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FOR AGRICULTURAL AND VETERINARY CHEMICALS
AUSTRALIA**

EXISTING CHEMICALS REVIEW PROGRAM

REVIEW OF THE MAMMALIAN TOXICOLOGY

AND

METABOLISM/TOXICOKINETICS

OF

DIAZINON

prepared by

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ABBREVIATIONS ABBREVIATIONS ABBREVIATIONS ABBREVIATIONS ABBREVIATIONS ABBREVIATIONS 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/day	mg/kg bodyweight/day
IV	Intravenous	ppb	Parts per billion
PO	Oral	ppm	Parts per million

ADI	Acceptable Daily Intake
A/G	Albumin/globulin ratio
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
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
MTD	Maximum Tolerated Dose
NOEL	No Observable Effect Level
OP	Organophosphorus pesticide
PEG	Polyethylene glycol
2-PAM	Pyridine-2-aldoxime methiodide
SU	Somogyi Units (amylase activity)
TGAC	Technical Grade Active Constituent
WBC	White blood cell/leucocyte
ACPH	Advisory Committee on Pesticides and Health
NHMRC	National Health and Medical Research Council
NDPSC	National Drugs and Poisons Scheduling Committee
NRA	National Registration Authority for Agricultural and Veterinary Chemicals

TOXICOLOGY HAZARD PROFILE

Absorption, distribution, metabolism and excretion in mammals	
Rate and extent of absorption	Oral: Almost complete with only 3% in faeces and a proportion of this being derived from biliary excretion. Bioavailability=35.5%
Distribution	Highest tissue concentrations were found in fat
Potential for accumulation	No evidence of accumulation
Rate and extent of excretion	Rapidly excreted (mainly) in urine ($t_{1/2}$ in plasma=2.9 h)
Metabolism	Well metabolised (at least 2 major metabolites in urine)
Toxicologically significant compounds (animals, plants and environment)	Parent compound & degradation products (O,S-, O,O-, S,S-TEPP)

Acute toxicity	
Rat oral LD ₅₀ (mg/kg bw)	76 – 466 (without stabiliser) 300 - 1350 (with stabiliser)
Worst oral LD ₅₀ in other species	82 in mice (without stabiliser)
Rat dermal LD ₅₀ (mg/kg bw)	200 - 900 (without stabiliser) 876 - >2150 (with stabiliser)
Worst dermal LD ₅₀ in other species	960 in rabbit (with stabiliser)
Rat inhalation LC ₅₀ (mg/m ³)	3500 (without stabiliser) 3100 - >5437 (with stabiliser)
Worst inhalation LC ₅₀ in other species	630 mg/m ³ in mice (without stabiliser)
Skin irritation	Slight irritant (with stabiliser)
Eye irritation	Slight irritant (with stabiliser)
Skin sensitization	Sensitizer (with stabiliser - Magnusson & Kligman Test)
T-value	7 (without stabiliser)

Degradation products		
Rat oral LD ₅₀ (mg/kg bw)		
Diazinon (with stabiliser) – non degraded	1350 (M), 1160 (F)	All performed in the same laboratory
O,O-TEPP	0.95 (M), 0.66 (F)	
O,S-TEPP	0.71 (M), 0.46 (F)	
S,S-TEPP	>10 (M), 3.5 (F)	

Short-term toxicity	
Target/critical effect	Plasma ChE inhibition
Lowest relevant oral NOEL (mg/kg bw/day)	0.02 (4-week rat feeding study)
Lowest relevant dermal NOEL (mg/kg bw/day)	No NOEL was established in a 21-day rabbit study. LOEL=1 mg/kg bw/day
Lowest relevant inhalation NOEL	0.05 (21-day rat nose-only exposure)

(mg/m ³)			
Genotoxicity	Non-genotoxic		
Long-term toxicity and carcinogenicity			
Target/critical effect	Plasma ChE inhibition		
Lowest relevant NOEL (mg/kg bw/day)	0.0037 – 1-year dog study 0.004 – 2-year rat study		
Carcinogenicity	No evidence of oncogenic potential		
Reproductive toxicity			
Reproduction target/critical effect	Minor effects (bodyweight loss) at parental toxic doses		
	Reduced pup survival during lactation		
Developmental target/critical effect	Minor effects (skeletal development) at maternotoxic doses		
Lowest relevant developmental NOEL	20 mg/kg bw/day		
Delayed neurotoxicity	No effects		
Immunotoxicity	No effects		
Dermal absorption	Less than 4% for diazinon. Markedly more for the degradation products (see discussion for details)		
Summary	NOEL (mg/kg bw/day)	Study	Safety factor
Current ADI (0.001 mg/kg bw/day) [Plasma ChE inhibition]	0.1	92-day feeding rat	100
New ADI (0.001 mg/kg bw/day) [Plasma ChE inhibition]	0.02	37-43 day human (capsule)	20 (safety factor increased 2-fold to account for closeness of NOEL and LOEL)
ARfD (acute reference dose)	0.005 mg/kg based on inhibition of RBC ChE activity in a 13 week human study with an LOEL of 5.0 mg/kg BW.		

[Note: Inhibition of erythrocyte and brain ChE activities are reported in the main toxicology report]

SUMMARY

Introduction

Diazinon is the common name for *O,O*-diethyl-*O*-(2-isopropyl-6-methylpyrimidin-4-yl)-phosphorothioate (IUPAC nomenclature), a contact organophosphorus insecticide with a broad range of insecticidal activity. It is effective against juvenile and adult forms of flying insects, crawling insects, acarians and spiders. Diazinon has been used in Australia for over 30 years and currently has over 100 products with over 1250 approved uses in Australia. Maximum residue limits (MRLs) have been set for diazinon in a wide range of crops and animal tissues. Diazinon is presently in Schedule 6 (in Schedule 5 for dust preparations containing 2% or less of diazinon) of the Schedule for the Uniform Scheduling of Drugs and Poisons (SUSDP). Following the removal of Appendix L from the SUSDP in 1994 which specified a maximum impurity limit of 2 g/kg for S,S-TEPP (sulfoTEPP) only, the NRA now routinely uses the FAO standard to establish impurity limits. In the current FAO specification (15/TC/S, 1988) for stabilised diazinon, the maximum permissible levels for the two main toxic impurities are 2.5 g/kg for S,S-TEPP and 0.2 g/kg for O,S-TEPP. The current Australian Acceptable Daily Intake (ADI), set by the NH&MRC in 1987, is 0.001 mg/kg bw/day.

Diazinon has been reviewed by the Joint FAO/WHO Meeting of Pesticide Residues (JMPR) in 1963, 1965, 1966, 1967, 1968, 1970 and 1993. In 1965, the JMPR established an ADI for diazinon of 0.0002 mg/kg bw/day based on plasma ChE inhibition in a 43-week dog study. In 1966, the ADI was amended to 0.002 mg/kg bw/day based on a NOEL of 0.02 mg/kg bw/day for inhibition of plasma ChE activity in a human 37-43 day study. The most recent review in 1993, that incorporated the change in JMPR policy to use inhibition of brain ChE (or erythrocyte ChE inhibition as a surrogate) as the toxicologically-relevant endpoint, has the same ADI based on the NO(A)EL in a different human study that detected significant plasma ChE inhibition at 0.025 mg/kg bw/day but with no corresponding ChE inhibition in erythrocytes.

Toxicokinetics and Metabolism

Intravenous Administration

Diazinon (10 mg/kg bw) administered IV to 16 male Wistar rats, followed by plasma sampling at various times up to 8 h after dosing, was rapidly cleared ($Cl = 4.69$ L/h/kg) resulting in a half life of 4.7 h in plasma. The volume of distribution at steady state was calculated to be 20.01 L/kg and the area under the curve, $AUC(0-\infty)$, was 2.27 mg.h/L. Thus, clearance was approximately equivalent to the hepatic blood flow in rats, however, the influence of differing doses on the linearity of these toxicokinetic parameters was not investigated. The extent of hepatic extraction, measured by simultaneous sampling the diazinon concentration in the carotid artery and hepatic vein after an intravenous injection into the left jugular vein, was 54.8% and 47.7% with a dose of 5 and 10 mg/kg bw respectively. The unbound fraction in plasma, determined by ultrafiltration over the range of 0.4-30 μ g/mL, was 10.9% (Wu et al, 1996).

Oral Administration

Diazinon administered to 16 male Wistar rats by stomach tube at a dose of 80 mg/kg bw was detected in blood by GC (fitted with an electron capture detector) after 30 min (first sampling time), with the maximum concentration (C_{max} 1.22 μ g/mL) being observed at 2 h (T_{max}). The

plasma AUC(0-∞) for oral dosing was 6.44 mg.h/L, clearance was 4.60 L/h/kg, and the volume of distribution was rather large at 22.93 L/kg. The apparent elimination rate was 0.25 h⁻¹, corresponding to a half life in plasma of 2.86 h. Oral bioavailability was calculated to be 35.5% and the oral clearance was 12.93 L/h/kg, suggesting a substantial hepatic first-pass uptake effect (Wu et al, 1996).

Percutaneous Absorption

Radiolabelled diazinon in tetrahydrofuran applied to a shaved dorsal skin area on Sprague-Dawley rats at a dose of 1 or 10 mg/kg bw was almost fully recovered in tissues, excreta and as volatiles in cage air (96.3-101.5%) after 144 h. Most of the applied radioactivity was detected in the urine (69-81%), followed by volatiles in cage air (17.7% at 1 mg/kg bw and 10.3% at 10 mg/kg bw), faeces (7.45% and 5.3% at 1 and 10 mg/kg bw respectively) and tissues (0.3% for both doses). Radioactivity detection in the tissues revealed that at 10 mg/kg bw, rats had up to 5.8% of the applied dose present in their stomach after 2 h, suggesting probable ingestion of some of the dermally applied dose (Williams & Marco, 1984). Given the rapid rate absorption of diazinon from the GI tract after an oral dose, it was not possible to exclude the possibility that some or all of the rats treated at the lower dose of 1 mg/kg bw also ingested a portion of the applied dose. Therefore calculation of any meaningful dermal absorption rates for OH&S purposes was not possible.

