not quantifiable. Limit of detection = 0.7 ng/g

Summary TCP concentrations (ng/g): Dams - blood

	Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NQ[c]	114 (24)	322 (13)	1974 (472)
PND1	NQ	111 (26)	395 (65)	2718 (1509)
PND5	NQ	143 (37)	537 (58)	1450 (95)
PND11	NQ	NQ	9.9 (5.1)[d]	71.4 (22.6)

[a] 5 dams/point. [b] numbers in parentheses are Standard Deviations.
[c] NQ = not quantifiable. Limit of detection = 10.0 ng/g
[d] 2 samples NQ replaced with 1/2 limit of quantitation (5 ng/g)

Summary chlorpyrifos concentrations (ng/g): Dams - milk

	Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NA	NA	NA	NA
PND1	NQ[c]	20.6 (8.5)[b]	139 (36)	3022 (1154)
PND5	NQ	13.5 (3.9)	81.8 (6.6)	1534 (192)
PND11	NQ	NQ	NQ	19.8 (6.5)

[a] 5 dams/point. [b] numbers in parentheses are Standard Deviations.
[c] NQ = not quantifiable. Limit of detection = 0.7 ng/g
NA = not applicable

Chlorpyrifos concentration in the blood of male and female pups showed a similar dose-related increase and time-related decrease; no chlorpyrifos was detected from PND5 onwards. Male pups recorded higher chlorpyrifos concentrations in blood than females. Oxon was present near the limits of detection in male and female pups at 5 mg/kg/d on GD20 only. TCP concentration in pups' blood showed a clear dose- and time relationship, starting from a peak concentration on GD20 in the 0.3 and 1.0 mg/kg/d groups and declining markedly with time; TCP was still detectable at PND11 in male and female pups at 5.0 mg/kg/d, but only female pups at 1.0 mg/kg/d.

Summary chlorpyrifos concentrations (ng/g): pups[a] - blood

	Male pups - Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NQ[c]	NQ	0.99[d] (0.41)	52.8 (25.2)
PND1	NQ	NQ	NQ	18.2[d] (24.6)
PND5	NQ	NQ	NQ	NQ
PND11	NQ	NQ	NQ	NQ
	Female pups - Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NQ[c]	NQ	1.19 (0.32)[e]	39.4 (13.0)
PND1	NQ	NQ	NQ	6.61[d] (8.0)
PND5	NQ	NQ	NQ	NQ
PND11	NQ	NQ	NQ	NQ

[a] 5 pups/point. [b] numbers in parentheses are Standard Deviations. [c] NQ = not quantifiable. Limit of detection = 0.7 ng/g. [d] 1 samples NQ replaced with 1/2 limit of quantitation (0.4 ng/g).
[e] 4 dams only - dam did not have enough fetuses and was excluded from the study

Summary chlorpyrifos-oxon concentrations (ng/g): pups[a] - blood

	Male pups - Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NQ[c]	NQ	NQ	0.97 (0.58)[d]
PND1	NQ	NQ	NQ	NQ
PND5	NQ	NQ	NQ	NQ

PND11	NQ	NQ	NQ	NQ
	Female pups - Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NQ[c]	NQ	NQ[e]	0.94 (0.37)[d1]
PND1	NQ	NQ	NQ	NQ
PND5	NQ	NQ	NQ	NQ
PND11	NQ	NQ	NQ	NQ

[a] 5 pups/point. [b] numbers in parentheses are Standard Deviations. [c] NQ = not quantifiable. Limit of detection = 0.7 ng/g. [d] 2 samples NQ replaced with 1/2 limit of quantitation (0.4 ng/g). [d1] 1 sample NQ replaced with 1/2 limit of quantitation (0.4 ng/g). [e] 4 dams only - dam did not have enough fetuses and was excluded from the study

Summary TCP concentrations (ng/g): pups[a] - blood

	Male pups - Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NQ[c]	93.9 (14.5)[b]	361 (64.1)	1680 (241.4)
PND1	NQ	NQ	137 (85.7)	842.7 (293.5)
PND5	NQ	NQ	NQ	47.4 (18.8)
PND11	NQ	NQ	NQ	42.3 (8.9)
	Female pups - Dose Group[a] (mg/kg/d)			
	0.00	**0.30**	**1.00**	**5.00**
GD20	NQ[c]	99.5 (13.7)	339.1[e] (93.8)	1884 (234)
PND1	NQ	50.0 (27.5)	133.9 (27.7)	433.3 (263)
PND5	NQ	NQ	NQ	50.2 (27.1)
PND11	NQ	NQ	9.5 (2.7)	47.0[d] (16.8)

[a] 5 pups/point. [b] numbers in parentheses are Standard Deviations. [c] NQ = not quantifiable. Limit of detection = 10.0 ng/g. [d] 2 samples NQ replaced with 1/2 limit of quantitation (5.0 ng/g.
[e] 4 dams only - dam did not have enough fetuses and was excluded from the study

Cholinesterase activity was decreased in a clear dose-related manner in all compartments in the dams. Forebrain and hindbrain showed a similar pattern of inhibition: activity was unaffected at 0.3mg/kg/d; activity was decreased slightly (generally <10%) but statistically significantly at 1.0 mg/kg/d with peak depression at PND5 followed by partial recovery PND22; activity was severely and statistically significantly inhibited at 5.0 mg/kg/d at all time points with peak depression at PND1. Heart samples showed peak cholinesterase activity depression at GD20 (5.0 mg/kg/d) or PND1 (0.3 and 5.0 mg/kg/d). Inhibition and recovery of heart cholinesterase activity were dose-related, and recovery was essentially complete by PND22 at all dose levels. Plasma cholinesterase activity was more severely affected than RBC cholinesterase but recovered more quickly. The decrease in cholinesterase activity was statistically significant at almost all points. Minimum plasma and RBC activity was recorded on PND1. Plasma activity had returned to, and overshot control levels on PND22 in all three dose groups, while RBC activity was still less than control values for the 1.0 and 5.0 mg/kg/d groups on PND22.

Summary cholinesterase activity (% of control[f]) - dams[a]

	Forebrain	Hindbrain	Heart	Plasma	Erythrocyte
	0.3 mg/kg/d				
GD20[c]	98 (3)	101 (7)	100 (20)	67[e] (10)**	74 (15)[e]**
PND1[d]	102 (4)	94 (20)	90 (7)*	48 (10)**	61 (9)**
PND5	102 (8)	101 (5)	96 (5)	68 (7)**	89 (17)**
PND11	99 (4)	105 (4)	88 (11)	84 (12)**	76 (8)**
PND22	99 (8)	98 (5)	110 (13)	117 (22)	93 (7)
	1.0 mg/kg/d				
GD20	92 (4)**	92 (2)**	51 (7)**	39 (6)[e]**	18 (7)[e]**
PND1	93 (3)**	93 (5)	58 (9)**	23 (4)**	13 (3)**
PND5	90 (4)*	88 (3)**	69 (10)**	31 (5)**	16 (3)**
PND11	91 (5)*	97 (7)	91 (14)	66 (10)**	23 (4)**
PND22	95 (6)	93 (8)	93 (21)	132 (25)	67 (10)**
	5.0 mg/kg/d				
GD20	13 (3)**	24 (5)**	17 (4)**	12 (3)[e]**	5 (3)[e]**
PND1	11 (4)**	20 (5)**	11 (3)**	6 (2)**	1 (2)**
PND5	16 (4)**	28 (7)**	17 (6)**	8 (1)**	3 (2)**
PND11	23 (1)**	43 (4)**	35 (6)**	49 (16)**	7 (4)**
PND22	58 (4)**	80 (5)**	94 (13)	120 (10)	54 (6)**

[a] 5 dams/point. [b] numbers in parentheses are Standard Deviations. [c] GD= gestation day. [d] PND = postnatal day. [e] 1 aberrant value removed. [f] results rounded to whole numbers for clarity

- & ** = $p<0.05$ & $p<0.01$ respectively

Cholinesterase inhibition in the pups was less severe than in the dams and recovered more quickly. Brain and heart activity was only decreased at the high dose and then only on GD20 and PNDs 1 and 5. Blood cholinesterase activity was decreased only at 5.0 mg/kg/d; RBC levels were more severely affetced than plasma and recovery to control values was complete by PND11 (plasma) and PND22.

Summary cholinesterase activity (% of control[f]) - pups - sexes combined[a]

	Forebrain	Hindbrain	Heart	Plasma	Erythrocyte
	0.3 mg/kg/d				
GD20[c]	104 (22)	107 (5)	112 (16)	104 (6)	102 (20)
PND1[d]	98 (9)	104 (11)	101 (20)	98 (6)	111 (16)
PND5	106 (27)	100 (8)	106 (10)	105 (12)	104 (15)
PND11	102 (40	93 (6)	96 (9)	93 (9)	101 (11)
PND22	103 (3)	100 (11)	102 (11)	96 (5)	102 (26)
	1.0 mg/kg/d				
GD20	91 (5)[e]	100 (6)[e]	94 (15)[e]	96 (7)[e]	106 (17)[e]
PND1	94 (50	101 (9)	97 (20)	94 (8)	101 (24)
PND5	124 (45)	96 (4)	108 (13)	108 (10)	105 (18)

PND11	102 (4)	93 (5)	100 (10)	96 (9)	97 (11)
PND22	99 (3)	102 (6)	102 (11)	97 (6)	110 (29)
	5.0 mg/kg/d				
GD20	40 (9)**	46 (9)**	18 (4)**	15 (4)**	8 (4)**
PND1	63 (9)**	67 (11)**	35 (13)**	40 (11**)	15 (5)**
PND5	103 (37)	88 (5)**	84 (13)**	82 (11)**	57 (15)**
PND11	97 (8)	96 (10)	94 (9)	91 (9)	86 (7)**
PND22	100 (8)	104 (6)	109 (21)	97 (15)	104 (16)

[a] 10 pups/point. [b] numbers in parentheses are Standard Deviations. [c] GD= gestation day. [d] PND = postnatal day. [e] missing data: only one pup in litter (N=9) [f] results rounded to whole numbers for clarity. * & ** = $p < 0.05$ & $p < 0.01$ respectively

Pups sacrificed at PND65 showed no cholinesterase activity depression compared to controls in any compartment or at any dose level.

There was some protection of the fetuses by the dams. The data from GD20 showed a higher blood level of chlorpyrifos in dams than in the fetuses at 1.0 mg/kg/d (dams 2.6 vs 1.0 ng/g) and 5.0 mg/kg/d (dams 110 vs 40-50 ng/g). This was consistent with the lack of any cholinesterase inhibition in fetuses from the 0.3 and 1.0 mg/kg/d dose groups, whereas dams showed significant cholinesterase inhibition in all compartments at 1.0 mg/kg/d and in blood at 0.3 mg/kg/d. The authors estimate that the high-dose pups ingested 120µg/kg/d of chlorpyrifos via the milk. Presumably the high levels of TCP recorded in pup blood arose from ingestion of TCP in dams' milk rather than metabolism of ingested chlorpyrifos; however there are no measurements of TCP levels in milk. Brain and tissue cholinesterase values recovered rapidly in the 5.0 mg/kg/d group despite the continuous dosing, indicating both rapid regeneration of endogenous cholinesterase and/or the maturation of detoxification systems. Results from this study do not indicate that rat pups are more sensitive than adults to cholinesterase inhibiton following chlorpyrifos exposure.

The NOEL for cholinesterase inhibition in pups was 1.0 mg/kg/d based on the significant inhibition seen in the fore- and hind-brain, heart, plasma and RBC compartments on gestation day 20 at 5.0 mg/kg/d. There was no NOEL for cholinesterase inhibition in dams as there was significant inhibition seen in the plasma and RBC compartments on gestation day 20 at 0.3 mg/kg/d and higher doses.

Bushnell PJ, Pope CN & Padilla S (1993). Behavioral and neurochemical effects of acute chlorpyrifos in rats: tolerance to prolonged inhibition of cholinesterase. J Pharmacol Exp Ther 266(2): 1007-17

This study was designed to determine whether the prolonged inhibition of ChE in rats produced by a single injection of chlorpyrifos (Pope et al, 1992) would also induce behavioural changes in similarly treated rats. ChE activity in blood and brain were measured to assess the degree and time course of direct effects. Evidence of tolerance was evaluated pharmacologically by challenges with the direct

muscarinic agonist oxotremorine, and neurochemically by assays of maximal tritiated QNB binding to measure downregulation of muscarinic receptors in the brain. Behavioural tasks were used to assess cognitive and motor function, and rats were also assessed for frank toxicity using selected parts of a functional observation battery.

Adult male Long-Evans rats (6-8/dose group) were trained to perform an appetitive test of memory and motor function and were then injected SC with single doses of 0, 60, 125 or 250 mg/kg of chlorpyrifos in peanut oil (2 ml/kg) and tested 5 days/week for 7 weeks. Unconditioned behaviour was also rated for signs of cholinergic toxicity. Cholinesterase activity was measured in whole blood (ca. Days 4, 15, 31, 52, 74 after dosing) and several brain regions (7 and 21 days after dosing). Muscarinic receptor density and the hypothermic effect of oxotremorine challenge were also measured.

Clinical signs of toxicity were seen in one rat at 250 mg/kg. Whole blood cholinesterase activity was inhibited by 60-75% (dose-dependent) at the first sample (day 4), and recovered to near control value after day 53 (60 mg/kg) and day 74 (125 mg/kg). Brain cholinesterase activity was strongly inhibited in a dose-dependent manner, and at the first sample (d 7) was up to 95% inhibited at the 250 mg/kg dose. By day 21, partial recovery of cholinesterase activity was seen in some brain regions at the 60 and 125 mg/kg dose levels, but no significant recovery was seen after 250 mg/kg. Muscarinic receptor density decreased with dose and time after dose. At 250 mg/kg receptor density in the hippocampus, frontal cortex and striatum fell to 70-75% of control after 7 days and to 60% of control 21 days after dosing; hypothalamic receptors were much less affected. Compared to controls, the hypothermia induced by oxotremorine challenge was significantly less pronounced in each dose group on days 8 and 32, but was comparable to controls by day 52.

Unconditioned behaviour was relatively unaffected by treatment, with a fine tremor in head and limbs peaking on day 9 and gone by 14 days after dosing the only sign of cholinergic overstimulation. Functional deficits (in working memory and motor function) appeared within 2 days after injection of chlorpyrifos and recovered within 3 weeks.

In summary, the behavioural effects of chlorpyrifos exposure recovered more quickly than did blood and brain cholinesterase activity, muscarinic receptor density or subsensitivity to oxotremorine. The authors cite this as evidence of the process of tolerance to organophosphate cholinesterase inhibitors, where functional recovery accompanies neurochemical compensations for the inhibited enzyme. The authors contrast these findings to those seen in rats dosed with the OP diisopropylfluorophosphate (DFP) (Bushnell et al, 1991). These rats showed progressive and persistent impairment of cognitive and motor function over a 3-week period of daily exposure, despite neurochemical and pharmacological evidence of tolerance to DFP-induced inhibition of ChE. This difference suggests that the DFP-induced behavioural changes cannot be attributed entirely to its effects on ChE activity and changes in muscarinic receptor binding.

Chanda SM, Harp P, Liu J & Pope CN (1995) Comparative developmental and maternal neurotoxicity following acute gestational exposure to chlorpyrifos in rats. J Toxicol Environ Health 44(2): 189-202

Pregnant rats (S-D, 5/dose/time) were injected SC with either the peanut oil vehicle or chlorpyrifos (98%) at 200 mg/kg as a single dose on gestation d 12 (GD12) and then sacrificed on either GD16,

GD20, or postnatal d 3 (PND3) for measurement of maternal and developmental indicators of toxicity. The day of birth was PND0. Clinical signs and body weights were recorded daily for all dams and pups.

On PND1 pups were weighed, sexed and randomised and culled to 7-8 pups per dam. Three cross-fostered treatment groups were established: a) CDCP: control pups cross-fostered to control dams; b) CDTP: treated pups cross-fostered to control dams; and c) TDCP: control pups cross-fostered to treated dams.

Maternal and foetal whole-brain samples were obtained on GD16 or GD20. Acetylcholinesterase was measured radiometrically using tritiated acetylcholine iodide (Johnson & Russell, 1975). Receptor densities were calculated after membrane fractions from the whole-brain homogenates were assayed for several activities: total muscarinic receptor binding was measured with tritiated quinuclidinyl benzilate (QNB) and specificity determined by binding in the presence/absence of atropine; the total binding of tritiated cis-methyldioxolane (CD), a high affinity muscarinic agonist, was assayed and the specificity determined by binding in the presence/absence of atropine; the total binding of the nicotinic agonist tritiated cytosine (CYT) was assayed and the specificity determined by binding in the presence/absence of nicotine.

Behavioural tests determined "righting reflex" and "cliff avoidance" performance in pups. Appropriate statistics were applied to the data, and the litter was used as the unit of analysis for all measures taken on foetuses or pups.

Results

While most treated dams exhibited no overt signs, a subset (4/28) showed moderate to severe cholinergic signs at 2-3 d after treatment, and these rats were omitted from further studies. Maternal body weight was initially decreased (15% reduction, 3 days after treatment) after which the body weight increase was comparable to controls. Chlorpyrifos exposure did not significantly affect foetal body weights or brain weights when assayed on GD16 and GD20. On PND1, pups were cross fostered to either treated or control dams as described; on PND3 there were no differences in body weights between these groups.

Extensive AChE inhibition (82-88%) was noted in maternal brain at all three time points (GD16, GD20 and PND3) following acute exposures. Foetal brain cholinesterase activity was less inhibited; ca. 60% of control at GD16 and 42% of control at GD20, and foetal blood levels of chlorpyrifos may have been much lower than in adults. This compared to values of 75-81% of control values seen on PND3 in treated pups cross-fostered to control dams and in control pups cross-fostered to treated dams following repeated exposures (25 mg/kg per day).

At GD16 and GD20, foetal brain AChE activity was inhibited 42-44%. While some degree of recovery in AChE activity was noted in pup brain by PND3, AChE activity was still inhibited (30%) in treated pups cross-fostered to control dams. *In vitro* inhibition of maternal and foetal (GD20) brain AChE activity by the active metabolite, chlorpyrifos oxon, suggested that the prenatal brain AChE activity was somewhat more sensitive than adult brain AChE (IC50 at 37.0^0C, 20 min: dam, 26.6 +/- 1.8 nM; foetus, 6.7 +/- 0.4 nM). Maternal brain muscarinic receptor binding was more extensively reduced (30-

32%) at GD20 and PND3 as compared to the developing brain at GD20 (16%) and PND3 (11%). A simple postnatal reflex test (righting reflex) was transiently (present at PND1 but not PND3) altered by chlorpyrifos exposure. The results suggest that acute chlorpyrifos exposure of dams during gestation produces more extensive neurotoxicological effects in the dam than in the developing foetus.

Chanda SM & Pope CN (1996) Neurochemical and neurobehavioural effects of repeated gestational exposure to chlorpyrifos in maternal and developing rats. Pharmacol Biochem Behav 53(4): 771-776

Pregnant rats (S-D, 5/dose/time) were injected SC with either peanut oil vehicle or chlorpyrifos (98%) at 25 mg/kg/d from gestation days 12-19 (GD12-19) and sacrificed either on GD20 or postnatal day three (PND3). The day of birth was PND0. In a separate dose-response study, rats were similarly exposed to chlorpyrifos at 6.25 or 12.5 mg/kg/d, from GD12-19 and sacrificed on GD 20 for analysis of various neurochemical markers. Clinical signs and body weights were recorded daily for all dams.

On PND1 pups were weighed, sexed and randomised and culled to 7-8 pups per dam. Three cross-fostered treatment groups were established: a) CDCP: control pups cross-fostered to control dams; b) CDTP: treated pups cross-fostered to control dams; and c) TDCP: control pups cross-fostered to treated dams.

Maternal and foetal whole-brain samples were obtained on GD16 or GD20. Acetylcholinesterase was measured radiometrically using tritiated acetylcholine iodide (Johnson & Russell,1975). Receptor densities were calculated after membrane fractions from the whole-brain homogenates were assayed for several activities: total muscarinic receptor binding was measured with tritiated quinuclidinyl benzilate (QNB) and specificity determined by binding in the presence/absence of atropine; the total binding of tritiated cis-methyldioxolane (CD), a high affinity muscarinic agonist, was assayed and the specificity determined by binding in the presence/absence of atropine; the total binding of the nicotinic agonist tritiated cytosine (CYT) was assayed and the specificity determined by binding in the presence/absence of nicotine.

Behavioural tests determined “righting reflex” and “cliff avoidance” performance in pups. Appropriate statistics were applied to the data; the litter was used as the unit of analysis for all measures taken on foetuses or pups.

Results

No maternal toxicity was seen at any dose. Treatment with 25 mg/kg/d of chlorpyrifos induced only an initial decrease in maternal body weight (2%, 3 days after treatment commenced), after which treated and control dams gained weight at the same rate. Treatment commencing on GD12 did not affect foetal weight at GD16 or GD20, but pup weight on PND1 was significantly reduced by treatment at 25 mg/kg/d.

Maternal brain cholinesterase activity measured at GD20 after treatment at 6.25, 12.5 and 25 mg/kg/d during GD12-19 was significantly inhibited in a dose-related manner ranging from ca. 30% of control value at 6.25 mg/kg/d down to ca. 10% of control at 25 mg/kg/d. Foetal brain cholinesterase was less inhibited and ranged from ca. 60% to 40% of control value at respective doses of 6.25 and 25 mg/kg/d,

possibly as a result of lower blood chlorpyrifos levels in foetuses than in adults.

In the next phase of the study, the dams were treated with 25 mg/kg/d commencing on GD12 and then sacrificed on GD 16, GD20 and PND3. Extensive acetylcholinesterase inhibition (down to 10-17% of control value) was noted in maternal brain at all three time points. Foetal brain cholinesterase activity was less inhibited; ca. 60% of control at GD16 and 42% of control at GD20. This compared to values of 75-81% of control values seen on PND3 in treated pups cross-fostered to control dams and in control pups cross-fostered to treated dams following repeated exposures (25 mg/kg per day).

A significant dose-related down-regulation of muscarinic receptors in maternal and foetal brain was noted at GD20 following the GD12-19 exposures of 6.25, 12.5 and 25 mg/kg/d. At 25 mg/kg/d, QNB binding on GD20 was reduced in maternal and foetal brain by 23% and 17% respectively. On PND3 maternal QNB binding was further reduced (31% reduction), CDTP pup brains recorded a 27% reduction compared to controls but TDCP pup values were comparable to controls.

At GD20, dams treated with chlorpyrifos at 25 mg/kg/d from GD12-19 recorded significantly reduced binding to CD (7%) and CYT (26%); on PND3 binding to CD was significantly higher than controls (26%), whereas binding to CYT was still reduced 15% compared to controls. The foetal-brain CD binding was reduced by 11% at GD20; pup-brain CD binding was comparable to controls at PND3 in CDTP pups, but was significantly higher than controls (22%) in TDCP pups. The foetal- and pup-brain CYT binding were not significantly different from controls at GD20 and PND3 respectively.

Righting reflex and cliff avoidance tests were markedly altered following repeated exposures. Chlorpyrifos exposure caused a significant increase in righting reflex time and a decrease in the percent cliff avoidance at both PND1 and PND3 when CDCP pups were compared to CDTP pups.

The results suggest that repeated exposures to chlorpyrifos cause extensive neurochemical and neurobehavioural changes in developing rats. Specifically, pups from dams which had not been exposed to chlorpyrifos but were then cross-fostered to treated dams on PND1 showed significant inhibition of brain cholinesterase activity, indicating that the pups were exposed to chlorpyrifos or its oxon during lactation. Generally, the neurochemistry of dams was more severely affected than the developing nervous system of the foetus, despite the higher sensitivity of foetal brain cholinesterase to chlorpyrifos inhibition. This probably arises from differences in biotransformation and/or delivery of the chlorpyrifos to the foetus. The significant dose-related down-regulation of muscarinic receptors (decreased QNB binding) seen on GD20 and PND3 in maternal and foetal brain following exposures of 6.25, 12.5 and 25 mg/kg/d may be indicative of a tolerance mechanism. The changes seen in the binding of the other cholinergic receptor ligands (ie. the muscarinic agonist CD and the nicotinic agonist CYT), and the overt foetal neurobehavioural decrements further suggests that repeated chlorpyrifos exposure induces changes in the foetal and adult cholinergic system. Comparison of these results with those from an earlier study which used acute exposure to chlorpyrifos (Chanda et al, 1995) reveals that repeated exposures caused greater down regulation of muscarinic receptors than the equivalent dose delivered acutely.