Diazinon (20%, w/v), the active ingredient in the product 'Dimpylate 20% Spot On', applied to a dorsal neck skin region of dogs at 20 mg/kg bw (as the product) was detected in the blood within 1 h. The maximal mean concentration of diazinon (C_{max}=19 ng/mL) was achieved after 8-12 h in 5/6 dogs. Probably owing to the large time intervals between blood sampling, the kinetic profiles were erratic so the half life in plasma could not be readily calculated for most dogs. However, in two dogs where the kinetics appeared somewhat less erratic, values of 25 and 84 h were calculated (Ferrandes, 1990).

During daily dermal application of ring-labelled [¹⁴C]diazinon (~ 40 mg/kg bw) in acetone to sheep for 3 days it was noted that the volatility of the preparation precluded an accurate assessment of the applied dose. However, quantitation and characterisation of the metabolites revealed that the highest concentration of radioactivity was present in the kidneys (13.3 µg eq/g). Other major sites of accumulation were in fat (11.3 µg eq/g), heart (6.4 µg eq/g), liver (5.9 µg eq/g) and leg muscle (2.2 µg eq/g). Apart from kidney and liver where diazinon metabolites predominated, most of the radioactivity (56-85%) present in these tissues was associated with unchanged diazinon (Capps, 1990 with an amendment by Carlin, 1994).

Tissue Distribution

In male ddy mice, kidney, liver and brain concentrations of diazinon after IP administration of 20 or 100 mg/kg bw were higher than in the blood (no other tissues examined). The calculated T_{1/2} in plasma for unchanged diazinon was approx. 2.5 h and the highest concentration was observed in the kidneys. In tissues other than kidneys, the concentration had declined to ≤ 8% of the peak concentration (T_{max} ~ 1 h) 8 h after dosing. Maximal tissue concentrations in kidney, liver and brain were 45, 20 and 2.5-fold respectively greater than that in blood, ie. 750 and 100 ng/mL for 20 and 100 mg/kg bw respectively at T_{max} (Tomokuni et al, 1985).

Similar kinetics occurred in male Wistar rats after IP dosing with 20 or 100 mg/kg bw; the maximal concentration of diazinon in blood was observed after 1-2 h and the half life was approximately 3 h. However, the diazinon concentration in the liver 8 h after dosing at 100 mg/kg bw was approximately 14-fold lower than in the blood (165 ng/mL) whereas brain and kidneys were about 3- and 37-fold respectively higher (Tomokuni et al, 1985 & Tomokuni & Hasegawa, 1985).

Consistent with a large volume of distribution, large hepatic first-pass effect and rapid elimination, more diazinon was found in the kidneys (0.95 µg/g), liver (0.47 µg/g) and brain (0.45 µg/g) than in plasma (0.17 µg/mL) 2 h after intravenous administration to male Wistar rats (Wu et al, 1996).

Unlabelled diazinon (dose not specified although was said to be 1/3 of an oral LD₅₀) administered to male Wistar rats by gavage and measured until complete elimination had been achieved (after 20 days), revealed that the rate of decline in the concentration of unchanged diazinon in plasma mimicked that observed in adipose tissue, muscle, brain and liver (the only tissues examined). After 4 days the tissue:blood ratio was 0.62, 0.13, 0.12 and 0.04 for adipose tissue, muscle, brain and liver respectively (Garcia-Repetto et al, 1995). A rat chronic feeding study summary also indicated some accumulation of unchanged diazinon in the fat (Hazleton Labs, 1955).

Radioactivity associated exclusively with unchanged diazinon was found (by paper chromatography) predominantly in perirenal fat (81 µg eq/g), pericardial fat (39 µg eq/g), adrenals (31.2 µg eq/g) and hind leg muscle (20.6 µg eq/g) in a dog given [³²P]diazinon by capsule 10 h earlier. Although slightly more radioactivity was present in the blood (86.6 µg eq/mL) than in perirenal fat, the ratio of unchanged diazinon to diethylphosphorothioate and diethylphosphate was not determined. Curiously, the lowest levels of radioactivity were found in kidney (3.2 µg eq/g) and brain (3.3 µg eq/g) despite there being extensive urinary excretion of diethylphosphorothioate and diethylphosphate (694 µg eq/mL, combined radioactivity) 5 h after dosing (Millar, 1963).

Oral administration of ring-labelled [¹⁴C] diazinon (150 mg/day) in gelatin capsules for 4 consecutive days resulted in substantial levels of radioactivity being present in the kidney (2.0 µg eq/g), liver (1.2 µg eq/g), leg muscle (0.29 µg eq/g), tenderloin (0.28 µg eq/g), omental fat (0.26 µg eq/g) and perirenal fat (0.23 µg eq/g) of lactating goats 24 h after the last dose. At the corresponding time the mean concentration in blood was 0.36 µg eq/mL. In the kidney, liver, leg muscle and tenderloin, most of the radioactivity was associated with diazinon metabolites whereas in omental and perirenal fat, unchanged diazinon accounted for between 64% and 68% of the detected radioactivity (Pickles; 1988, Simoneaux, 1988a, b, c).

Unlabelled diazinon (1000 mg/kg bw) administered to sheep by stomach tube accumulated in fat (624 µg/g) at 127-fold the concentration found in blood (4.9 µg/mL), 48 h after dosing. Liver (18 µg/g), brain (15 µg/g), muscle (14 µg/g) and kidney (12 µg/g) concentrations of unchanged diazinon were also greater than that found in blood. The only metabolite measured, namely hydroxydiazinon appeared to accumulate in these tissues in the same approximate ratio relative to blood but at about a 7-fold lower concentration (Janes et al, 1973).

Claborn et al (1963) found that after 16 weekly sprayings with solutions of 0.05% or 1.0% diazinon, omental fat in Hereford cattle had detectable levels of diazinon (up to 0.75 µg/g) that gradually declined so that 14 days after cessation, the levels were undetectable (<0.05 µg/g).

Excretion

Although about 70% of the total radioactivity found in the urine of rhesus monkeys was excreted during the first 24 h after intravenous dosing, faecal excretion of radioactivity continued at a steady rate over a 7-day monitoring period suggesting a enterohepatic recirculation (Wester et al, 1993). Biliary excretion is also an important elimination pathway in rats; individual [$2\text{-}^{14}\text{C}$] labelled metabolites, G27550, GS31144 & i (see Figure 1, section 2.2 for structures), injected intravenously (to ascertain the formation sequence) were excreted in faeces (7-16% of injected dose) and urine (Mücke et al, 1970).

After oral dosing, diazinon and its metabolites were mainly excreted in the urine in all species tested, namely rat, dog, goat and cow. Capps (1989) reported that rats excreted 95.7-96.6% of a single 10 or 100 mg/kg bw dose or after preconditioning with 10 mg/kg bw/day for 14 days within 24 h. An earlier study by Mücke et al (1970) reported slightly lesser quantities in urine after a 4 mg/kg bw dose, ie. 75%. Most of the remaining dose in each study was excreted in faeces, ie. 2.5-2.8% and 20% for the Capps and Mücke, et al studies respectively. Owing to the further metabolism of the cleaved diethylphosphorothioate group from diazinon to carbon dioxide, there was less radioactivity found in both faeces (17.5%) and urine (65.4%) for the ethoxy-labelled diazinon (Mücke et al, 1970). For doses ranging between 4-20 mg/kg bw, dogs, goats and cows excreted 85%, 64% and 74% of the administered dose respectively in the urine (Iverson et al, 1975; Simoneaux, 1988a, b, c; Robbins et al, 1957).

Radioactivity excreted in goat milk accounted for 0.31% of the administered dose (150 mg/day for 4 days) with the mean concentration ranging between 0.33 and 0.46 $\mu\text{g eq/mL}$. This concentration was similar to that found in whole blood, namely 0.36 to 0.43 $\mu\text{g eq/mL}$ (Pickles, 1988; Simoneaux, 1988a, b, c). In the lactating cow, approximately 0.16% of a single 20 mg/kg bw oral dose was excreted in the milk over 168 h. During the first 24 h after dosing, approximately a quarter to a third of the radioactivity in milk appeared to be associated (co-migrated) with unchanged diazinon on paper chromatography (Robbins et al, 1957).

Metabolite Profiles in Rat, Dog, Goat and Sheep Excreta, and in Goat Milk

The low amounts of radioactivity associated with unchanged diazinon in urine and faeces indicate that diazinon is extensively metabolised in rats, dogs, goats and sheep. Five important conversion processes for diazinon have been identified (see Figure 1, section 2.2 for pathway and chemical structures). These involve:

- transformation of the P-S moiety to the P-O derivative (ie. formation of O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphate, ie. metabolite G24576 or diazoxon)
- multifunction oxidase/hydrolase mediated cleavage of the ester bond leading to the hydroxy pyrimidine (2-isopropyl-6-methyl-4(1H)-pyrimidone, ie. metabolite G27550)
- oxidation of the methyl substituent leading to the corresponding alcohol (metabolite vii in Figure 1)
- oxidation of the isopropyl substituent leading to the corresponding tertiary alcohol (O,O-diethyl O-[2-(α -hydroxyisopropyl)-4-methyl-6-pyrimidinyl] phosphorothioate, ie. metabolite CGA14128)
- glutathione mediated cleavage of the ester bond leading to the glutathione conjugate (eg. glucuronide conjugate of G27550, ie. metabolite vi)

Significant amounts of the pyrimidinol derivative, G27550 are oxidised at the isopropyl moiety thereby giving rise to the hydroxy derivatives, GS31144 & i. This was demonstrated by injecting these individual [2'-¹⁴C]-labelled metabolites intravenously in rats. Apart from some conversion to several uncharacterised aqueous polar metabolites for GS31144 (approx. 17% of recovered radioactivity in urine), GS31144 & i were excreted unchanged whereas G27550 was excreted in combination with approximately equal amounts of GS31144 & i. These results have been substantially confirmed in a recent study by Capps (1989). The absence of any exhaled radioactive carbon dioxide after oral administration of [2'-¹⁴C] diazinon in rats suggests that no pyrimidine ring cleavage occurs. By contrast, approximately 6% of the recovered radioactivity was found in expired air after ingestion of ethoxy-labelled diazinon, indicating that only a small proportion of the diethylphosphorothionate and diethylphosphate, formed after esterase activity and excreted predominantly in urine, is further metabolised (Mücke et al, 1970).