10.2 Human/exposure studies

Pennington JY & Edwards NH (1971) Comparison of cholinesterase depression in humans and

rabbits following exposure to chlorpyrifos. The Dow Chemical Company (TA-477). [Dow; submission 238, part 2 vol 1. A3162/5 Box 42]

Four humans and three rabbits were exposed for 5 minutes to chlorpyrifos formulation M-2995 (61.5% chlorpyrifos, 34.5% xylene; lot 042 7032) via an ULV cold aerosol fog generator delivering 3.8 litres/h. The humans were exposed at a distance of 8 m wearing plastic coveralls but with heads and hands exposed for 2 subjects, and heads, hands and arms exposed for 2 subjects. The rabbits were exposed at a distance of 8 m at 1.3 m height (2 rabbits) or 0.8 m height (1 rabbit). Plasma and erythrocyte cholinesterase determinations were made pre- and at several intervals post-dose.

For the humans, air sampling was conducted using 2 shoulder height filters, 3.7 cm^2 in area, one of which measured passive skin deposition and the other sampled air at one litre/min. Air samples for rabbit exposures were taken by positioning filters directly under the noses of rabbits and sampling air at one litre/m. Exposure was terminated at 5 minutes due to the eye and lung irritation induced by the formulation.

The air sampling recorded breathing-space concentrations of chlorpyrifos of about 108 mg/litre (range 83-133) for humans and rabbits, with passive deposition accounting for 2-6% of filter load. There was no depression of plasma or RBC cholinesterase in 24-h post-exposure samples from the human subjects. At 24 h post-dose, rabbits recorded a decrease in plasma cholinesterase (up to 33%) and RBC cholinesterase (up to 12%), but by 72 h post-dose these values had recovered to near control values.

Kilian DJ, Edwards HN, Benge MC & Tabatabai Z (1970) Results of human skin exposure to Dowco 179. The Dow Chemical Company, July 20, 1970. Adapted from Intra-company Report No. TMD-70-2 Date March 18, 1970.[Dow; Submission 11462, 238; reference 122]

Dursban 6 (6 lb/gallon EC, formulation M-2995, 61.5% chlorpyrifos, 35% xylene) was applied to the skin of the back and abdomen of human volunteers (one subject/dose; skin area not stated) and 2 rabbits/dose at 5, 10, 25 and 50 mg/kg for 20 (5 d/week), 4, 3, and 1 applications respectively. The applied dose was covered with occlusive patches for 12 h, and after patch removal each site was scored for irritation prior to washing. Each site was used only once. Plasma and erythrocyte cholinesterase determinations (pH-Stat method) were made pre- and at several intervals post-dose (exact time not stated). An initial study used 5 humans at 12-h dermal doses ranging from 93.5 - 622.5 mg/kg and reported generally identical RBC and plasma cholinesterase levels at 24, 48 and 72 h post exposure even at the highest dose.

No skin irritation was recorded. The table below indicates that plasma cholinesterase values were significantly inhibited under two of the dose regimens, activity reaching a minimum at around 3 days after the last dose and then recovering to control values in 4-10 days; RBC cholinesterase values were not inhibited under any regimen.

Cholinesterase activity after dermal dosing of humans* (% of predose value)

	End of dosing	**End of dosing**	**Day 1**	**Day 1**	**Day 3**	**Day 3**
Dermal dosing	Plasma	RBC	Plasma	RBC	Plasma	RBC
10 mg/kg for 4 x 12 h	99	102	97	103	97	100
25 mg/kg for 2 x 12 h	100	100	100	100	102	100
25 mg/kg for 3 x 12 h			67	98	48	98
5 mg/kg for 20 x 12 h	67	103	64	104	74	104

*one subject/dose regime

Coulston F, Golberg L & Griffin T (1972). Safety evaluation of Dowco 179 in human volunteers. Institute of Experimental Pathology and Toxicology, Albany Medical College, Albany New York. Dated March 1972. [Dow; Submission 238; A3162/5, Box 43]

Sixteen healthy adult male volunteers were assigned to four experimental groups; one served as a placebo control and the others received chlorpyrifos (DOWCO 179; technical chlorpyrifos; purity and source not stated) in tablet form at dose levels of 0.014, 0.03 and 0.10 mg/kg/d for up to 27 days. The period of dosing was different for each dose level, and ranged from 9 days at 0.1 mg/kg/d, through 20 days at 0.3 mg/kg/d to 27 days at 0.014 mg/kg/d. Control subjects received the placebo for 48 days. Although not specifically stated, it appears that the high-dose treatment was terminated due to the degree of plasma cholinesterase inhibition on day 9. No rationale was given for the termination of treatment at the lower dose levels. The test material was administered each day with breakfast. Heparinised blood samples were obtained from subjects twice-weekly for plasma and erythrocyte cholinesterase determinations, and these determinations continued until all values had returned to pre-administration levels. The experimental protocol did not state whether blood samples were taken at the same time on each sample day, and so it was not clear what the time interval was between test material administration and blood sampling. No indication was given for the frequency of observations made for clinical signs following treatment.

After cessation of treatment, subjects were monitored for recovery periods of 7 days (controls and low dose), 30 days (0.03 mg/kg/d), or 35 days (0.10 mg/kg/d). At weekly intervals, separate blood samples were collected for haematology and routine clinical chemistry determinations. Serum chemistry parameters measured were calcium, phosphorous, glucose, urea nitrogen, uric acid, cholesterol, protein, albumin, bilirubin, alkaline phosphatase, lactate dehydrogenase, and SGOT. Haematological parameters were white cell count, haemoglobin, haematocrit, basophils, eosinophils, neutrophils, lymphocytes, and monocytes. Urinalysis was also performed weekly (specific gravity, colour, turbidity, pH, cellular content), and portions of the urine samples were retained for determination of the test material and metabolites.

Results

No clinical signs associated with test material administration were reported during the study. However, no indication was given as to the frequency of observations for clinical signs of intoxication. If the study depended upon self-reporting of clinical effects, such effects may have been under-reported, or poorly defined. A single subject in the 0.1 mg/kg/d group reportedly suffered from a runny nose, blurred vision

and a feeling of faintness on the final day of dosing. The subject was treated for a cold, and was asymptomatic by the end of the day. However, these symptoms were also consistent with cholinesterase inhibition, and blurred vision (consistent with cholinesterase inhibition following exposure to organophosphate compounds) was not a symptom typically associated with a simple cold. The subject's plasma cholinesterase activity was inhibited by 70% compared with pretest levels. No decrease in erythrocyte cholinesterase activity was noted in any test subjects during the study. With the exception of plasma cholinesterase inhibition, clinical chemistry parameters were not affected by treatment, and neither were urinalysis nor haematology determinations.

As summarised in the table below, rapid and marked depression of plasma cholinesterase activity was observed in subjects at the high dose of 0.1 mg/kg/d chlorpyrifos. Within-day comparisons with placebo controls revealed that the group mean depression of activity was greater than 40% by day 6 and greater than 60% by day 9, at which time the treatment was suspended. Plasma cholinesterase activity did not return to control levels until about day 34 in these high dose subjects. Similar results were observed when the plasma cholinesterase activity in the high-dose subjects was compared with the baseline activity of the group prior to treatment.

At 0.03 mg/kg/d, mean plasma cholinesterase activity was inhibited by greater than 20% compared with placebo controls on days 16-20 of treatment. When compared to baseline levels pre-treatment, the mean plasma cholinesterase activity in this group was reduced by up to 34% on day 18, and activity was still inhibited by more than 20% compared with baseline levels on day 34. Individual subject plasma cholinesterase inhibition (relative to each subject's mean predose activity) was as high as 53% at day 18 of administration. This inhibition of plasma cholinesterase activity was considered to be biologically significant, and treatment related. At this dose level, treatment was suspended after day 20, and so it was not possible to determine whether continued treatment at 0.03 mg/kg/d would have resulted in further inhibition of plasma cholinesterase activity, but plasma cholinesterase activity did not return to control levels for several weeks after cessation of treatment.

At 0.014 mg/kg/d, plasma cholinesterase activity was inhibited by 20% at a single sample interval only (day 13), and plasma cholinesterase levels were similar to baseline activity from day 20 to day 27, even with continued chlorpyrifos administration. As there was no consistent biologically-significant depression of plasma cholinesterase activity at 0.014 mg/kg/d during the treatment period, this dose represents the NOEL for this study.

In the study report, it was stated that statistical analysis of plasma cholinesterase inhibition was performed by the investigators for each dosage group, using curve-fitting procedures to calculate linear regression (least squares method), and analysis of variance of the resulting slopes. The analysis revealed that the difference between the mean of the slopes of the control group and the low dose group was not significant, but the investigators did not report the results of this statistical analysis for the high dose groups.

A separate statistical analysis was conducted by the investigators to determine the significance of plasma cholinesterase activity at the higher dose levels. At 0.03 and 0.1 mg/kg/d, two-way analysis of variance by the investigators indicated that the inhibition of mean plasma cholinesterase activity was significant ($p<0.05$) compared with controls at the high dose level (0.1 mg/kg/d) only. The investigators stated that the 0.03 mg/kg/d dose level was “a threshold dose for plasma cholinesterase inhibition”, but that the

inhibition was not statistically significantly different from controls. However, as the inhibition of plasma cholinesterase activity in individual subjects at 0.03 mg/kg/d was as high as 53% compared with pre-test levels, this dose appears to be more than a threshold level for such effects, as it induces biologically significant inhibition.

The NOEL for inhibition of erythrocyte cholinesterase activity was 0.1 mg/kg/d, with no effect on activity at any dose level.

Under the conditions of this study, the NOEL was 0.014 mg/kg/d, based on biologically significant, dose-related inhibition of plasma cholinesterase activity at 0.03 and 0.1 mg/kg/d. The statistical significance of the inhibition at 0.03 mg/kg/d was equivocal, and depended upon the statistical tests used. This was a reflection of the small group size (n=4), assay variability, the experimental design (using different treatment periods for each test group), and the inherent variability in plasma cholinesterase activity between individuals.

Note: As part of the Australian regulatory process, advice was sought from the Advisory Committee on Pesticides and Health (ACPH) regarding the NOEL for this study, as changes in the NOEL would result in changes to the Australian ADI. The Committee noted that the statistical significance of plasma ChE inhibition at 0.03 mg/kg/d in humans was considered to be equivocal and agreed that the NOEL for this study should remain at 0.03 mg/kg/d.

Group mean percentage change in plasma cholinesterase activity compared with placebo controls (n=4)

	Sample time (days)	**1**	**3**	**6**	**9 or 10**	**13**	**16**	**18**	**20**	**23**	**27**	**30**	**34**	**37**	**41**	**44**	**48**	**51**	**55**
Dose mg/kg/d																			
0.014			**0**	**-2**	**-16**	**-27**	**-17**		**-11**	**0**	**-10**	+5	+6						
0.03		**-3**	**-4**	**-14**	**-5**	**-13**	**-22**	**-28**	**-23**	-11	-19	-8	-11		+11	-4	-4	+3	
0.1		**-6**	**-4**	**-42**	**-66**	-49	-33		-21	-21	-21	-10	+23	+29	+15	+4			

Group mean percentage change in plasma cholinesterase activity compared with intra group baseline activity pre-treatment (n=4)

	Sample time (days)	**1**	**3**	**6**	**9 or10**	**13**	**16**	**18**	**20**	**23**	**27**	**30**	**34**	**37**	**41**	**44**	**48**	**51**	**55**
Dose mg/kg/d																			
Placebo		**+11**	**+8**	**+10**	**+19**	**+10**	**0**	**+3**	**+6**	**-4**	**+6**	**-5**	**-8**	**-9**	**-4**	**+10**	**+6**	**+10**	**+8**
0.014			**+6**	**+7**	**-10**	**-21**	**-18**		**-7**	**-5**	**-6**	+14	+13						
0.03		**-5**	**-8**	**-16**	**0**	**-15**	**-31**	**-34**	**-28**	-24	-24	-23	-28		-6	-7	-10	0	
0.1		**-9**	**-9**	**-44**	**-64**	-50	-41		-27	-33	-27	-25	-1	+3	-3	0			

Figures in bold type indicate measurements made during treatment periods.

Nolan RJ, Rick DL, Freshour NL & Saunders JH (1982) Chlorpyrifos: pharmacokinetics in human volunteers following single oral and dermal doses. The Dow Chemical Company. Study No.: HEB-DR-0043-4946-4, dated August, 1982 [Dow; submission 939, November 1988, part 4, vol 1, pp 4.299-4.313. Dow; Submission 238 (1987) Part 4, pps 4.228-4.242]

Nolan RJ, Rick DL, Freshour NL & Saunders JH (1984) Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol Appl Pharmacol 73:8-15 (1984) [Dow; Submission 1080. Dow; Submission 1053, part 3, pp 3.213-3.220. Dow; Submission 11462, reference 114. Public Domain]

Six male Caucasian volunteers, aged 27-50 years, were used in this study. Volunteer A served as a pilot, and was given a single oral dose (0.5 mg/kg) of chlorpyrifos (Dow; 99.8% purity; Lot AGR 166043) in the form of a lactose capsule, administered approximately 30 minutes after food. This dose was administered approximately one month prior to the treatment of other volunteers. Volunteer A was also given a dermal dose of chlorpyrifos (0.5 mg/kg, dissolved in methylene chloride) when the other volunteers were given their oral dose. Two weeks after the first dermal dose, Volunteer A was given a further dermal dose (0.5 mg/kg) in dipropylene glycol methyl ether (DPGME). The remaining volunteers were given a dermal dose of 5.0 mg/kg chlorpyrifos in DPGME four weeks after the administration of their oral dose.

Dermal applications were made to sites on the forearms of volunteers (approximately 100 cm^2), and the test material was not covered or occluded. The volunteers were encouraged to follow normal routine, and each took a bath or shower 12 to 20 h after application of the dermal dose. All urine was collected from volunteers from 24-48 h prior to dosing, through 120 h post-dosing. Separate collections were made at 0, 6, 12, 24, 36, 48, 60, 72, and 96 h post-dosing. Two additional 12-h urine collections were made starting 156 and 180 h post dosing, following the 5.0 mg/kg dermal treatment. Blood samples were collected prior to, and at discrete intervals following, treatment for determination of chlorpyrifos and its principal metabolite 3,5,6-trichloro-2-pyrinidol (3,5,6-TCP), and for the determination of cholinesterase activity.

No signs or symptoms of chlorpyrifos toxicity were reported in any volunteers during the study. Following the oral administration of 0.5 mg/kg chlorpyrifos, plasma cholinesterase was inhibited by 71% (compared with predose levels) in volunteer A, and by 85% (mean) in the other volunteers within 12-24 h after treatment. The range of individual values was not provided. Plasma cholinesterase activity had returned to >80% of the mean predose value by day 30 after oral treatment. Following dermal application at day 30, plasma cholinesterase activity decreased to approximately 70% of predose levels, and returned to about 80-90% of these levels by day 40 of the study. The intra-group variation was stated to be considerable by the authors of the study, but these figures were not provided in the report. Erythrocyte cholinesterase inhibition was not significantly inhibited following either oral or dermal doses of chlorpyrifos.

No unchanged chlorpyrifos was detected in the urine (analytical limit 10 ng/ml). Following the 0.5 mg/kg oral dose of chlorpyrifos, 3,5,6-TCP was first detected in the blood after 1-2 h, after which time the concentration increased rapidly (half life of 0.5 h), reaching a mean maximum concentration of 0.93 µg/ml, six hours after ingestion of the dose. Following the 5.0 mg/kg dermal dose of chlorpyrifos,

the mean half-life for appearance of 3,5,6-TCP in the blood was 22.5 h, with the highest mean concentration of 0.063 µg/ml observed 24 h after dosing. The mean predicted absorption following oral administration was 72 ± 11%, and following dermal administration was 1.35 ± 1.0%. This was in agreement with the percentage of the administered dose recovered from the urine following oral (70%) and dermal (1.28%) administration.

Kisicki JC, Wilkinson Seip C & Combs ML (1999) A rising dose toxicology study to determine the No-Observable-Effect-Levels (NOEL) for erythrocyte acetylcholinesterase (AChE) inhibition and cholinergic signs and symptoms of chlorpyrifos at three dose levels. Dow Agrosciences LLC Study ID: DR#K-044793-284 dated 19 April 1999. [Dow submission] GLP

This was a well-conducted study designed to determine a NOEL for RBC cholinesterase inhibition in healthy volunteers (6/sex/dose) following a single oral dose of 0, 0.5, 1.0 or 2.0 mg/kg chlorpyrifos (Lot # MM930503-17, purity - 99.8%; lactose powder was the diluent/placebo). The study design was double-blind, randomised, placebo control and conducted in two phases separated by 14 days. Separate groups of volunteers were dosed with 0, 0.5 or 1.0 mg/kg in the first phase and the results assessed prior to initiating a study of 0 or 2.0 mg/kg in the second phase.

Volunteers (18-55 years old) were screened for general good health according to set criteria, and instructed to refrain from alcohol, strenuous exercise and prescription medications before and during the study. Doses were taken by capsule after an overnight fast. The health status of subjects was closely monitored; vital signs (blood pressure, pulse, respiration and temperature) were assessed prior to dosing and at 1, 2, 4, 8, 12, 24, 48 and 168 h post-treatment. Subjects were questioned regarding their well being at each sample time, and clinical evaluation of the symptomology was recorded. Subjects were aware of the signs and symptoms of cholinergic toxicity and were instructed to inform the study physician of any adverse effects experienced. The subjects were blind as to their treatment group and the assessment and treatment of these signs and symptoms was perfomed by a physician also blind to the treatment group of the subject.

Blood samples were collected at –10 and 0 h pre-treatment and at 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 h post-treatment; these samples were analysed for RBC cholinesterase activity and chlorpyrifos and metabolites. In addition all urine voided during the period –48 h through to 168 h post-dose was collected in a series of 12 h or 6 h time spans and analysed for chlorpyifos and metabolites. A clinical laboratory evaluation (haematology, clinical chemistry and urinalysis) and a brief physical examination were performed at the completion of the study. The paraoxonase status of each subject was measured.

Results

No data were provided for the analysis of paraoxonase status or analysis of blood and urine for chlorpyrifos and metabolites. There were no significant deviations from the study protocol. One male (phase 1 control) and one female (subject 56, phase 2, 2.0 mg/kg) did not provide a complete series of blood and urine samples.

No signs of toxicity were observed in any subject. All symptoms reported were mild and similar between treatment groups.

Signs and symptoms frequency by treatment group

Dose	**0.0[a]**	**0.0[b]**	**0.5**	**1.0**	**2.0**
No. of subjects	12	12	12	12	12
No. with symptoms (%)	8 (67)	3 (25)	5 (42)	9 (75)	5 (42)

[a] Phase 2 control [b] Phase 1 control

No treatment related differences were seen in systolic or diastolic blood pressure, pulse, respiration, body temperature or 12-lead ECG information during or after the study.

Extensive statistical analysis was perfomed for the RBC cholinesterase activity data seeking relationships between sample time, gender and treatment group. The study design using two pre-exposure measurements as a baseline meant that a minimum decrease of 17.3% in RBC cholinesterase activity compared to pre-exposure levels was required to achieve statistically significance. No statistically significant differences in RBC cholinesterase activity were seen between groups. The only statistically significant effect of treatment were recorded for the female volunteer who withdrew from the study (subject 56, 2.0 mg/kg). This female had decreased RBC cholinesterase levels compared to her pre-treatment values at most of the sample times (98.4%, 77.2%, 71.8%, 74.1%, 81.4% and 79.5% of pre-treatment value at 4, 8, 12, 24, 36 and 48h post-dose). If the data for this subject are removed from the analysis, then the 2.0 mg/kg female group-means are indistinguishable from the concurrent control values.

Mean Female RBC cholinesterase activity as percent of mean baseline value

Dose	-10	0	4	8	12	24	36	48
0.0[a]	99.5 (2.1)	100.5 (2.1)	98.3 (2.0)	102.1 (2.4)	100.7 (1.8)	99.7 (3.9)	98.4 (1.7)	100.2 (2.3)
0.0[b]	99.2 (4.4)	100.8 (4.4)	102.5 (5.9)	103.1 (5.4)	98.4 (3.2)	99.4 (3.7)	94.6 (5.3)	97.7 (5.1)
0.5	101.2 (6.7)	98.8 (6.7)	96.3 (6.5)	98.1 (2.1)	95.8 (3.9)	97.0 (3.6)	94.7 (4.7)	97.1 (4.1)
1.0	98.2 (0.9)	101.8 (0.9)	104.6 (4.2)	100.8 (3.5)	98.9 (3.7)	100.9 (5.6)	96.0 (4.1)	98.9 (2.1)
2.0	99.8 (1.8)	100.2 (1.8)	100.3 (3.2)	96.6 (6.81)	91.1 (9.7)	95.2 (10.6)	95.9 (7.4)	94.5 (7.7)

Mean Male RBC cholinesterase activity as percent of mean baseline value

Dose	-10	0	4	8	12	24	36	48
0.0[a]	100.6 (1.8)	99.4 (1.8)	99.1 (2.2)	99.6 (4.6)	99.8 (4.3)	98.4 (4.3)	97.6 (2.8)	98.2 (3.1)
0.0[b]	96.1 (5.1)	103.9 (5.1)	101.6 (4.0)	102.5 (5.2)	102.9 (5.8)	102.6 (2.3)	98.2 (3.8)	95.9 (6.8)
0.5	98.7 (1.1)	101.3 (1.1)	102.0 (3.0)	103.4 (4.9)	99.7 (3.0)	101.9 (3.3)	98.1 (3.0)	92.8 (4.7)
1.0	98.6 (1.9)	101.4 (1.9)	104.5 (7.8)	101.1 (4.2)	101.1 (4.7)	101.7 (4.7)	98.5 (4.6)	92.1 (2.0)
2.0	101.2 (2.3)	98.8 (2.3)	99.2 (3.1)	98.9 (3.5)	98.7 (4.0)	99.2 (2.6)	99.5 (5.1)	98.4 (3.1)

[a] Phase 2 control [b] Phase 1 control

There were no effects of treatment on the post-study clinical chemistry findings. Any results sufficiently

outside the normal range to be clinically significant were retested, and with few exceptions were found to be within normal limits on retest.

In this study of the effects of a single oral dose of chlorpyrifos to fasted human males and females, the NOEL for clinical signs or symptoms in this study was 2.0 mg/kg, the highest dose tested. The NOEL for RBC cholinesterase inhibition was 1.0 mg/kg based on statistically significant inhibition in 1/12 subjects exposed at 2.0 mg/kg. There were no effects of treatment at any dose level on general health measures during the study nor on clinical chemistry parameters measured at 7d after dosing.

Ishikura H (1988) Evaluation of airborne chlorpyrifos in the living area of Lentrek-termiticide-treated houses at US Air Force Base, Okinawa during and after application and evaluation of operators' exposure to chlorpyrifos. Dow Chemical Pacific Laboratory Report Code: GHF-P 783, September 1988 [Dow; Submission 1080]

An evaluation of airborne chlorpyrifos in the living areas of treated houses at US Air Force Base, Okinawa (in summer) and an evaluation of operator exposure was conducted during and after application. The test houses had slab floors with holes drilled in them to enable the termiticide to be pumped into the soil, and the attics were also sprayed with chlorpyrifos. The concentration of chlorpyrifos in houses 24 h after application was in the range of 0.001 to 0.009 mg/m³ in the kitchen, living room and bedrooms. At 48 h the maximum detectable concentration was 0.002 mg/m³. During the application process the concentration reached a level of 0.018 mg/m³ when spraying the attic. It was estimated that the amount of chlorpyrifos that came in contact with skin (applicator) ranged from 153 to 513 µg/treatment, with protection. Without protective clothing, 11 mg/treatment was the contact dose.

Ishikura H (1989) Evaluation of airborne chlorpyrifos in the living area of Lentrek* 20 MC treated house during and after application, and evaluation of operator exposure to chlorpyrifos. Dow Chemical Pacific, Laboratory Report Code: GHF-P 826, 17 February, 1989. [Dow; Submission 1080]

An evaluation of airborne chlorpyrifos in the living areas of houses (in winter) during and after application of the insecticide Lentrek 20MC termiticide (micro-capsule formulation) was conducted in a Japanese house at Nagoya-shi. These test houses had raised wooden floors and the test substance was sprayed onto the soil and structures under the house. Air sampling resulted in the detection of chlorpyrifos levels of 0.0198 mg/m³ under the floor of the house, and 0.0011 to 0.0061 mg/m³ in the house during application. Concentrations 1 and 24 h after application were less than the detectable limit (0.0005 mg/m³). Skin contact was estimated as 660 to 1540 µg/treatment (with protective clothing).

Kawakita T (1987) Evaluation of Lentrek* termiticide applicator exposures to chlorpyrifos during house treatment in Japan. The Dow Chemical Company, Laboratory Report Code: HEH 2.1-80-1(1), 9 April 1987. [Dow; Submission 1080]

Evaluation of Lentrek termiticide applicator exposures to chlorpyrifos during under house treatment in Japan (in winter). Applicators wore protective clothing and respirators. The estimated total amount of chlorpyrifos to come in contact with the skin (with protective clothing) was in the range of 165 to 1430 µg/treatment of a house. Working without protective clothing could result in skin exposure of up to 100 mg/application. Cholinesterase depression was generally low, although inhibition of plasma cholinesterase reached a maximum of 18% in workers plugging drilled holes, over exposure periods of 2.5 h, with

protective clothing worn. The maximum concentration of airborne chlorpyrifos detected during the study was 1.26 mg/m³ in the house.