Efficient cleavage of the ester bond was also observed in a lactating cow orally dosed with [³²P]diazinon. Approximately 70% of the radioactivity excreted in urine was found to be in the form of diethylphosphorothionate and diethylphosphate (Robbins et al, 1957). The same two urinary metabolites were also observed in a dog given [³²P] diazinon orally by capsule (Millar, 1963).

Some minor metabolic pathways were apparent in sheep given large oral doses of diazinon or with *in vitro* liver microsome preparations where metabolites retained the pyrimidinyl phosphorus ester bond. Janes et al (1973), administering a large oral dose of unlabelled diazinon (1000 mg/kg bw by gavage) to sheep that caused frank toxicity, detected 3 major esterified phosphorothionate metabolites in urine after 72 h as judged by co-migration with control standards on TLC and their reactivity with esterases. Spectroscopic techniques, ie. NMR, mass and infrared, confirmed their identity as hydroxydiazinon (CGA14128), isohydroxydiazinon (vii) and dehydrodiazinon (v). By contrast, using much smaller doses of approximately 40 mg/kg bw, only ester cleaved diazinon metabolites were detected in the urine of sheep 6 h after dermal exposure (Capps, 1990).

Liver microsome preparations from adult sheep of various breeds catalysed the *in vitro* production of several fully esterified metabolites from unlabelled diazinon (23-33 µM), ie. in rate order, isohydroxydiazinon (vii) > hydroxydiazinon (CGA14128) >>> dehydrodiazinon (v) > G24576 (diazoxon) > (ii). Other species such as rat, guinea pig, turkey, chicken, and pig had differing formation rates for these metabolites but with the exception of the chicken no other metabolites were formed; metabolite viii was formed in chickens (Machin et al, 1975). In another *in vitro* study, analysis of the metabolites formed during the incubation of rat microsomes with [ethoxy-¹⁴C]diazinon suggested that diazoxon (G24576) was rapidly produced and subsequently hydrolysed to diethylphosphate as determined by Dowex ion exchange chromatography analysis (Nakatsugawa et al, 1969). This result was essentially confirmed by Yang et al (1971), where the incubation of rat liver microsomes with [ethoxy-¹⁴C] diazinon gave rise to both diethylphosphate and diethylphosphorothioate whereas with [ethoxy-¹⁴C] diazinon only diethylphosphate was formed. No direct evidence of any desethylation was found.

Radioactivity in goats milk was predominantly associated with metabolites GS31144 and G27550 and they collectively accounted for about 77% of the total radioactivity found in the milk (Pickles, 1988; Simoneaux, 1988a, b, c).

Acute Toxicity

The acute toxicity of technical diazinon in mammals is moderate. The oral LD₅₀ of stabilised diazinon in rats ranged from 300 to 1350 mg/kg bw in a variety of vehicles. Non-stabilised diazinon, ie. in pre-1969 studies, also had moderate acute oral toxicity (LD₅₀ ranging between 76 - 466 mg/kg bw).

The acute dermal toxicity of stabilised diazinon was moderate in rats (LD₅₀ 876 - >2150 mg/kg bw) and rabbits (LD₅₀ 960 - 3500 mg/kg bw).

The acute inhalation toxicity of stabilised diazinon was low in rats. Whole-body inhalation toxicity (LC₅₀) ranged between 3100 and 4370 mg/m³, whereas for nose-only exposure the lethal concentration was in excess of 5437 mg/m³. In mice, death from whole-body inhalational exposure to stabilised diazinon occurred at a markedly lower concentration relative to rats, ie. the LC₅₀ was determined to be 1600 mg/m³.

Signs of acute toxicity (oral, dermal, inhalation, IP) were those typically seen in organophosphate intoxication and included muscarinic effects (diarrhoea, salivation, pupil constriction), nicotinic effects (muscle fasciculations and fatigue) and CNS effects (ataxia, convulsions).

Technical diazinon was a slight eye and skin irritant. Similarly, there was evidence of skin sensitisation.

The acute oral toxicity of formulated products varied, with LD₅₀s ranging from 293 to >5050 mg/kg bw. Dermal toxicities were generally low (>1000 mg/kg bw). Microencapsulated formulations also have very low acute oral (LD₅₀ >5000 mg/kg bw) and low dermal toxicity (LD₅₀ >2000 mg/kg bw). Generally, the products were slight eye and skin irritants and did not sensitise skin. However, there was an exception in which severe ocular irritation was observed with one product. This product is not currently registered in Australia.

Effect of Ageing on Acute Toxicity

During the late 1950s and early 1960s it became apparent that 'aged' technical diazinon could cause laboratory animal deaths at lower concentrations than had been observed using freshly prepared diazinon TGAC or products. Further investigation into the cause of this increased toxicity indicated that the presence of a small volume of water and oxygen promoted the formation of diethylphosphorothionate, which in turn was further hydrolysed to diethylphosphate (Margot & Geysin, 1957). However, these intermediates are able, under catalytic influence of poorly characterised factors, to combine with itself or the other to form highly toxic tetraethylpyrophosphate (O,O-TEPP or TEPP), monothiono-tetraethyl pyrophosphate (O,S-TEPP) or dithiono-tetraethyl pyrophosphate (S,S-TEPP or sulfoTEPP). Median lethal dose studies, performed in 1989 but not reported until 1995, have confirmed the markedly increased toxicity of these 3 compounds in female rats, ie. 0.66 mg/kg bw for O,O-TEPP, 0.46 mg/kg bw for O,S-TEPP and 3.48 mg/kg bw for S,S-TEPP (Kuhn, 1995a, b, c). Many of the median lethal dose studies performed on diazinon before 1969, when this marked propensity for degradation became well known, were generally much lower than found in later studies using a stabilised formulation (see Table in section 3.1.1, ie. Bruce et al, 1955; Gaines, 1960, 1969; Boyd & Carsky, 1969).

Formation of O,O-TEPP, O,S-TEPP and S,S-TEPP has been claimed to be reduced by the addition of a stabiliser, epoxidised soybean oil, immediately after synthesis of the TGAC (Spindler, 1969; Sterling, 1972). However, a 1993 survey sponsored by the NRA found that among a random sample of 157 unopened off-the-shelf liquid diazinon products available from retail outlets throughout Australia, 26 (or 16.5%) contained some S,S-TEPP, and 13 (or 8.3%) of these exceeded a benchmark impurity limit of 2 g/kg a.i. (for S,S-TEPP). All 7 (or 4.4%) of the batches that were found to contain O,S-TEPP also exceeded the FAO benchmark impurity limit of 0.2 g/kg a.i. established for O,S-TEPP. The third potential impurity in diazinon, namely tetraethylpyrophosphate (O,O-TEPP), was not quantified due to the absence of an appropriate reference standard. Five of the 7 batches in which O,S-TEPP was detected also contained S,S-TEPP in excess of the benchmark limit. Hence, a total of 15 batches (or 9.5%) exceeded either one or both impurity limits. Although there did not appear to be a clear relationship between water content and the degree of degradation found in the samples tested, it was suggested that there still may be a link between the integrity of the container (to prevent introduction of moisture) and the initiation of diazinon degradation. The number of tested batches that were found to exceed the label-stated diazinon concentration by $\pm 10\%$ was 35 (or 22%) (McDonald, 1993, 1994; Allender & Britt, 1994).

The acute oral LD₅₀ in rats (strain and source not specified) of HPLC-purified diazinon, 90% technical diazinon or 90% technical diazinon stored for 1 year was 470, 170 and 30 mg/kg bw respectively. The composition of 'new' and 'aged' 90% diazinon used in this study is shown in Appendix I and highlights increases in sulfoTEPP (4x) [S,S-TEPP], TEPP (7x) [O,O-TEPP] and isodiazinon (19x) concentration with ageing. The acute oral LD₅₀ of pure isodiazinon was 65 mg/kg bw and together with sulfoTEPP and TEPP may have been responsible for the increased toxicity of 'aged' diazinon (Nichol et al, 1982).

SulfoTEPP (S,S-TEPP) in 3 commercial and 3 'military' diazinon formulations, ie. as a dust, EC and in an oil solution, was detected by GC/MS. Although the percentage of sulfoTEPP ranged between 0.2-0.71%, the lowest levels were detected in the dust formulations. On the basis that the oldest EC formulation tested had one of the lower levels of sulfoTEPP, it was reasoned that the formulation age was not a useful predictor for the estimating sulfoTEPP content in formulations. The authors concluded that sulfoTEPP was most likely to be formed during the synthesis of diazinon with diethyl thiophosphorylchloride (Meir et al, 1979).

Several different diazinon formulations (EC, LC and dust) at various strengths and available in Canada were tested by GC to determine their S,S-TEPP content. The maximum content found in these products was 0.53% and there was no apparent relationship between the date of manufacture and the S,S-TEPP content (Turle & Levac, 1987).

Antidote Studies.4

The effects of atropine-oxime therapy on ChE activity after acute diazinon poisoning were investigated in rats and rabbits. Rats treated with atropine and 2-PAM demonstrated significant reactivation of diaphragm ChE levels, ie. 45% and 35% after IV and oral administration respectively relative to 11% in controls. In rabbits, 2-PAM administration resulted in a reactivation of inhibited blood ChE activity with a concurrent decrease in signs of toxicity. However, clinical signs and blood ChE inhibition reappeared within 2 h of 2-PAM administration (Harris et al, 1969).

2-PAM administration alone was effective in treating mild and moderate diazinon intoxication in cattle, sheep and goats. In severe intoxication, 2-PAM alone was ineffective for the immediate relief of poisoning effects, whereas combination therapy with atropine was effective (Younger & Radeleff, 1964).