Vaccaro JR (1983) Evaluation of applicator exposure to airborne chlorpyrifos during short-term application of chlorpyrifos based partial release aerosol in local dwelling, Midland, Michigan, October 19, 1982. The Dow Chemical Company, Laboratory Report Code: HEH 3.5-5-28(3). 5 January, 1983. [Dow; Submission 1080]

An evaluation of applicator exposure to airborne chlorpyrifos during short-term (1.9 to 2.9 min) application of chlorpyrifos-based partial-release aerosol in houses (in autumn) was conducted. The formulation containing chlorpyrifos was sprayed on the floor and floor coverings to control a flea infestation. Exposure of the applicators to chlorpyrifos ranged from 0.05 to 0.12 mg/m³ during application.

Ludwig PD, Kilian DJ, Dishburger HJ & Edwards HN (1970) Results of human exposure to thermal aerosols containing Dursban insecticide. Mosquito News, 30:3, 346-354. [Dow; Submission 238, A3162/5, B43; Submission 11462, reference 123]

Male and female adult volunteers (Dow employees) were exposed to a thermal fog under simulated conditions approaching expected exposure patterns. The tests were conducted at a dosage recommended for adult mosquito control (1.5 oz DOWCO 179/gallon of oil). The cholinesterase activity in erythrocytes and plasma was determined at various time intervals after exposure. Under the conditions of use there was no sign of toxicity or cholinesterase depression.

Eliason DA, Crammer MF, von Windeguth DL, Kilpatrick JW, Suggs JE & Schoof HF (1969) Dursban premise applications and their effect on the cholinesterase levels of spraymen. Mosquito News, 29:4, 591-595. [Dow; Submission 238, A3162/5, B43]

Kenaga EE (1967b) Comments on data concerning cholinesterase effects of Dursban in man following U.S. public health service experimental yellow fever mosquito eradication tests in Florida during 1966 and 1967. Dow Chemical Company report A1A-394, TK 2-1684, dated 20 October 1967. [Dow; Submission 11462, reference 121]

A monitored field test was used to determine the effect that chlorpyrifos (Dow; Dursban; purity not stated) had on spraymen applying treatment at the rates used for programs for eradication of mosquitoes. This study was conducted following two earlier studies that were of limited success. These studies were conducted in 1966, 1967 and 1968, respectively. The three reports on the exposure of humans following spraying with DOWCO 179 were:

1. Dursban 0.5% emulsion in a 9-day spray program using power spray equipment, with the average application of 29.1 gallons per premises, involving five spraymen and one foreman.

2. Dursban 0.25% emulsion in a 5-day program with four spraymen, one foreman, and two controls, with an average application of 0.5 gallons/premises.

3. Dursban 0.5% emulsion in a 14-day program, where the spraymen wore protective clothing and were given explicit safety instructions, using power spray equipment, with seven spraymen and hosemen applying an average 4.6 gallons per premises. The foreman and three control subjects were expected to have no contact with any anti-cholinesterase compounds during the program.

Cholinesterase activity measurements were conducted, and individual exposure was terminated if ChE inhibition reached 50% of baseline values.

Results

In the first study, three spraymen showed a marked depression in plasma ChE activity, with reductions of 68 to 82% compared with baseline levels. In the two remaining spraymen, where baseline values were not available, there was a reduction in plasma ChE activity of 52-56% over the 9-day period. The study was discontinued after two weeks due to the significant decrease in ChE activity.

In the second study, there was reportedly no treatment-related effect on cholinesterase activity during the short study period.

In the third study, RBC ChE activity was not affected by treatment. Plasma ChE activity was reduced by 50-80% in spraymen using a suspension formulation at the first sample interval. All workers in this group had plasma ChE activity reduced by 50% or more , and activity had generally returned to pre-exposure levels within 2 months following cessation of use. The four spraymen using an emulsion formulation did not display a reduction in plasma ChE activity during the exposure period.

Kenaga EE & Lembright (1967) Cholinesterase levels and exposure history of a man using Dursban M (M-3019) for mosquito control. Dow Chemical Company report A1A-174 TK 2-1683, dated 23 October 1967. [Dow; Submission 11462, reference 120; submission 238, A3162/5, B43]

In this report, a single individual mixed and applied chlorpyrifos as part of mosquito control programs. The subject reportedly used no other insecticides, and wore no safety equipment, and the concentration of test material used was considered to be high. The subject's serum cholinesterase levels were reportedly unaffected during this exposure period. The lack of exposure data, and the fact that a single individual was tested, means that this study was not of any use for regulatory purposes.

Ballard JB (1986) Chlorpyrifos concentration measured in the air of homes treated with Dursban TC termiticide. The Dow Chemical Company, Laboratory Report Code: GH-P 1295, 16 September 1986. [Dow; Submission 1080]

Chlorpyrifos concentrations were measured in the air of homes previously (up to 18 weeks) treated with Dursban TC termiticide. The data were gathered over many years and all seasons. A total of 16 homes were treated and tested for chlorpyrifos levels. When application instructions were followed rigorously, the air levels of chlorpyrifos ranged from 0.0003 to 0.0011 mg/m^3. Based on the information obtained from this study, and other reported data collected from a total of 68 homes, the study authors concluded that the location of the home, home type, length of time since treatment, the number of applied gallons, and concentration of finished solution did not appear to influence the resulting concentration of

chlorpyrifos in the air of the home.

Kaplan JG, Kessler J, Rosenberg N, Pack D & Schaumburg HH (1993) Sensory neuropathy associated with Dursban (chlorpyrifos) exposure [published erratum appears in Neurology 1994 Feb; 44(2): 367]. Neurology 43(11): 2193-6. Luxembourg Industries Dossier file no. 5.9.2/01. [David Gray; Submission 11467]

In this study, the authors reported 8 case studies where chlorpyrifos exposure was claimed to cause sensory neuropathy. The cases each presented with a range of symptoms, and exposure characterisation was poor. Only 1 patient (case 3) reported signs of cholinesterase inhibition, including lacrimation, muscle twitching and diarrhea, and this individual was a pesticide applicator who was reportedly exposed to chlorpyrifos in a closed environment for 6 months. The patient's RBC cholinesterase activity was reportedly “low” initially, but recovered to “normal” levels within two months. The actual ChE activity in this patient was not stated, and was only performed at the onset of symptoms. No neurological evaluations were conducted until 6 weeks after the other symptoms were reported, and at this time the evaluation reported sensory loss of all modalities in a stocking-glove distribution test, mild distal weakness and areflexia in the lower extremities. Nerve conduction studies and quantitative sensory threshold studies reportedly revealed changes consistent with peripheral neuropathy of the distal anonopathy type. The decreased reflexes and mild distal weakness were consistent with polyneuropathy. Follow-up examination at one year revealed remission of all symptoms. In the other 7 patients (4 from the same family), symptoms were non-specific, and the absence of symptoms of cholinesterase inhibition suggested that these patients were not acutely exposed to chlorpyrifos to the extent of case 3.

Case 2 was a woman who noted burning, numbness, cramps, and a sensation of vibration in her hands and feet 4 weeks after her house was sprayed with chlorpyrifos by an exterminator, but was only evaluated 6 months after these initial symptoms. This patient was also found to have mildly attenuated reflexes in the ankles. Three months later, this patient was normal, except for borderline sural nerve action potential amplitudes, but these effects were not seen three months after this examination.

Case 8 reported impaired memory and slow thinking three weeks after pesticide application in her house (8 applications over a three week period), but no other clinical symptoms were noted. Examination revealed a sensory loss in the feet, and ankle reflexes were depressed. Neurologic examination performed 6 months later was normal. Eighteen months after this time, the patient's neurological examination was normal, but neuropsychological evaluation revealed reduced intellectual functioning, and impaired performance on memory tests. The decreased reflexes and/or loss of sensation in cases 2 and 8 were consistent with clinical signs of polyneuropathy, but the evidence was not conclusive.

Cases 4 to 7 (same family) reported short-term memory impairment, but neuropsychological evaluation was declined, making assessment of cognitive functional impairment difficult. The patients initially complained of headaches, nausea, and painful muscle cramps, and, one month later, numbness and paresthesia, mainly in the lower extremities. Six months later, there were no more complaints of cognitive function effects, and nerve conduction studies were generally without abnormal findings.

The lack of immediate electrodiagnostic testing, the poor exposure characterisation, and the variability in the reported clinical findings makes interpretation of the case studies generally difficult. Additionally,

the method of questioning used to obtain information from the patients was subjective. No follow up examinations were conducted for a range of parameters on a number of patients, and not all patients were subjected to the same initial testing regimen.

Based on the information provided in this report, there was evidence that one patient (case 3) was exposed to chlorpyrifos occupationally, with exposure sufficient to decrease cholinesterase activity and cause cholinergic symptoms. This patient also presented evidence of mild, reversible polyneuropathy, possible in the presence of ongoing decreased RBC cholinesterase activity. Two other patients (cases 2 and 8) had limited evidence of mild polyneuropathy

Nolan RJ, Freshour NL, Rick DL & Dittenber DA (1981) Chlorpyrifos: Probe study to determine plasma and erythrocyte cholinesterase depression and blood chlorpyrifos levels in male Fischer 344 rats following a single oral or dermal dose. Dow Chemical Company report A1A-143, dated 4 December 1981. [Dow; Submission 11462, reference 93]

No GLP statements were made regarding the design and/or conduct of this study.

Analytical grade chlorpyrifos (Dow; Lot no. AGR 166043; stated purity 99.8%) was prepared as a 20% w/v stock solution in methylene chloride. In the oral toxicity component of this study, the stock solution was diluted in corn oil (1% v/v) then given to male Fischer 344 rats (Charles River USA) by oral gavage at a dose of 5 mg/kg (dose volume 2.5 ml/kg). In the dermal component of this report, the 20% w/v stock solution was diluted with methylene chloride to provide solutions containing 10, 40, and 60 mg/ml chlorpyrifos. Aliquots of the test dilutions (0.1 ml) were applied to the shaved intact skin between the shoulders, giving doses of 5, 20, and 80 mg/kg. The test sites were not occluded. Three animals/group were treated in the oral and dermal test groups, and two animals/group in the oral vehicle (corn oil) and dermal vehicle (methylene chloride) groups. Following dosing, animals were observed for 24 h for clinical signs of toxicity before being killed. Blood samples were obtained for cholinesterase determinations.

To determine blood chlorpyrifos levels, two animals were given a single oral dose of 5 mg/kg, and two animals were given single dermal doses of 20 mg/kg. After 4 h, the animals were killed, and blood collected.

Results

No clinical signs of toxicity were observed. At oral and dermal doses of 5 mg/kg, plasma cholinesterase activity was inhibited by 30 and 36%, respectively. The dermal doses of 20 and 80 mg/kg resulted in plasma cholinesterase inhibition of 78 and 90%, respectively. RBC cholinesterase activity was reduced by 46% after the oral dose of 5 mg/kg. Following the dermal application of chlorpyrifos, RBC cholinesterase inhibition was 5, 83, and 64%, at 5, 20, and 80 mg/kg, respectively. The oral and vehicle control compounds did not decrease ChE activity.

The mean concentration of chlorpyrifos in blood was 28 ng/ml following the oral dose of 5 mg/kg, and 13 ng/ml following the dermal dose of 20 mg/kg.

Fenske RA, Black KG, Elkner KP, Lee C-L, Methner MM and Soto R (1990) Potential

exposure and health risks of infants following indoor residential pesticide applications. American J. Public Health, 80: 689-693.

This study examined the use of chlorpyrifos in the treatment of flea infestation inside a residence. Application of chlorpyrifos was by the broadcast technique, which involved the hand spraying of an aqueous mixture directly onto target surfaces such as rugs, floors and furniture. The test formulation (Dursban LO, 41.5% chlorpyrifos) was applied approximately 40 cm above the carpet as a 0.50% aqueous spray (40 ml/3.785 litres of water) to three identical rooms (two ventilated, one not) covering the entire floor and spraying for 5-7 minutes per room. Application of the test substance was carried out by a licensed pest control applicator using standard spraying techniques. The aim of this study was to make a preliminary risk assessment for crawling infants (9-10 months of age) for exposure to the residual air and surface concentrations. Air and surface chlorpyrifos residues were measured at regular intervals, ie. prior to application and then post-application at 0-0.5 h, 0.5-1 h, 1-1.5 h, 1.5-3 h, 3-5 h and 5-7 h, and then for a two-hour interval 24 h after application.

Chlorpyrifos residues were collected on aluminium foil collection strips (surface) from each room immediately (5 minutes) after spraying, and levels averaged 13.6 µg/cm^2 ($\pm$ 6.1), with no statistically significant differences between rooms. This measured deposition corresponded well with the value of 13.6 µg/cm^2 that was calculated from the label recommendations. Surface residues obtained by wipe-sampling in the non-ventilated room (3.90 µg/cm^2) were significantly higher (double) than in the ventilated rooms (1.69 µg/cm^2) at 30 minutes after application, and at all sampling times through to the end of the study (at 24 h).

Chlorpyrifos vapours were measured at the sitting adult breathing zone (100 cm above ground) and the infant breathing zone (25 cm above ground), and results showed significantly higher chlorpyrifos levels in the infant breathing zone in both ventilated and non-ventilated rooms. Vapor levels in the adult breathing zone peaked at around 20 µg/m^3 in ventilated rooms and >60 µg/m^3 in the non-ventilated room at 3 to 5 h post-application.

Substantially higher chlorpyrifos concentrations were measured in the infant breathing zone with 94 µg/m^3 detected 5 to 7 h post-application in the non-ventilated room and >60 µg/m^3 at 3 to 5 h in ventilated rooms. After 24 h post-application an equilibrium concentration of approximately 30 µg/m^3 was detected in all rooms. It would appear that the treated carpet served as a source of volatilised chlorpyrifos.

Time-weighted averages for the 24-hour post-application period in the infant breathing zone were 41.2 and 66.8 µg/m^3 for ventilated and non-ventilated rooms, respectively. These values were significantly higher than the values of 12.3 and 45.7 µg/m^3 for the adult breathing zone. All these concentrations exceeded the National Academy of Sciences (US) guideline value of 10 µg/m^3 for chlorpyrifos in indoor air.

A risk assessment (over 24 h exposure) was conducted based on a scenario of a 9-10 month old infant playing in a carpeted room beginning at least one hour after a 0.5% chlorpyrifos broadcast application. It was assumed that the infant respiratory volume was 2.1 litres/min resting (16 h/day) and 6.3 litres/min active (8 h/day), that respiratory absorption of chlorpyrifos vapour was 100%, that total carpet contacted in one day was 2.3 m^2, that there was a 100% transfer of available residue to skin, and that

dermal absorption of chlorpyrifos was 3%.

Under these conditions, the combined absorption by the inhalational and dermal routes was estimated at 0.075 and 0.158 mg/kg for ventilated and non-ventilated rooms, respectively. Exposure estimates for 24 to 48 h (second day) were approximately 2-3 times lower. Dermal exposure represented approximately 68% of the total absorbed dose. Absorption via the oral route was not included even though infants will continually put their hands (or objects) in their mouth while playing.

While the risk assessment calculations did not include some worst-case assumptions (such as 100% dermal absorption), it does include some major uncertainty factors, including the rate of transfer of pesticides from treated surfaces to the skin and the amount of surface contacted over time. Also, the permeability of infant skin to chlorpyrifos might differ from that of adult skin.

The exposure values obtained from this risk assessment indicate the potential for the no-observed effect level (based on plasma cholinesterase inhibition) to be exceeded. Strategies to minimise such potential exposure were suggested by the authors, and included adequate ventilation, extended re-entry periods following application, special warnings regarding contact between treated surfaces and small children, and rigorous training and licensing of applicators.

Currie KL, McDonald EC, Chung LTK & Higgs AR (1990) Concentrations of Diazinon, Chlorpyrifos and Bendiocarb after application in offices. Amer. Ind. Hyg. Assoc. J. 51(1): 23-27

Airborne and surface concentrations of chlorpyrifos were measured at intervals up to 10 days after broadcast spray application onto the carpeted floors of several offices. Chlorpyrifos airborne concentrations, measured at 1m above the floor, peaked 4 h after application at 27 $\mu g/m^3$ in one office. In empty offices, airborne chlorpyrifos concentrations increased until 4 h after spraying; in furnished offices, levels decreased after spraying, and stabilised at low levels for 2 days, after which time the levels declined further. Insecticide deposition measured on aluminium plates ranged from 0.2-4.86 ng/cm^2. Prior to application of the insecticide, aluminium collection strips were placed on horizontal furniture surfaces at heights of 0.4, 1.5 and 2.1 metres above the floor. Surface concentrations of chlorpyrifos were greater at 0.6 m from the floor when compared with a zone 1.5 to 2.1 metres above the floor. Peak surface residue concentrations of chlorpyrifos were 5.9 ng/cm^2 at 48 h after application.

Lean S & Cantrell P (1992) Airborne chlorpyrifos levels inside dwellings following a soil barrier termiticide treatment using Dursban PC termiticide and insecticide. DowElanco Australia and Workcover Authority of NSW.

The Workcover Authority of NSW conducted this airborne monitoring study. DowElanco supplied the chemicals and the dwellings while the Authority conducted and reported all analytical work; the Authority has previously been involved in organochlorine and chlorpyrifos air-monitoring studies, together with the NSW Department of Agriculture.

Airborne chlorpyrifos levels inside dwellings were determined at 1, 7, 30, 100 and 180 days following a soil barrier treatment against termites using Dursban PC in the subfloor space of seven dwellings. Application was conducted by a registered pest control company (Rentokil) according to Australian

Standard 2178 at the label dilution of 1 % active ingredient (200 ml of 500 g/litre EC in 10 litres water). 100 Litres was applied per cubic metre of soil by trenching and incorporation into the backfill soil.

Seven homes in the northern suburbs of Sydney were chosen; details were as follows:

No	Location	Construction	Space (cm)	Soil Type	Airflow
1	Avalon	weatherboard	50 - 75	clay	fair - good
2	Avalon	weatherboard	150 - 225	sandy loam	good
3	St Ives	brick veneer	50 - 150	sandy loam	fair - good
4	Elanora Hts	double brick	50 - 200	sandy loam	good
5	Frenchs Forest	weatherboard	50 - 150	loam	fair
6	St Ives	brick veneer	50 - 250	sandy loam	good
7	Pennant Hills	double brick	50 - 75	loam	fair - good

Air sampling used a sampling pump at 1 litre air/min (calibrated each time) for 2 h at each sampling time, with sample collection using an ORBO 42 or 44 sampling tube; the pump was located 1 to 1.5 metres off the floor. Chlorpyrifos was assayed by GC with an electron capture detector.

Results were as follows:-

Day	Mean chlorpyrifos concentration (µg/m³)	Range
Prespray	0.03	0 - 0.11
1	0.58	0.24 - 0.95
7	0.40	0 - 0.82
30	0.29	0 - 0.63
100	0.11	0 - 0.30
180	0.04	0 - 0.14

Measurements were taken in 3 rooms per dwelling. Data were averaged for 6 houses as house no. 6 had a sub-floor chlorpyrifos spray for spider control 2 weeks prior to the trial, giving pre-spray levels of 0.5 µg/m³ and therefore it was left out of mean calculations.

Thus when the 3-room samples in each of the 6 homes used in this study were averaged, no chlorpyrifos level exceeded 1 µg/m³ at any time. The only individual measurement which exceeded this level after day 1 was the kitchen of house 1 on day 7 (1.1 µg/m³).

It was concluded that under-slab treatment would be unlikely to give rise to levels higher than those found in the above study.

Cantrell P (1992) Exploratory Air Monitoring Studies to Determine the Fate of Domestic Chemicals. Masters Thesis, the University of NSW.

In a 12-month monitoring study, 3 homes in the Sydney area (Ashfield, Newport and Kiribilli) were given sub-floor treatments with 1% chlorpyrifos at 50 g/m^2 as per label directions (3 rooms monitored in each house). The maximum individual level recorded was 15.4 µg/m³, in the subfloor space of one of the homes on the day after treatment. Mean indoor airborne levels did not exceed 0.44 µg/m^3 at any

time.

In a 6-month monitoring study, 7 homes in the Sydney area were monitored (3 rooms/house) following subfloor treatment. The following results were obtained:-

Airborne Chlorpyrifos Levels (µg/m³) ± SD

Time	Indoor	Subfloor
Pretreatment	0.10 ± 0.41	2.03 ± 12.0
1 day	0.69 ± 0.90	70.9 ± 172.1
1 week	0.41 ± 0.56	37.4 ± 28.6
1 month	0.41 ± 0.58	15.4 ± 14.6
3 months	0.13 ± 0.31	7.62 ± 20.9
6 months	0.06 ± 0.17	3.0 ± 3.0

These data were from the same experiment reported by DowElanco and WorkCover (Lean & Cantrell, 1992), except that data for all 7 houses were averaged (not 6 as in the above report).

Note: No Australian data on indoor chlorpyrifos levels after underslab treatment were generated, although in studies of homes built on concrete slabs treated with sub-slab cyclodienes, it was found that there was little or no cyclodiene in indoor air.

Vaccaro J, Bohl R, Skowronski B & Moribito P (1987) Airborne Chlorpyrifos Concentrations Measured During and Following Applications of Dursban TC Insecticide to Residential Dwellings. the Industrial Hygiene Laboratory of the Dow Chemical Company, Michigan (Lab Project GH-P 1310).

'Dursban TC' was used in this study, containing, (in g/litre), chlorpyrifos (480), xylene (295), Casul 70 (157), T-Det (190) and EPO-61 anti-foam (5); this was ostensibly equivalent to 'Dursban Micro-Lo'.

Airborne chlorpyrifos was monitored in 32 dwellings in the USA, pre-, during and post-application (2, 4, 8 and 24 h, 1 week, 3 months and 1 year). Eight (8) of each type of building were monitored; plenum space dwellings, crawl-space dwellings, basement dwellings and slab-type constructions.

Comment: While it was not clear what a plenum space dwelling is, it appears that there was venting from the house into the sub-floor space; such constructions were not very common in the USA. It was stated that previous studies had shown that airborne concentrations of chlorpyrifos were higher in this type of construction than in basement or conventional crawl-space constructions.

The dwellings were each treated with a 1 % emulsion according to label directions, by trenching, injection and/or surface band. Air sampling used glass absorber tubes packed with 'Chromosorb 102' and calibrated air pumps operating at approx. 1 litre/min (2 h sampling times). Duplicate samples were taken in the centre of each of two rooms, at a height of 1 - 1.5 metres; in basement constructions, samples were also taken in the centre of the basement. Assays were by GC, utilizing electron capture detection.

Airborne chlorpyrifos concentrations within the dwellings only once exceeded 10 µg/m³, in the plenum

space of one dwelling during application (10.4 µg/m³). At 7 days post-application, concentrations ranged from 0.07 - 8.1 µg/m³ (plenum dwellings), 0.03 - 2.7 µg/m³ (basement constructions), 0.08 - 0.87 µg/m³ (crawl-space dwellings) and 0.08 - 1.0 µg/m³ (slab-type constructions).

Slab dwellings were treated with a combination of techniques including drilling through the slab around the inside perimeter of the dwelling and outside, using trenching or rodding techniques, to create a chemical barrier. At 24 h post-treatment in 6/8 slab-floor dwellings, airborne chlorpyrifos had dropped below 1 µg/m³ and at 30 days, all measured concentrations were at or below this level.

In crawl-space dwellings, all measured levels were below 1 µg/m³ at 24 h.

In basement dwellings (walls drilled from the inside to treat wall voids, with trenching or rodding around exterior walls), highest measured levels were in the basement; 2/8 houses had levels still just exceeding 1 µg/m³ at 7 days (1.13 and 1.15 µg/m³) while at 90 days 1 house had 1.4 µg/m³ (spray spilled on floor of basement during treatment); highest levels detected in the latter case were 4.37 µg/m³ on day 4.

In plenum dwellings, 3/8 had levels which exceeded 1 µg/m³ on day 7 (means of two rooms, 1.09, 1.05 and 7.0 µg/m³). In the latter house, mean 2-room levels were 4.7 and 0.85 µg/m³ at days 30 and 90 respectively. The reason for the highest levels in this house were not stated although the highest level ever found in this study viz. 10.4 µg/m³ occurred during treatment of this house.

Wright CG, Leidy RB & Dupree HE (1991) Chlorpyrifos in the air and soil of houses four years after its application for termite control. Bull. Environ. Contam. Toxicol (1991), 46, p 686-689. [Dow Elanco, Submission 6005]

This study examined the air and soil (around exterior walls) levels of chlorpyrifos four years after standard spraying (no details) for termite control. Included in the study were comparison between soil and construction types.