Enzyme Activity Effects

Technical diazinon administered by intraperitoneal injection to 8 female Wistar rats at a dose of 40 mg/kg bw resulted in tremors and convulsions. Treated rats, sacrificed after these effects became maximal, ie. after 2 h, had significantly reduced ($p<0.05$) ChE (by 57%) activity but elevated fructose 1,6 diphosphatase activity (by 28%) in the brain relative to a corresponding number of untreated controls. Brain phosphoenolpyruvate carboxykinase or glucose-6-phosphate dehydrogenase activities measured at the same time were unchanged. Glycogen content in the brain was also significantly reduced (by 30%; $p<0.05$) after treatment together with a significant rise in lactate (by 40%; $p<0.05$) concentration. Pyruvate concentrations appeared unchanged whereas blood glucose was elevated (by 50%; $p<0.01$). It was speculated that these changes were a compensatory mechanism to provide extra energy to cerebral tissue as a result of the stimulatory effects of diazinon (Matin & Husain, 1987).

Very little regional variation (ie. cerebellum, cerebral cortex, striatum and hippocampus) in the degree of ChE inhibition was apparent in the brain of Sprague-Dawley rats following exposure to technical diazinon at 0, 2.5, 150, 300, or 600 mg/kg bw. Significant ChE inhibition ($>28\%$) occurred throughout the brain and spinal cord at doses in excess of 2.5 mg/kg bw. In contrast, significant ChE inhibition was observed in plasma at the lowest tested dose of 2.5 mg/kg bw but not in erythrocytes where significant inhibition became apparent at the next higher dose of 150 mg/kg bw. Irrespective of its location (brain, spinal cord, plasma or erythrocytes), ChE activity became inactivated (significantly) after 3 h but the maximum inactivity was observed 9 h after dosing. Little or no recovery of ChE activity was evident 24 h after dosing (Potrepka, 1994).

A single application of Dotton Flea Control (Dimpylate 20% Spot On) at 0, 20, 60, or 100 mg/kg bw applied directly to the skin of Beagle dogs caused no deaths, clinical signs, changes in food or water consumption, bodyweight or in any of the measured haematology, clinical chemistry or urinalysis parameters except for reduced ChE activity in plasma. Similarly, there were no treatment-related changes in organ weight, gross pathology, or histopathology (Woehrle, 1990).

Pancreatitis

Two published studies from the same laboratory described the effects of a single dose of diazinon on the canine pancreas, when administered in the absence or presence of secretin. Diazinon injected IV to anaesthetised dogs caused a 2-fold rise in mean intraductal pressure, an increased secretory rate (4-fold), acinar cell vacuolisation, hyperamylasemia (7-fold) and hyperlipasemia (21-fold); effects that were prevented with IV atropine (Dressel et al, 1979). No such effects were observed in cats after acute exposure to diazinon and this was attributed to the paucity of tissue-fixed butyrylcholinesterase in the pancreatic acinar cells and ampullary-sphincteric smooth muscle. In guinea pigs, another species with abundant tissue-fixed butyrylcholinesterase, pancreatic lesions similar to those observed in dogs were found. It was concluded that inhibition of pancreatic butyrylcholinesterase leads to cholinergic hyperstimulation of the acinar cells, resulting in acute pancreatitis (Frick et al, 1987). Enzyme histochemistry of the major and minor

pancreatic sphincters of the dog indicated that diazinon exposure inhibits the activity of butyrylcholinesterase present in periaampullary nerves and pancreatic smooth (sphincter) muscle whereas acetylcholinesterase activity in the periaampullary nerves was only slightly attenuated; this outcome suggests a possible mechanism for pancreatic ductal hypertension (Dressel et al, 1980).

Human pancreatic fragments incubated *in vitro* in the presence of echothiophate (an OP) or acetylcholine had a similar number of acinar cell cytoplasmic vacuoles though both were significantly greater than controls but the mean number of zymogen granules per cell for the combination treatment was substantially less than for controls or for each individual treatment (Goodale et al, 1993).

Short-Term Repeat-Dose Toxicity

Wistar rats fed diazinon in the diet at 2 ppm for 7 days or at 25 ppm for 30 days had no clinical signs although significant ($p \leq 0.05$) inhibition of ChE in plasma and erythrocytes at 25 ppm was apparent in both sexes. Females though, appeared to be more sensitive to ChE inhibition with a significant reduction being observed in the plasma after a 7-day exposure at 2 ppm (equivalent to 0.2 mg/kg bw/day) (Davies & Holub, 1980a).

This gender difference in sensitivity for ChE inhibition was confirmed in a comprehensive investigation that monitored ChE inhibition in blood and in the regional areas of the brain of Sprague-Dawley rats during and following a 28-day dietary exposure at 0.3, 30, 300, or 3000 ppm (equal to 0.02, 2.3, 23 or 213 mg/kg bw/day in males and 0.02, 2.4, 23, or 210 mg/kg bw/day in females). Significant ($p \leq 0.01$) dose-related reductions in erythrocyte and plasma ChE activity were observed at 30 ppm in both sexes, however, significant dose-related ChE inhibition ($p \leq 0.05$) was observed in all tested regions of the brain, ie. cerebellum, cerebral cortex, striatum, hippocampus and thoracic spinal cord, of females at 300 ppm but only in the cerebellum of males. The greater incidence of treatment-related muscle fasciculations among females (14/15) relative to males (3/15) at the highest tested dose of 3000 ppm is additional support for a gender difference in sensitivity (Chang, 1994).

Tif:RAI rats exposed to an aerosol of diazinon in whole-body chambers at concentrations of 151, 245, or 559 mg/m³ for 6 h/day, 5 days/week for 21 days were found to have clinical signs of poisoning (exophthalmus and diarrhoea) and a significant ($p \leq 0.01$) reduction in brain and plasma ChE activity at concentrations ≥ 245 mg/m³. Recovery of diazinon-inhibited ChE activity in blood and brain to control values after 25 days was also demonstrated. Although a significant reduction in brain ChE activity was also observed at the lowest concentration tested, namely 151 mg/m³, this cannot be considered to be biologically plausible due to the absence of a corresponding significant inhibition of blood ChE activity (ie. in plasma and/or erythrocytes). However, another (though nose-only) inhalational study by Hartmann (1990; see below) indicates that brain and blood ChE were inhibited in males and females at concentrations below that used in this study, suggesting that the absence of ChE inhibition in blood at 151 mg/m³ is, for some obscure reason, incorrect (Zak et al, 1973).

A published report described the detection of diazinon in the atmosphere following the application of 36 diazinon-impregnated polymeric strips (25 sq. cm) onto the walls of a room housing Sprague-Dawley rats. The concentration in air gradually increased from 0.32 µg/m³ after 1 day to a maximum of 1.21 µg/m³ after 30 days (no further measurements taken), however, no

corresponding inhibition of ChE activity was detected in either erythrocytes or plasma of the rats during this time (Hinkle et al, 1980).

Wistar rats exposed to an aerosol of diazinon at concentrations of 15, 97, or 710 mg/m³ in whole-body chambers for 6 h/day, 5 days/week for 28 days had clinical signs of cholinergic intoxication (tremors, abnormal respiratory movements, salivation and gasping) at 710 mg/m³ and significant dose-related reductions in erythrocyte and plasma ChE activities at all tested concentrations (ie. 15, 97 and 710 mg/m³). Relative to males, females tended to have more marked clinical signs together with a correspondingly increased degree of ChE inhibition in plasma, erythrocytes and brain. Other changes, possibly related to treatment at the highest concentration of 710 mg/m³, were significantly ($p < 0.05$) reduced total protein (M 6%, F 11%), globulin (M 15%, F 11%), creatinine (M 17%) together with an elevated A/G (M 20%, F had reduced albumin - 11%) and AP (M 25%, F 43%) in plasma (Hardy et al, 1984).

Tif:RAIf rats exposed by the nose-only route to an aerosol of diazinon at concentrations of 0.05, 0.46, 1.57, or 11.6 mg/m³ for 6 h/day, 5 days/week for 21 days had no clinical signs of toxicity but had significant dose-related reductions in brain, erythrocyte and plasma ChE activities in females at concentrations ≥ 0.46 mg/m³. Other findings at higher concentrations that may possibly be related to treatment were reduced serum glucose concentration in males at 1.57 and 11.6 mg/m³ and some erythrocyte parameters, ie. reduced RBC and Hb, and increased MCV in females at 11.6 mg/m³ (Hartmann, 1990).

Diazinon applied with partial occlusion to the skin of New Zealand White rabbits at 1, 5, or 100 mg/kg bw for 5 days/week for 3 weeks caused the death of 4/5 males at 100 mg/kg bw within 6 days. The dose was reduced to 50 mg/kg bw/day on day 7 resulting in a reduction in the number and severity of clinical signs among survivors. Whereas anorexia, hypoactivity, ataxia, fasciculations, muscular hypotonia, tremors, salivation and diarrhoea were apparent at 100 mg/kg bw/day, only anorexia and diarrhoea were observed among survivors at 50 mg/kg bw/day and males at 5 mg/kg bw/day. Well-defined erythema (grade 2) and hyperkeratosis at the application site for all survivors at 50/100 mg/kg bw/day were clear treatment-related effects. Similarly, a significant reduction in platelet count, increased albumin and A/G ratio at 100/50 mg/kg bw/day together with reduced ChE activity in plasma at all doses and in erythrocytes at 100/50 and 5 mg/kg bw/day was observed among females. Irritation of the stomach and reduced ChE activities in plasma and erythrocytes were the major findings for the sole surviving male at 50/100 mg/kg bw/day (Tai & Katz, 1984).