Chlorpyrifos levels detected in the air of kitchens and bedrooms, four years after treatment, ranged between 2 and 6 µg/m³. Concentrations of chlorpyrifos were slightly higher in dwellings (slabs or crawl) built over sandy soil (4 to 6 µg/m³) when compared with dwellings (slab or crawl) built over clay (2 to 3 µg/m³).

Soil levels of chlorpyrifos were greater in clay (473 to 499 ppm) than in sand (177 to 256 ppm) for crawl or slab-crawl construction types. It was stated that the air levels of chlorpyrifos determined at 4 years after spraying were greater than those measured at 6 months and 2 years after spraying. Values for these earlier sampling times were not given and the reason for this difference was stated as unknown.

Wright CG, Leidy RB & Dupree HE Jr (1994) Chlorpyrifos in the air and soil of houses eight years after its application for termite control. Bull Environ Contam Toxicol 52(1): 131-4.

In this study, sampling and analysis were conducted on the residences as reported in an earlier paper by the same authors (Wright et al, 1991). Chlorpyrifos levels detected in the rooms ranged from <0.1 to 0.7 µg/m³. The chlorpyrifos levels in the ambient air were lower than the levels detected at 1, 2, and

4 years, both for the maximum levels seen in any house and for the average level for all houses combined. There were no differences in chlorpyrifos levels present in the air of houses built over sand rather than clay soils, by house construction type (slab, crawl, or slab-crawl) or room type (kitchen or bedroom). There was a general decrease in residues of chlorpyrifos in soil between 4 and 8 years from many of the houses.

Dow Chemical Company (1990) Evaluation of dislodgable residues and absorbed doses of chlorpyrifos to crawling infants following indoor broadcast applications of a chlorpyrifos based emulsifiable concentrate. Dow Chemical Company, Study ID DECO-HEH 2.1-1-182 (IDI), 1990 [Set 14, B32; Dow Elanco 6005]

This study simulated exaggerated behavioural patterns of an active, scantily clad child on a treated floor surface (eight rooms), and examined physicochemical dissipation of chlorpyrifos on a treated surface and the absorption of chlorpyrifos in adult human volunteers. Dursban LO insecticide was diluted with water and applied (by a certified applicator) as a 0.5% chlorpyrifos emulsion at a rate of one gallon/1,600 ft^2 in accordance with the label. Once the application was completed, a period of two hours was allotted for drying with natural ventilation (at least one window in each room was open) used to facilitate drying. A total of eight rooms from two dwellings, six bedrooms and two living rooms, were treated in the previously described manner.

Air sampling started immediately upon completion of application and at 1, 2, 4 ,8, 12, 24 and 48 h after application. The sampling height was 15 inches off the floor because this represents the breathing zone of a child sitting on the floor. The distribution of chlorpyrifos onto the floor was measured by the placement of gauze strips (represents carpet floor) and aluminium strips (represents non-porous surfaces) randomly around the treated rooms. Levels of chlorpyrifos, that had settled onto these strips, were determined at 0, 1, 2, 4, 8, 12, 24 and 48 h post-application by collecting and measuring the chlorpyrifos on 4 strips (2 gauze, 2 aluminium) at each time interval. Wipe tests of the carpet were carried out using a drag system consisting of a 3x3x3/4 inch block of wood loaded with a 8.5 pound lead weight, and a pad of denim placed under the block of wood. The weighted block was dragged 48 inches in 10 to 15 seconds with four trials conducted at each time interval (0, 1, 2, 4, 8, 12, 24 and 48 h).

Human activity in the treated rooms commenced immediately following the drying period and was carried out by six male volunteers, each wearing a bathing suit, who followed activities that simulated the movement of a child on the floor. The dwellings were completely closed during the activity periods which lasted for 4 h. Blood and urine samples were collected from the subjects at 24 h after the start of the study.

The basis for calculations of indirect contributions to total exposure were as follows.

A. Inhalation exposure calculation (24 h cycle of rest and activity)

Total respiratory volume at rest (child) 1.5 litres/min
Total respiratory volume during activity (child) 4.2 litres/min
Child's weight (1 year old) estimated 10.2 kg
Time Weight Average (TWA) chlorpyrifos concentration 14.5µg/m^3

TWA x Total Respiratory Volume = µg/kg (24 h)
Child's weight

For both at rest and active situations. TWA's ranged from 11.9 to 19.9 ug/m³.
The average inhalation exposure in the child was estimated to be 4.9 µg/kg over a 24 h period.

B. Dermal exposure calculation (drag values at 4 h after spraying)

Drag (wipe) value 29.3 µg/ft²
Magnitude of area of contact 43.1 ft²
Skin absorption factor 3% assumed Child weight 10.2 kg
Drag value x contact surface area x dermal absorption = µg/kg Child's weight
Surface to body weight ratio for child to adult taken into account with a correction factor of 1.52.
The average dermal exposure in the child was estimated to be 4.0 µg/kg.

C. Oral exposure calculation based on chlorpyrifos levels in hand washes collected during activity period.

Chlorpyrifos in hand rinse 291.1 µg
Hand size of infant 25% of adult hand
Child's weight 10.2 kg
Chlorpyrifos levels x Hand size factor = µg/kg
Weight
The average oral exposure in the child was estimated to be 11.6 µg/kg.

The oral value was calculated from the hand rinses following the 4-h activity period. The assumption was made that all the material rinsed from the hands would be ingested and absorbed. The hand exposure was included in the dermal exposure. The total systemic exposure to chlorpyrifos by a child was estimated to be 20.5 µg/kg.

Calculation of estimated absorption of chlorpyrifos in a child based on urinary levels of chlorpyrifos metabolite 3,5,6-TCP.

A direct measurement of the amount of chlorpyrifos absorbed was achieved by using the urinary levels of 3,5,6-TCP and performing a calculation to determine the concentration of the parent compound. The urinary data does not allow a determination of the contribution of each route of absorption, but by using the percentage contribution of each route as presented in the indirect measurement of absorption an estimate was made. These estimates were presented in the following table: -

Amount of chlorpyrifos absorbed (µg/kg) in a child

Indirect measurement		Direct measurement (urinary data)	
Inhalational	4.9	Inhalational	15.3
Dermal	4.0	Dermal	

Hand/oral	11.6	hand/oral	11.6
Total	20.5		26.9

The value generated for the dermal (in inhalation/dermal) exposure component also took into account the difference in surface to body weight ratio (factor of 1.52) between adult males and crawling infants.

Berteau PE, Knaak JB, Mengle DC & Schreider JB (undated) Insecticide absorption from indoor surfaces. Biological Monitoring For Pesticide Exposure, ACS Symposium Series 382, Chap 24. Washington DC, American Chemical Society. [Dow Elanco, 6005]

This paper reviewed articles on insecticide absorption from surfaces. Data for this review comes from US Poison Control Centres with a great majority of the exposures described occurring in the home. This paper suggested that young children clad in little more than diapers were at risk playing on previously sprayed surfaces such as floors, carpets, furniture and bedding. The higher body surface area to volume ratios of children compared to adults increases the likelihood that children will received a toxic dose.

A calculation of a worse case exposure of an infant to chlorpyrifos sprayed in a kitchen took into account inhalation (0.18 mg), dermal (19.9 mg) and oral (1.91 mg) exposures. Assumptions made were that there was 100% absorption from the skin after a 0.5% chlorpyrifos spray was used with a one hour period of drying and all chlorpyrifos on the skin of the hands was licked off (oral exposure). The average air concentration was 0.0015 mg/m^3 and the average surface concentration was 0.0433 mg/100m^2. The total exposure dose for the infant was estimated as 2.68 mg/kg.

Blondell J & Dobozy VA (1997) Review of chlorpyrifos poisoning data. US EPA report. [Dow; Submission 11460, reference 5]

In this document, the US EPA summarised the case reports, case series, statistical surveys, and epidemiological studies of acute and chronic health effects reported to be related to chlorpyrifos. The report noted certain limitations, including inadequate documentation of exposure and effects, reporting biases, and absence of denominator information on the population at risk. However, where consistent patterns of risk factors were identified, the report also made recommendations to mitigate such risk. Some of the conclusions and recommendations arising from this report have been outlined below. An independent assessment of the report has not been conducted by Australian authorities.

Report conclusions

The report concluded that chlorpyrifos was one of the leading causes of acute insecticide poisoning incidents in the United States. This finding was based largely on an examination of Poison Control Centre reports. The high frequency of poisoning incidents was attributed to the widespread use of chlorpyrifos inside and outside the home, and chlorpyrifos was stated to be the fourth most common insecticide found in US homes in a 1990 US EPA survey.

Certain types of uses were considered to pose greater health risks, while other types of use were associated with little or no significant health impacts. The report suggested that this finding may have been biased by problems associated with surveying certain user groups (such as agricultural fieldworkers), and that additional surveys of poisonings are needed to confirm the health risks associated with

chlorpyrifos use.

The main concern was associated with the use of chlorpyrifos liquid formulations used by homeowners or Pest Control Operators (PCOs) indoors or outdoors, termite treatments, and liquid sprays and dips applied to domestic animals. Information on the number of homes and applications involving PCOs was considered to be necessary information to better characterise the population at risk.

Most of the more serious poisonings were associated with misuse or inappropriate use (spills, inadvertant contamination) by a PCO.

Report recommendations

The report recommended that the sponsors of chlorpyrifos arrange for a more detailed analysis of the Poison Control Centre data, and a prospective epidemiological study or statistically valid survey was recommended to determine the extent, circumstances, and persistence and severity of chronic health effects attributed to chlorpyrifos exposure. The health effects to be surveyed included chronic neurobehavioural effects, symptoms of peripheral neuropathy, multiple chemical sensitivity, and reactive airway disease or asthma.

It was recommended that registrant-sponsored training and education programs be developed and implemented for PCOs using liquid chlorpyrifos indoors or for termite treatment. The potentially hazardous applications involving broadcast or fogger treatments indoors were recommended for cancellation, and it was recommended that the termiticide treatment of existing structures be restricted and require the on-site presence of a certified applicator.

A number of further recommendations were made, mainly regarding the addition of precautionary warning statements, changes in use patterns, restriction on formulation concentrations for particular uses, and the rewriting of literature to warn of the hazards associated with the use of chlorpyrifos products.

Shurdut BA, Chen WL, Burns CJ, McCormick RA, Nolan RJ & Racke KD (1997) Critical assessment of report entitled "Review of chlorpyrifos poisoning data" (by J. Blondell and V. Dobozy, January 14, 1997). DowElanco Study GH-C 4359, dated 31 March 1997. [DowElanco; Submission 11460; reference 6]

In this review, DowElanco responded to the above US EPA review on chlorpyrifos poisonings (Blondell & Dobozy, 1997), and stated that there were deficiencies in the EPA report which invalidated its use for reaching any conclusions about the safety of chlorpyrifos. These alleged deficiencies have been outlined below. An independent assessment of this review has not been conducted by Australian authorities.

The review stated that there has been a mis-interpretation of Poison Control Centre data, and that data from state and national poison control centres supported the relative safety of products containing chlorpyrifos. It was stated that according to the American Association of Poison Control Centre's (AAPCC) Toxic Exposure Surveillance System (TESS) database, chlorpyrifos-containing products (which account for 25% of the urban insecticide market) represented less than 10% of all insecticide-

related inquiries to poison control centres. This number of inquiries was reportedly less than the number of inquiries for other common household substances).

The review also claimed that the US EPA report:

- failed to respect the limitations of anecdotal information,
- failed to consider the extensive testing and long use history for chlorpyrifos products with regard to neurological injury,
- made an inappropriate use of anecdotes and studies of organophosphates to characterise the safety of chlorpyrifos,
- failed to consider the extensive database on exposures following use of chlorpyrifos products, and
- selectively presented the data.

Aiuto LA, Pavlakis SG & Boxer RA (1993a) Life-threatening organophosphate-induced delayed polyneuropathy in a child after accidental chlorpyrifos ingestion. The Journal of Paediatrics 122(4): 658-660.

Gutmann L & Bodensteiner JB (1993) Organophosphate-induced delayed polyneuropathy [letter; comment]. J Pediatr 123(5): 837

Aiuto LA, Pavlakis SG & Boxer RA (1993b) Organophosphate-induced delayed polyneuropathy [letter; comment]. J Pediatr 123(5): 837

In this medical case-report (Aiuto et al, 1993a), a previously well 3-year-old boy was found playing near an open, spilled bottle of insecticide containing chlorpyrifos (Dursban; Dow Chemicals; concentration not stated). The boy went to sleep but awoke a short time later (time period not stated) and was described as crying, salivating and in respiratory distress. Upon admission to a medical facility, the patient was comatose, miotic, and unresponsive to deep pain, and had frothy white nasal and oral secretions. Fasciculations of the eyelids and twitching of the extremities were also noted, and ECG examination revealed sinus tachycardia of 160 beats/minute.

In the absence of other foreign substances in serum and urine, a diagnosis of acute organophosphate poisoning was made, and the patient was administered a gastric lavage, and given iv doses of atropine (0.02 mg/kg), 2-PAM (500 mg), and a loading dose of phenytoin (dose not stated). The patient opened his eyes shortly after treatment, and the muscle fasciculations ceased. Physical examinations were conducted, and revealed white secretions in the patient's nose, mouth and endotracheal tube. Neurologic examination revealed globally decreased tone with inability to follow commands, and deep tendon reflexes were present bilaterally. Treatment with mechanical ventilation, atropine and 2-PAM were continued, and the patient's secretions decreased and muscle tome improved. Therapy with 2-PAM was discontinued after 72 h.

Between days 3 and 6 of hospitalisation, the patient suffered from stridor and/or respiratory distress, and these effects were treated with atropine, aerosolised racemic epinephrine, and/or intubation. On day 3, the plasma cholinesterase activity was 8000 IU/litre (normal range 7700 to 17500 IU/litre). On day 9, the patient was free of stridor and was transferred to a general pediatric unit.

On hospital day 11, the patient had an acute episode of severe stridor that did not respond to repeated

treatments with aerosolised racemic epinephrine. The patient was intubated, and during direct laryngoscopy and broncoscopy the airway appeared patent, without any swelling or obstruction, but bilateral vocal cord paralysis was observed. The patient was found to have areflexia, with electromyography on day 18 revealing normal latencies and conduction velocities for age, but there was a complete lack of F latencies. This finding was considered to be consistent with proximal polyneuropathy. Bilateral vocal cord paralysis persisted on day 19. By day 23, neurologic examination revealed some return of deep tendon reflexes, and by day 27, deep tendon reflexes were easily elicited, and the patient was able to stand. By day 31, the results of electromyography, nerve conduction studies, sensory evoked potentials, and brain-stem auditory evoked potentials were normal. Increased vocal cord movements were observed on day 38, and the patient was fully ambulatory prior to discharge on day 52.

Discussion

The authors of this paper noted that clinical manifestations of organophosphate-induced polyneuropathy (OPIDN) begin 1-3 weeks after the acute cholinergic crisis, and that examinations are consistent with distal, symmetric, predominantly motor polyneuropathy. In this patient, the symptoms of cholinesterase inhibition, along with the manifestations of weakness and areflexia 11 days after ingestion and the electromyographic findings, confirmed the presence of an acute transient polyneuropathy, with a more proximal distribution than usually seen in adults.

The acute, reversible bilateral vocal cord paralysis reported in this patient has not previously been seen as part of OPIDN, but the authors believed that the onset of stridor and vocal cord paralysis coincided with the onset of muscle weakness, and resolved at a similar time to the normalisation of peripheral neuropathy.

Gutmann and Bodensteiner (1993) responded to the Aiuto et al article, and suggested that this case may not represent a clear example of OPIDN. They noted that chlorpyrifos has been associated with OPIDN only after severe intoxication, characterised by extensive cholinesterase inhibition. The inhibition of plasma cholinesterase activity in this case was instead suggestive of mild chlorpyrifos intoxication. It was also noted that the clinical findings associated with OPIDN are predominantly motor polyneuropathy, with flaccid weakness and atrophy primarily in the distal limb muscles, and recovery usually requires months to years, and is often incomplete. The diagnosis of the patient in this case implied that evoked compound muscle action potentials and sensory nerve action potentials had normal amplitude, which would run counter to a diagnosis of OPIDN.

In response to these comments, Aiuto et al (1993b) noted that the patient's symptoms were consistent with cholinesterase inhibition of >90%, and that the plasma cholinesterase levels did not necessarily correlate with the severity of the ingestion. The authors concluded that the patient suffered from a delayed, life-threatening neuropathy, but were unable to determine the mechanism of such a neuropathy.

Shemesh I, Bourvin A, Gold D & Kutscherowsky M (1988) Chlorpyrifos poisoning treated with ipratropium and dantrolene: a case report. J Toxicol Clin Toxicol; 26(7): 495-8.

In this case report, a 38-year-old man drank an undefined quantity of chlorpyrifos 25% solution. The

patient was treated with atropine 3 mg iv every two h for six days (total 400 mg), but any attempt to remove atropine treatment resulted in reappearance of respiratory distress. After this time, the patient was stuporous, catatonic and suffering from respiratory distress, with white mucous secretions, and his serum cholinesterase activity was undetectable. Ipratropium, 0.5 mg, was administered endotracheally as an aerosol mist over a ten minute period, and there was an improvement in the patient's clinical condition, though the salivary secretions continued. The patient continued to receive ipratropium (between 1 and 2 mg daily), and by the fifth day the respiratory support was discontinued. A significant amount of catatonia and coarse tremor were Relieved following administration of dantrolene 10 mg iv, followed by 25 mg po, three times daily. Serum cholinesterase activity was still undetectable one month after discharge.

Osterloh J, Lotti M & Pond SM (1983) Toxicologic studies in a fatal overdose of 2,4-D, MCPP, and chlorpyrifos. J Anal Toxicol; 7(3): 125-9.

In this case report, a 26-year-old man intentionally ingested approximately 360 ml of a 6.7% chlorpyrifos formulation, 360 ml of a 2,4-D (10.8%) and MCPP (11.6%) formulation, and a few granules of a warfarin (0.025%) concentrate. The patient's major clinical findings on admission were coma, myoclonus, miosis, cardiac arrythmias, and a progression into hypotension, oliguria and death after several episodes of asystole. The authors stated that such symptoms were consistent with those seen in other patients following ingestion of chlorofenoxy acetic acids such as 2,4-D and MCPP. The patient ingested a total of 1200 mg/kg of chlorfenoxy acetic acids, well above the reported range of fatal doses of 80-714 mg/kg. The patient did not display many of the usual signs of organophosphate poisoning, such as lacrimation, salivation, respiratory paralysis, or muscle fasciculation. The red blood cholinesterase activity was inhibited by about 70% at 13h, and by up to 90% by 26 h. Plasma cholinesterase activity was inhibited completely at all time intervals. Lymphocyte neurotoxic esterase (NTE) activity was inhibited by about 50% after 13 h but was within normal limits after 19 h. Cerebral cortex NTE activity was within normal limits, but peripheral nerve NTE activity was inhibited by about 70% compared to normal control levels. This inhibition of NTE activity was measured in the absence of any significant signs of organophosphate intoxication.

Lotti M, Moretto A, Zoppellari R, Dainese R, Rizzuto N & Barusco G (1986) Inhibition of lymphocytic neuropathy target esterase predicts the development of organophosphate-induced delayed polyneuropathy. Arch Toxicol, 59: **176-179.**

In a case where a forty-two year old male attempting suicide by drinking a 41% commercial formulation of chlorpyrifos (estimated dose 300 mg/kg bw), the patient showed coma, respiratory insufficiency, lachrymation, salivation, sweating, miosis, fasciculations and bronchorrhea upon hospital admission 18 hours post-exposure. EEG signs of diffuse irritation were recorded and. Gastric lavage was performed (a chest X-ray showed right basal bronchopneumonitis) and chlorpyrifos was detected in the analysis. Plasma cholinesterase activity was inhibited almost 100% by 36 hours. Coma and cholinergic signs persisted for 17 days, during which time atropine, pralidoxime, mechanical respiration and antibiotics were used. The patient was asymptomatic on day 24 post-exposure and drug administration was stopped; no signs of PNS or CNS involvement was observed. On day 30, lymphocytic NTE, blood acetylcholinesterase and plasma butyrylcholinesterase were markedly below the normal range. Thereafter enzyme activities recovered slowly. On day 43, weakness and paraesthesia in the legs, with tendon reflexes and sensory

conduction velocity reduction were noted. By day 62, leg weakness was more severe, gait was impaired and tendon reflexes were absent. Electromyography of leg muscles showed symmetrical signs of denervation with signs of spontaneous activity, and motor conduction velocity was reduced. Electrophysiological studies of the arms confirmed a symmetrical reduction of sensory conduction velocity of the ulnar nerves. Sural nerve biopsy on day 63 revealed a few altered myelinated fibres. Aspects of axon and myelin degeneration were also found occasionally. The changes were consistent with mild distal axonopathy. Chlorpyrifos was still detectable 10 days post exposure, and NTE inhibition was still 60% 4 weeks post-exposure (Lotti et al., 1986).

Sherman JD (1996) Chlorpyrifos (Dursban) – Associated birth defects: Report of four cases. Archives of Environmental Health 51(1): 5-8. [DowElanco; Submission 11461; reference 1]

In this paper, the author presented four incidents of birth defects allegedly associated with exposure to chlorpyrifos. In this paper, the four children (2 boys and two girls; ages not stated) were reported with a range of birth defects including ventricular, eye, and palate defects and growth retardation (all children), hydrocephaly, microcephaly, mental retardation, blindness, hypotonia, wide-spread nipples, and deformities of the teeth, ears and external genitalia. The mothers of the affected children were reportedly exposed to chlorpyrifos in either the workplace or the home during pregnancy. Two of the children were born to the same mother.

The exposure of the mothers to chlorpyrifos was very poorly characterised, but the paper does not indicate that exposure was severe or sustained during organogenesis. The range of birth defects reported in the children was not consistent with results of studies in laboratory animals, where similar effects have not been demonstrated.

On the basis of the findings in this report, and the poor characterisation of exposure, it was not possible to determine whether the reported effects were associated with chlorpyrifos exposure.

Gibson JE (1996) Critical review of allegations associating Dursban with human teratogenicity. DowElanco laboratory Study ID JEG122396, dated 23 December 1996. [DowElanco; Submission 11461; reference 3]

In this report, DowElanco reviewed the previous paper by Sherman (1996), and disputed the association of the reported defects with chlorpyrifos exposure. This review states that the medical records of the cases indicated that the effects seen in the children were not the same in all children, and that the effects in some of the children were consistent with a specific diagnosis of an autosomal recessive birth defect syndrome of the brain and eye known as cerebro-oculio-facio-skeletal syndrome (COFS), or a closely related syndrome known as MICRO syndrome.

This review also reported that exposure to chlorpyrifos in one case occurred after the first trimester of pregnancy, and that the effects seen in the children were inconsistent with results of animal studies.

On the basis on the information provided in this review, and the lack of information in the original case report paper (Sherman 1996), it appears as though there was no association between the cases reported and chlorpyrifos exposure.

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APPENDIX I

List of Clinical Chemistry, Haematology & Urinalysis Parameters

Clinical Chemistry	Haematology	Urinalyses
albumin alkaline phosphatase bilirubin (total) calcium chloride cholesterol (total) cholinesterase activity creatinine (blood) gamma-glutamyl transpeptidase globulin glucose (blood) LDH (serum lactate dehydrogenase) phosphorus potassium protein (total) SGPT (serum alanine aminotransferase) SGOT (serum aspartate aminotransferase) sodium triglycerides urea nitrogen (blood) CPK (creatinine phosphokinase)	clotting parameters (clotting time, prothrombin time) erythrocyte count haematocrit (packed cell volume) haemoglobin leucocyte differential count leucocyte total count platelet count reticulocyte count MCH MCHC MCV blood smear	appearance specific gravity glucose ketones sediment (microscopic) occult blood pH protein volume bilirubin urobilinogen

APPENDIX II

List of organs for organ weight determination and for histopathological examination

Organs Weighed	Tissues Examined		
adrenals	adrenals	heart	prostate
brain	aorta	ileum	rectum
gonads	blood smear	jejunum	salivary gland
heart	bone	kidneys	seminal vesicle
kidneys	bone marrow	lacrimal gland	skin
liver	brain (3 levels)	liver	spinal cord (cervical
spleen	cecum	lungs	thoracic, lumbar)
thyroid	colon	lymph nodes	spleen
(w/parathyroid)	duodenum	mammary gland	sternum
	epididymes	muscle (smooth)	stomach
	eyes	muscle (skeletal)	testes
	eyes (optic nerve)	nerve	thymus
	gall bladder	(peripheral)	thyroid (w/parathyroid)
	Harderian glands	oesophagus	trachea
	head - 3 sections (nasal	ovaries	urinary bladder
	cavity, para-nasal	pancreas	uterus
	sinus, tongue, oral	pituitary	vagina
	cavity, naso-pharynx,		zymbal's gland
	inner-ear)		gross lesions