Subchronic Toxicity

A series of 3 published reports from one laboratory described the effects of gavage administered diazinon on catecholamine (noradrenaline, adrenaline, dopamine, and serotonin) concentrations in blood and brain, amino acid neurotransmitter (Asp, Glu, Tau, Gln, and GABA) concentrations in the brain, phosphofructokinase, hexokinase, lactate dehydrogenase, and succinate dehydrogenase activities in the left ventricle, soleus, medial gastrocnemius and plantaris muscles, and ChE activities in the blood and brain, in Wistar rats. Diazinon administration, performed twice weekly with 1.75 mg/kg bw for 28 weeks so that the average daily dose was 0.5 mg/kg bw/day, resulted in an absence of clinical signs, a reduction in bodyweight, elevated dopamine and reduced amino acid neurotransmitter concentrations in the brain, an elevation in serotonin concentration in plasma and hexokinase activity in the plantaris muscle. It was speculated that although ChE activities were reduced in the blood and brain of rats, it did not necessarily reflect the degree of neurological impairment (for humans) and this may be explained by the reduction in

both brain excitatory neurotransmitters, ie. Asp and Glu, and inhibitory neurotransmitters, ie. Tau and GABA (Rajendra et al, 1986; Anthony et al, 1986; Wilkinson et al, 1986).

Technical diazinon fed daily to male Wistar rats at 0.5 or 5 mg/kg bw/day for 26 weeks resulted in no clinical signs but a significantly ($p < 0.001$) reduced bodyweight gain (by approx. 20%), and attenuated ChE activity in blood. Plasma ChE activity was reduced by approximately 70% relative to controls throughout the study at both 0.5 or 5 mg/kg bw/day whereas erythrocyte ChE activity was reduced by more than 20% relative to controls from week 2 at 5 mg/kg bw/day and from week 20 at 0.5 mg/kg bw/day. The erythrocyte ChE activity was reduced to approximately 20% of control values by both doses at the end of the study. Since there was no change in the amplitude of the action potential in EMG examinations throughout the study, it was suggested that measurement of this parameter in rats may not be a useful indicator of exposure to diazinon. Hence, because significant inhibition of plasma and erythrocyte ChE activities were observed at the lowest tested dose of 0.5 mg/kg bw/day, a NOEL for this 26-week feeding study could not be established (Hussain et al, 1981).

In order to increase the accuracy for determining a NOEL based on ChE inhibition in plasma, erythrocytes and brain, diazinon was fed to female Wistar rats in a semi-purified diet at 0, 5, 10, or 15 ppm for 92 days, or 1, 2, 3, or 4 ppm for 42 days, or 0.1, 0.5, 1.0, or 2.0 ppm for 35 days. Female rats were selected for study because a previous short-term study had shown them to be more sensitive than males to ChE inhibition (Davies & Holub, 1980a). There were no clinical signs observed at any dose or duration, and treated rats had no treatment-related changes in food consumption or bodyweight gain. ChE inhibition in the brain did not achieve significance ($p \leq 0.05$) at any dose, whereas significance in erythrocytes was achieved after 42 days at concentrations in excess of 1 ppm, and in plasma after 35 days in excess of 0.1 ppm. Therefore a NOEL for this study can be established at 0.1 ppm (equivalent to 0.01 mg/kg bw/day) based on a statistically significant inhibition of plasma ChE activity at the next higher dose of 0.5 ppm (Davies & Holub, 1980b).

A high incidence of respiratory disease among Sprague-Dawley rats in a colony meant that clinical signs attributable to the presence of diazinon at 0, 0.5, 1, 2, or 4 ppm in the diet for 13 weeks were difficult to assign. Clinical signs associated with the infection were rapid and laboured respiration, wheezing, rough fur, bloody discharge from the eyes and nose. The only sign clearly attributable to treatment occurred at 4 ppm where 2/15 males had slight tremors during the third week of treatment. Food consumption and bodyweight gain were unaffected by treatment. Although statistical significance was not calculated, the presence of a clear dose-response relationship for the inhibition of plasma ChE activity among females suggests that the NOEL for this study is 1 ppm (approx. equivalent to 0.1 mg/kg bw/day) based on substantial inhibition of plasma ChE activity (37%) at 2 ppm (Weir, 1957a).

Diazinon fed to Sprague-Dawley rats in the diet at concentrations of 0, 0.5, 5, 250, or 2500 ppm for 13 weeks caused no treatment-related deaths. Clinical signs that appeared to be treatment-related occurred only at 2500 ppm where soft faeces and a degree of hypersensitivity to touch and sound were intermittently observed throughout treatment in both sexes (10/15 males and 15/15 females; 12/15 males and 15/15 females, affected respectively); aggressive behaviour was also noted in 3/15 males. Bodyweight loss resulting from treatment was observed at 2500 ppm where a reduced weight gain was evident (significant; $p < 0.01$) from day 14 to 42 in males and day 7 to 49 in females, so that at the end of treatment males and females were 6% and 13% respectively lighter than concurrent controls. Although no significant changes in water consumption among groups occurred, food consumption was reduced for rats at 2500 ppm, but only during the first

week of treatment in males (17%; $p < 0.01$) and for the first two weeks in females (31% and 13% respectively; $p < 0.01$). Calculations involving the diazinon concentration in food, the weight of food consumed and the average group midperiod bodyweight, enabled the dosage level in each treatment group to be calculated. For males this was 0.03, 0.3, 15 and 168 mg/kg bw/day respectively whereas for females it was slightly higher at 0.04, 0.4, 19 and 212 mg/kg bw/day.

Ophthalmoscopic examination did not reveal any changes attributable to treatment. The haematological assessment in females revealed dose-dependent changes in erythrocytic parameters (ie. erythrocyte count, 1.3, 1.8, 2.8 and 9.5% respectively; Hb, 1.5, 2, 3.6 and 4.1%; Hct, 1.8, 1.8, 4.4 and 7.7%), although only Hct at 250 ($p < 0.05$) and 2500 ppm ($p < 0.01$) achieved significance. A corresponding significant ($p < 0.01$) increase in reticulocytes (3.3-fold) was also observed among females at 2500 ppm (changes at lower concentrations were not examined). Increased WBC ($p < 0.05$) in females at 2500 ppm and eosinophil count ($p < 0.05$) in males at 0.5 ppm were probably incidental findings as there did not appear to be any dose-response relationship. Clinical chemistry changes were also characterised by a lack of any dose-response relationship. However, there was significant ($p \leq 0.01$) inhibition of ChE activity in the erythrocytes of females and the plasma of males and females at 5 ppm whereas ChE activity in the brain was significantly inhibited only once the diazinon concentration in the diet reached 250 ppm in females and 2500 ppm in males. Apart from a significantly increased specific gravity of the urine (M 2.2%, F 1.6%; $p < 0.01$ for both) at 2500 ppm that was associated with non-significant reductions in urine volume (M 20%; F 17%) and water consumption (M 17%; F 12%), urinalysis was similar among treatment groups.

Macroscopic inspection of organs at necropsy revealed no gross abnormalities, although the absolute (15%; $p < 0.05$) and bodyweight-relative (20%; $p < 0.01$) weight of the liver was significantly increased in females at 2500 ppm. Males in the same group also had increased absolute (4%) and bodyweight-relative (7%) liver weights though both these changes were not significant. Although neither males nor females at 2500 ppm had significantly increased liver weights relative to brain weight, an increase of 6.5% and 12% respectively suggests a physiological adaptation, an assertion consistent with centrilobular hepatocellular hypertrophy observed in 13/15 females at 2500 ppm (and 3/15 at 250 ppm). The only other significant ($p < 0.05$) organ weight change was observed for the body-weight relative increase in weight of the kidneys (12%) in females at 2500 ppm. However, the NOEL for the study was established at 0.5 ppm (equal to 0.03 mg/kg bw/day in males and 0.04 mg/kg bw/day in females) based on significant plasma ChE inhibition in both sexes and erythrocyte ChE inhibition in females at 5 ppm (Singh et al, 1988).

A published report indicated that diazinon fed to groups of 10 male Wistar rats in the diet at concentrations of 0, 1, 5, 25, or 125 ppm (equal to 0.102, 0.44, 2.26, or 11.7 mg/kg bw/day; calculated as a.i.) for 16 weeks resulted in no treatment-related deaths or clinical signs. At the conclusion of the study erythrocyte ChE activity was depressed to 90% and 81% of controls at 1 and 5 ppm respectively. At 25 ppm and 125 ppm, erythrocyte ChE activity decreased to 54% and 20% respectively of the controls after 4 weeks and remained low throughout the remaining study period. Plasma ChE activity was reduced to 83% and 48% at 15 and 125 ppm respectively but remained unchanged at 1 and 5 ppm. At all concentrations, the plasma ChE activity was increasing toward the end of the study period. Brain ChE activity was only marginally reduced (6%) at the highest tested concentration of 125 ppm. Thus, the NOEL can be set at 5 ppm (approximately 0.44 mg/kg bw/day), based on significant inhibition of erythrocyte ChE activity in male rats at 25 ppm (Edson & Noakes, 1960).

Diazinon with Fuller's earth (1% or 10% w/w) administered orally to groups (2/sex) of mongrel dogs in gelatin capsules at 0.02, 0.04 or 0.08 mg/kg bw/day for 6 days/week for 90 days caused no deaths or changes in bodyweight, appetite or behaviour (though none of these parameters were reported with any detail). A pregnant female at 0.04 mg/kg bw/day uneventfully delivered 6 pups on day 26 of the study (although it is unclear as to whether this pregnancy was known prior to the commencement of the study). Another dog at 0.04 mg/kg bw/day had a sore throat while a third was treated with 400000 U of penicillin/streptomycin for a cold. Three of four dogs at 0.08 mg/kg bw/day had respiratory infections; apparently attributable to the rather high incidence of kennel cough in the colony. The maximal mean ChE inhibition in plasma ranged from 28% after 16 days at 0.02 mg/kg bw/day to 38% after 8 days at 0.04 mg/kg bw/day and 62% at day 33 at 0.08 mg/kg bw/day. In erythrocytes, ChE activity was in excess of the pretest value at all doses and all sampling times except for 25% inhibition at 0.08 mg/kg bw/day on day 8. This study is not considered adequate for regulatory purposes for the following reasons: i) low number of dogs per group (2/sex); ii) no concurrent control group; iii) a number of the dogs had respiratory infections; iv) one dog was pregnant (Weir, 1957b).

In a published study designed to monitor plasma and erythrocyte ChE inhibition, diazinon at 0, 0.25, 0.75, or 75 ppm (as a.i.) was fed to mixed-breed dogs [1/sex/group and 5 in the control group (gender not stated)] in their regular ground dog chow for 12 weeks. However, treatment-related effects other than ChE inhibition were not measured or reported. Although the results for plasma and erythrocyte ChE inhibition were presented only as graphs, it appears that for treatment at 0.75 and 75 ppm, activity was significantly inhibited in plasma by up to 35% and 95% respectively. In erythrocytes, the activity was only significantly inhibited (by 55%) at 75 ppm. Recovery in plasma was complete after about 4 weeks whereas the activity in erythrocytes was still less (by about 10%) than control values (Williams et al, 1959).

In a study designed to establish the maximum tolerated dose and a NOEL, diazinon was fed to Beagle dogs (4/sex/group) in the diet at concentrations of 0, 0.1, 0.5, 150, or 300 ppm (equal to 0.0034, 0.020, 5.9, or 10.9 mg/kg bw/day) for 13 weeks. There were no treatment-related deaths, however, reduced bodyweight gain resulting from treatment was observed in females (by 34%) at 150 ppm and in both sexes at 300 ppm (M, 33%; F, 45%). This difference in bodyweight gain was insufficient to cause a significant difference in mean bodyweight at the conclusion of treatment. Similarly, food consumption among treatment groups did not appear to be significantly different from controls.

Ophthalmoscopic examination did not reveal any changes attributable to treatment. Clinical chemistry and haematology changes revealed no dose-related changes and all significant differences observed were transient. However, one change in clinical chemistry that was probably related to treatment involved a reduced serum protein concentration at 300 ppm. Males had reduced albumin concentrations on days 29, 56 and 86 (12%, 16%, 11%) and although significance ($p < 0.01$) was achieved on days 29 and 56, the increased variability at day 86 (ie. SD=0.2 relative to 0.03 for control) suggests that with a greater number of dogs per group significance would have been achieved. This assertion is further supported by the observation that the total protein concentration on days 29, 59 and 86 was significantly ($p < 0.01$) reduced (by 8.4%, 15% and 10% respectively) at 300 ppm and by 1.4% ($p < 0.05$) at 150 ppm on days 29 and 56 of treatment. Clear treatment-related changes were observed for ChE activity in erythrocytes, plasma and brain with significant inhibition ($p \leq 0.01$) being achieved among all dogs at 150 and 300 ppm. Males at 0.5 ppm also had significant ($p \leq 0.05$) ChE inhibition in plasma. Urinalysis revealed a slightly increased specific gravity for both males and females at 300 ppm throughout

treatment, however, these increases only achieved significance at day 92 for females (2.2%: $p < 0.05$) treated at 150 and 300 ppm.

Macroscopic examination of the tissues removed at the terminal kill revealed no changes attributable to treatment. Histopathology identified atrophy of the pancreatic acini in one male at 300 ppm, a result consistent with similar lesions observed after a single dose (25 mg/kg bw) in a published study (see under pancreatitis). Thus, in conclusion, based on the reduced bodyweight gain, 300 ppm in the diet is probably the maximal tolerated dose. However, the NOEL for the study was established at 0.1 ppm (equal to 0.0034 mg/kg bw/day) based on significant plasma ChE inhibition in males at 0.5 ppm (Barnes et al, 1988).

In a published study, diazinon was administered in capsules to Beagle dogs (3/sex/group) at a concentration of 0, 2.5, 5, 10, or 20 mg/kg bw/day for 8 months. Dose selection was based on a dose-ranging study in which survival, necroscopy and histopathology at doses up to 500 mg/kg bw/day were assessed. At a dose of 25 mg/kg bw/day or greater, 1 or both dogs at each of the tested doses died. Necropsy, performed on dogs surviving more than a day (ie. at ≤ 100 mg/kg bw/day), revealed haemorrhage on the dura mater and congestion or haemorrhage of the intestine that ranged from petechial and paint brush haemorrhage of the mucosal and serosal surface to frank haemorrhage on the mucosal surface. Histopathology revealed several minor findings among the 8 examined dogs, however, the major findings were haemorrhage in the colon, and a yellow-brown pigment in the cytoplasm of hepatic parenchymal cells and cortical renal epithelium.

At the highest dose of 20 mg/kg bw/day, all 3 males and a female died or were sacrificed *in extremis* during treatment. The males died on days 14, 24 and 166 and the female on day 19. Reduced food intake, emesis with occasional diarrhoea and body fasciculations was observed among the dogs prior to death or sacrifice. Similar cholinergic signs were noted in a dog (1/6) at 10 mg/kg bw/day during the first 45 days but it survived. Although bodyweight losses occurred in some dogs, the number, dose group and extent were not reported. In view of these deaths and a lack of appetite at 20 mg/kg bw/day, an additional 3 dogs were recruited to determine the effects of emaciation on haematological, biochemical, and pathological parameters. Treatment duration (with diazinon) for these additional dogs was the same as for the original group.

There were no significant changes in any of the measured haematology parameters although there was a marked increase in myeloid elements (100-150 fold) in the dogs that died or were sacrificed. Clinical chemistry changes apart from serum amylase were limited to a male dog that died after 166 days at 20 mg/kg bw/day and a second male that died 5 days before the completion of the study (232 days) following exposure at 10 mg/kg bw/day. Although the extent of the increases were not reported, alkaline phosphatase, lactate dehydrogenase and ornithine carbamoyl transferase concentrations were claimed to be elevated in these 2 male dogs. Markedly increased serum amylase were also observed in these 2 dogs that died as well as in the 3 surviving dogs at 10 mg/kg bw/day and another at 5 mg/kg bw/day (extent and dog gender not reported).

Gross pathological and histopathological changes were observed mainly among dogs treated at 20 mg/kg bw/day where lesions affecting the GI tract and associated organs were evident. In 5 of 6 dogs, marked oedematous thickening of the jejunum or duodenum occurred with one having a ruptured duodenum resulting in peritonitis while yet another had rupture of the pyloric portion of the stomach. Moderate cirrhosis of the liver was observed in 3/6 dogs. The only other probable treatment-related changes occurred in an emaciated dog at 10 mg/kg bw/day in which the thyroids were soft, the pancreas, spleen and testicles had atrophied and the liver had a yellow

fatty appearance. Histopathological examination confirmed atrophic changes in the acinar cells and interstitial fibrosis of the pancreas, and in the pulp of the spleen; spermatogenesis had been completely arrested. The liver had parenchymal atrophy and hepatic cell dissociation. Thus, in the absence of any determination of ChE inhibition, the NOEL can be set at 2.5 mg/kg bw/day based on an elevated amylase activity in the serum at the next higher tested dose of 5 mg/kg bw/day.

In the second part of this published comparative study, diazinon was administered in capsules to Hormel-Hanford pigs (3/sex/group) at concentrations of 0, 1.25, 2.5, 5, or 10 mg/kg bw/day for 8 months. Dose selection was based on a dose-ranging study in which survival, necroscopy and histopathology of 4 pigs (2/sex) at doses up to 25 mg/kg bw/day were assessed. Groups were reduced to one male at 50 and 100 mg/kg bw/day, and two for each at 300 (1/sex) and 500 (2 males) mg/kg bw/day. At a dose of 25 mg/kg bw/day or greater, pigs at each of the tested doses either died or were sacrificed *in extremis*, except for one in the 500 mg/kg bw/day group that survived treatment. Necropsy on pigs treated at 5, 10, 25, or 50 mg/kg bw/day, revealed similar lesions as for dogs (see above), ie. haemorrhage on the dura mater and congestion or haemorrhage of the intestine that ranged from petechial and paint brush haemorrhage of the mucosal and serosal surface to frank haemorrhage on the mucosal surface. Additionally haemorrhage of the heart, large fatty areas in the pancreas and congestion of the stomach were observed. Histopathology revealed several minor findings probably unrelated to treatment, however, a major finding that was related to treatment was the marked depletion of rib marrow cells in 6 of 8 pigs in which sections were prepared and examined.

In the main study at the highest dose of 10 mg/kg bw/day only 1 female survived treatment. The other 5 pigs in the group that died on days 12, 16, 20, 25, and 38 had characteristic cholinergic signs prior to death, though there was no apparent relationship between time to onset of these clinical signs and time to death. The surviving pig, although having similar signs for 15 days, was remarkable by virtue of the delayed onset, ie. after 4 months of treatment. At 5 mg/kg bw/day there were no deaths and only 1 pig had signs that persisted for the first 6 months of treatment, whereas at 2.5 mg/kg bw/day the only pig that developed clinical signs after 19 weeks was sacrificed *in extremis* during week 20. In 4 of the 5 pigs that died at 10 mg/kg bw/day, oedematous thickening of the jejunum wall was observed, and 1 pig had localised mucosal erosion that penetrated into the muscular layers resulting in a marked mucosal seepage throughout the intestines. Three of the 5 pigs had also formed ulcers in the duodenum and all had somewhat firm livers that were apparently difficult to cut. This liver hardening was probably in part the result of interlobular connective tissue thickening in 3/6 pigs at 20 mg/kg bw/day as revealed by histopathological examination. For the sacrificed pig at 2.5 mg/kg bw/day, ascites harvested from its abdominal cavity clotted after exposure to air; it also had a mottled liver. It is unclear though as to whether the thickening of the interlobular connective tissue and lobular congestion observed during histopathological examination in 1 pig were associated with this sacrificed pig (no individual animal data).

Haematological parameters were normal except for a slight elevation in the myeloid:erythroid ratio (2-fold) and a reduced reticulocyte count (4-fold) among the pigs that died at 10 mg/kg bw/day. Apparently (though not reported in detail) erratic elevated levels of ornithine carbamoyl transferase, creatinine phosphokinase and amylase were observed in some pigs from all treatment groups. Noteworthy gross pathological changes in pigs treated at 5 mg/kg bw/day were limited to oedema in the wall of the jejunum of a pig and a friable liver with focal subcapsular haemorrhage in another. It is unclear (because individual animal data is not present) whether the friability was associated with the lobular congestion observed under microscopic examination in a pig from this

treatment group. The absence of the individual amylase activities (and other clinical chemistry parameters) indicates that a NOEL cannot be established for pigs in this study (Earl et al, (1971).

Chronic Toxicity

Technical diazinon fed to B6C3F1 mice or (Fischer) F344 rats in their diet for 103 weeks at concentrations of 0, 100, or 200 ppm in mice and 0, 400, or 800 ppm in rats did not affect survival or bodyweight, however, hyperactivity was evident among all diazinon-treated mice and among rats at 800 ppm. Dose selection was based on a dose-ranging study that indicated no change in survival or bodyweight at concentrations up to 200 ppm in mice and 800 ppm in rats.

In mice the only treatment-related non-neoplastic lesion observed was an increase in cystic hyperplasia of the uterus/endometrium. The incidences were 22/46 at 200 ppm and 1/44 at 100 ppm relative to 0/22 in controls. Apart from an increased incidence of hepatocellular carcinoma at 100 ppm, (ie. 4/21, 20/46 ($p=0.046$) and 10/48 for control, 100 and 200 ppm groups respectively) that was not associated with a corresponding increase in hepatocellular adenoma (2/23, 0/47, 3/49 for control, 100 and 200 ppm groups respectively) liver adenomas, there was no treatment-related increase in the total number of tumours in treated mice relative to controls. Therefore, under conditions of this assay, the NOEL for carcinogenicity in mice was at the maximal tested of 200 ppm (30 mg/kg bw/day). However, a NOEL for clinical signs could not be established.

In rats, palpable tissue masses were said to be more prevalent among females at 400 ppm and males at 800 ppm relative to controls, though the histopathology examination revealed an approximately equal incidence of neoplastic and non-neoplastic lesions among all groups. There was no specific neoplasia type that appeared to be attributable to treatment, although the lymphoma incidence increased in males at 400 ppm (25/50; $p=0.011$) relative to controls (5/25) and males at 800 ppm (12/50). The biological significance of this observation is uncertain. Endometrial stromal polyp formation in females is possibly related to treatment in that for the 400 and 800 ppm groups the incidence was 8/43 (19%) and 11/49 (22%) respectively, whereas in concurrent controls only 2/23 (9%) were affected. These increased tumour incidences, although not statistically significant, were also accompanied by a shorter time to onset (of a palpable mass), ie. 62 and 72 weeks for the 400 and 800 ppm groups respectively whereas for controls it was 104 weeks. The investigators dismissed these observations on the basis that this lesion is common among F344 rats and fall within the historical control incidence. However, this assertion could not be verified because historical control data were not supplied. Similarly, it was not possible to investigate a possible link between the vaginal bleeding and/or discharge, and the presence of endometrial polyps because the individual animal data for clinical signs were not available.

Thus, under the conditions of this assay, diazinon has an uncertain carcinogenic potential in rats. The NOEL for the study was established at 400 ppm because of hyperactivity observed at the next higher concentration of 800 ppm (Wheeler et al, 1979).

Technical diazinon added to dry rodent chow and fed to Swiss CD-1 white mice at concentrations of 0, 4, 20, or 100 ppm (equivalent to 0.6, 3, or 15 mg/kg bw/day) was prepared in batches at intervals ranging between 7 and 157 days, however, a stability analysis showed that by day 7, 2.7% had degraded and by day 157 the reduction was 39%. After this problem was recognized,

batches were prepared weekly and stored frozen, although the impact of the preceding 8 months on the study outcome could not be determined.

During the first 11 months of treatment, daily monitoring (excluding weekends and holidays) was limited to reporting deaths. Thereafter more detailed observation and reporting of clinical signs, that included palpation to detect tumors, was initiated. After month 14, the frequency of palpation and clinical signs monitoring was reduced to monthly intervals.

Apart from a marked increase in the number of deaths following a 3-week episode of elevated humidity in the room housing the mice, there was no treatment-attributable increase in mortality. Since this episode caused a higher death rate among mice in all groups (during weeks 80-81), the median survival were comparable in all groups, ie. at 0, 4, 20 and 100 ppm it was 459, 500, 501 and 510 days respectively in males, and 546, 539, 507 and 538 days respectively in females. Since clinical signs were monitored during the latter stages of the study, those noted were generally associated with aging mice, ie. alopecia, skin lesions, and skin irritation and piloerection.

Significant ($p < 0.05 - 0.01$) loss of bodyweight was observed among all treated males during the first 2 months and then (transiently) at months 7, 9 and 14 for the 20 and 100 ppm groups. In contrast, a significantly ($p < 0.05 - 0.01$) reduced bodyweight was observed among all treated females (ie. 13%, 11% and 12% at 4, 20 and 100 ppm respectively at month 18) throughout the study except for months 6, 13 and 19. Macroscopic examination of tissues taken from decedents and those at the scheduled kill revealed mainly isolated incidences of kidneys with a discoloration or a granular surface, and enlarged spleen among males and females; urine stained perineum and subcutis oedema among males and missing eyes, ovarian cysts and enlarged uterine horns or uteri among females. However, none of these findings were related to dose and many occurred with equal frequency as for controls. There also appeared to be a good correlation of gross lesions with histopathological findings. In summary, there was no evidence of any inflammatory, degenerative, proliferative or neoplastic lesions among treated males and females.

In view of the unknown doses actually administered, this study can only be considered as supplementary data for to assess carcinogenicity potential. However, rats fed diazinon in their diet at a concentration that caused significant bodyweight loss showed no increase in tumour incidence (Kung et al, 1980).

Technical diazinon fed to B6C3F1 mice in the diet at concentrations of 0, 100, 200, or 300 ppm in males (equivalent to 15, 30, or 45 mg/kg bw/day) and 0, 100, 200, or 400 ppm (equivalent to 15, 30, or 60 mg/kg bw/day) in females for 104 weeks did not cause an increase in mortality or clinical signs. Food consumption was significantly ($p < 0.05$) reduced on 33 of 58 occasions throughout treatment in males at 300 ppm and on 11 occasions in females at 400 ppm. The report, however, indicates that no precautions had been taken to minimise food spillage so that estimates made before week 17 were unlikely to be accurate, hence for males, significance was still achieved on 25 of 43 occasions in males and 7 in females. However, although the overall food consumption in males was approx. 13% less than controls, the concomitant bodyweight loss was only 4%, whereas for females whose overall consumption was reduced by 2% the loss was 7%. Actual dosages achieved appear to have exceeded targets because no adjustment to the diazinon concentration in the diet was made during treatment and because food spillage was not controlled, the actual mean daily dose cannot be accurately determined.

There were few haematological changes that appeared to be related to treatment except for a reduced number of segmented neutrophils in females after 24 months. This reduction, ie. 41%, 43% and 51% at 100, 200 and 400 ppm respectively achieved significance ($p < 0.05$) only at the highest dose. Absolute heart weight was increased for all treatment mice after 12 and 24 months, however, significance ($p < 0.05$) was not achieved except for females at 400 ppm (16%) after 12 months and in males at 100 (7%) and 400 ppm (9%) after 24 months. Although significance was abolished when compared with changes in bodyweight except for females at 400 ppm after 24 months, the consistent trend for increased absolute heart weight suggests a treatment-related effect. Other significant organ weight reductions, ie. liver (by 12%) and kidney (by 11%) in females at 400 ppm after 24 months are also possibly treatment related.

No consistent treatment-related increase in incidence of palpable masses among mice, the number per mouse or percent with masses over the duration of the study. A statistical analysis of the tumor incidence data did not reveal any increase in incidence for any treatment group relative to controls. Hence, under the conditions of this study, there was no evidence that diazinon at dietary concentrations up to 300 ppm in males and 400 ppm in females causes an increase in the number of tumors in B6C3F1 mice (Goldsmith & Craig, 1983).

In a study for which only a summary was available it was reported that diazinon fed to albino rats in the diet at concentrations of 10, 100, or 1000 ppm for 104 weeks did not cause any treatment-related deaths, clinical signs or changes in food consumption relative to controls. Apparently the only haematological change at the terminal kill was a reduced haematocrit among males at 1000 ppm. Reductions in ChE activity in brain, erythrocytes and plasma were apparently dose related, with the greatest reduction being observed in plasma followed in order by that in erythrocytes and the brain.

However, the reporting of the gross and histopathological observations was so brief that no independent evaluation of the study was possible. Apparently though, there was no evidence of any treatment-related tumour formation. Therefore, a NOEL for this study cannot be established in the absence of any data for independent evaluation (Anon, 1955).

Technical diazinon fed to (Fischer) F344 rats in the diet at concentrations of 0, 0.1, 1.5, or 22.5 mg/kg bw/day in one study and 0 or 0.025 mg/kg bw/day in a second for 104 weeks was judged to have not altered longevity. However, although it was claimed that survival among groups in the first study was not significantly affected by treatment, the cumulative number of deaths, namely 67, 59 and 76 relative to 61 in concurrent controls (combined sex) suggests that the increased mortality (ca. 20%) at the highest dose of 22.5 mg/kg bw/day is a treatment-related effect. There was no apparent gender difference in mortality at any dose and for the females in the second study there was no apparent difference in cumulative deaths between the treatment (21) and concurrent control groups (20). Apart from an increased number of lesions in the stomach, ie. ulceration (M, 12; F, 9 relative to 4 and 3 respectively in controls), acanthosis (15, 12 relative to 6 and 9), hyperkeratosis (15, 14 relative to 6 and 9), granulated submucosal tissue (16, 11 relative to 6 and 6) and hyperplasia of epithelium (7, 6 relative to 0 and 1), among rats of either sex at 22.5 mg/kg bw/day that died between weeks 105 and 120, macroscopic and microscopic investigations did not reveal an increased incidence of neoplastic or non-neoplastic lesions attributable to treatment among the rats that had died or had been killed *in extremis* before the terminal or interim kills. Whilst all lesion incidences were elevated relative to controls, only mucosal granulation and the epithelial hyperplasia among males achieved significance ($p < 0.05$).

There were numerous significant ($p < 0.05$ or 0.01) weekly increases in the incidence of periorbital staining (or ocular discharge), urogenital and perianal staining among females in the 22.5 mg/kg bw/day group. Time to onset for periorbital and urogenital staining was 17 and 16 weeks respectively whereas significance for the incidence of perianal staining was delayed until week 43. For males, the incidence of perianal staining also achieved significance, however unlike females, all treatment groups were affected. Somewhat surprisingly, the incidence among males in the control group was initially significantly higher than those in the treatment groups, though after 10 weeks this reversed and remained significantly less than observed at 0.1 and 1.5 mg/kg bw/day for the next 14 weeks and for 23 weeks at 22.5 mg/kg bw/day. The time to onset (for significance to be achieved) in all treatment groups was 48 weeks, ie. slightly delayed relative to females at 22.5 mg/kg bw/day. No signs were reported for the second study.

Food consumption for males and females at 22.5 mg/kg bw/day increased progressively throughout treatment and this increase achieved significance during weeks 53-104 in males and 105-120 in females. The overall food consumption, ie. for weeks 1-120 was increased by 5% ($p < 0.01$) in males and 4% ($p < 0.05$) in females. This progressive increase in food consumption also resulted in a slightly increased bodyweight over the duration of treatment (3% in males, 4% in females; not significant) that only achieved significance ($p < 0.05$) during weeks 26-52 in females and weeks 26-78 in males. Despite the increased food consumption, the achieved mean dosage (because of adjustments) was only marginally increased for the 22.5 mg/kg bw/day group, ie. 0.025, 0.01, 1.5 and 22.6 mg/kg bw/day respectively. Water consumption recorded for weeks 1, 12, 25, 51, 77 and 101 revealed a reduced consumption for the 22.5 mg/kg bw/day group rats on most occasions with significance being achieved for week 12 in females ($p < 0.001$) and week 1 in males ($p < 0.05$). The increased water consumption for males ($p < 0.05$) relative to controls during weeks 51 (12%) and 101 (22%) but not relative to other treatment groups suggests a non-treatment related effect.

Probable treatment-related changes in haematology were observed for the erythrocytic parameters and lymphocytes in males after 12 weeks of treatment. The RBC, Hb and Hct were reduced in an approximate relationship with dose, ie. at 0.1, 1.5 and 22.5 mg/kg bw/day respectively, RBC was 3%, 6%** and 7%**, whereas Hb was 5%*, 6%[†] and 5%[†], and Hct was 2%*, 6%** and 6%** (* $p < 0.05$; [†] $p < 0.01$; ** $p < 0.001$). The lymphocyte counts were similarly reduced for the 3 respective treatment groups, ie. 10%, 20%* and 20%*. All erythrocytic and lymphocytic counts had recovered to be within the normal range by week 25 of treatment. There were no clear treatment-related effects in urinalysis, however, at 22.5 mg/kg bw/day, alkaline phosphatase activity was significantly reduced (M 11%, $p < 0.05$; F 22%, $p < 0.01$) after 12 weeks and maintained until week 51. Other changes in clinical chemistry that were possibly related to treatment was a significant increase ($p < 0.001$) in the concentration of uric acid at 25 weeks, ie. 2-fold in males and 1.7-fold in females. However, there were clear treatment-related changes in ChE activity in plasma at all dose levels with females being appreciably more sensitive to the enzyme inhibition. In contrast, acetyl-ChE inhibition in erythrocytes achieved significance ($p \leq 0.001$) at 1.5 mg/kg bw/day in both sexes. In the brain, acetyl-ChE activity was significantly inhibited in both sexes only at the highest dose tested, ie. 22.5 mg/kg bw/day.

No consistent treatment-related increase in incidence of palpable masses, macroscopic changes and organ weight were observed at 0.5, 1.5, or 22.5 mg/kg bw/day at the terminal kill. Similarly, there was no increase in the incidence of neoplasms attributable to treatment. However, at 22.5 mg/kg bw/day there was an increased incidence of submucosal granulation tissue (8 relative to 0 in controls), acanthosis (9 relative to 0 in controls) and hyperkeratosis (8 relative to 0 in

controls) of the keratinised cells in the stomach that achieved significance ($p < 0.05$) among females.

In conclusion, there was no evidence of carcinogenicity in rats treated with diazinon at doses that caused increased mortality, cholinergic clinical signs, increased bodyweight and food consumption, and ulceration of the keratinised region of the stomach. However, a NOEL for this study could not be established because significant ChE inhibition was observed in the plasma of female rats at the lowest tested dose of 0.025 mg/kg bw/day (Ashby & Danks, 1987)

A study that investigated effects of technical diazinon in the diet of Sprague-Dawley rats at concentrations of 0, 0 (ie. with 26.5 ppm of epoxidized soybean oil (ESO); the stabiliser present in the technical formulation), 0.1, 1.5, 125, or 250 ppm a.i. (equal to 0.004, 0.06, 5 and 10 mg/kg bw/day for males and 0.005, 0.07, 6 and 12 mg/kg bw/day for females) was terminated prematurely after 98/99 weeks because of a reduced survival among males at 0.1 and 125 ppm (30% and 35% respectively) that was unrelated to treatment. Most deaths in all groups, irrespective of gender, were the result of pituitary adenoma and/or senile nephropathy. Both these conditions are apparently associated with senescence in this strain of rats.

Auditory and ophthalmologic examinations did not reveal any treatment-related findings. However, there was a progressive increase in the incidence of foot sores among 250 ppm males after 56 weeks so that by week 98, 6/11 (54%) survivors were affected, whereas at 0, 0 (ESO), 0.1, 1.5 and 125 ppm, 2/12 (17%), 1/9 (11%), 1/6 (17%), 3/10 (30%) and 1/7 (14%) respectively were affected. However, the absence of a dose-incidence relationship for the increased incidence of skin ulceration suggests that this observation has a doubtful relationship to treatment. These foot sores among males at 250 ppm may possibly be secondary to a treatment-related increase in bodyweight despite there being a greater bodyweight gain at lower concentrations (ie. 34, 32, 17, and 23% at 0.1, 1.5, 125, and 250 ppm respectively). Though it seems that most of the increase in bodyweight at the end of treatment was attributable to the presence (or palatability) of ESO in the formulation because the gain relative to the ESO-control group (ESO concentration is equal to that present for the 250 ppm group) was reduced to 17, 15, 0, and 6% at 0.1, 1.5, 125, and 250 ppm respectively. A corresponding increase in food consumption among males approximately matched the observed increase in bodyweight. There was no change in water consumption among treatment groups and females did not have any treatment-related foot sores or corresponding increased food consumption with accompanying weight gain.

Significant inhibition ($p \leq 0.01$) of plasma ChE was observed in females at 1.5 ppm and in males at 125 ppm, whereas inhibition for erythrocyte and brain ChE in males and females was observed at concentrations in excess of 1.5 ppm. Rats at 250 ppm that were allowed to recover for 4 weeks after 52 weeks of treatment had reduced inhibition of plasma (to [6%] in males and 10% in females), erythrocyte (1% in males and 7% in females) and brain ([5%] in males and 9% in females) ChE. Despite the increased activity, significance ($p \leq 0.01$) was still achieved for impaired brain and erythrocyte ChE activities in females. There was no inhibition of ChE activity in controls fed the diet supplemented with ESO.

No treatment-related change in the incidence of neoplasms, histopathological lesions or tissue weight was observed. However, following literature reports of possible retinal degeneration following administration of organophosphates, eyes and optic nerves (where available) from this study were re-assessed 2 years later by different pathologist from an independent laboratory. The conclusion drawn after examining sections from control, vehicle control and the 250 ppm group

was that there were no significant ocular lesions in either the interim or interim recovery necropsy, and despite the presence of cataracts (unilateral and bilateral), inflammatory changes (iridocyclitis, hypopyon, phthisis bulbi and keratitis) and diffuse, bilateral retinal degeneration (a male and female in the control group and a female in the vehicle control) at the terminal kill, none were increased relative to the control incidence.

In conclusion, there was no evidence of carcinogenicity in rats at concentrations that caused a significant reduction of ChE activities in brain, erythrocytes and plasma of both sexes. The NOEL was 0.1 ppm (equal to 0.004 mg/kg bw/day for males and 0.005 mg/kg bw/day for females) based on significant plasma ChE inhibition at the next higher dose of 1.5 ppm (Kirchner et al, 1991 & Mann, 1993).

Technical diazinon fed to Beagle dogs in their diet at concentrations of 0, 0.1, 0.5, 150, or 300 ppm a.i. (equal to 0.0032, 0.015, 4.7, or 7.7 mg/kg bw/day for males and 0.0037, 0.02, 4.5, or 9.1 mg/kg bw/day for females) for 52 weeks caused a marked reduction in bodyweight gain among dogs at 300 ppm, (ie. 10% and 24% that for controls in males and females respectively) that indicated a dose in excess of the MTD and therefore necessitated a reduction to 225 ppm after 14 weeks (day 99) of treatment. Significant reduction ($p < 0.05$ or 0.01) in bodyweight gain was also observed at among males at 150 ppm from day 14 to day 70. This reduced weight gain for males at 150 ppm resulted in a mean bodyweight difference of 16% by the end of treatment. Although food consumption was significantly reduced ($p < 0.05$ or 0.01) at day 21, 28 and 35 for 150 ppm males, the total mean consumption was reduced by 23% over the duration of the study. Females at 150 ppm also had reduced food consumption that achieved significance on days 35, 42, 70, 84, 98, 140, 168, 308 and 336, however, the overall mean reduction in consumption (22%) relative to controls was similar to males.

Apart from significant plasma ChE inhibition among females at 0.5 ppm, all dogs had significant ChE inhibition in plasma and erythrocytes at the next higher tested concentration of 150 ppm. In keeping with the enhanced ChE sensitivity observed among females, brain ChE activity was also significantly inhibited at a lower concentration (150 ppm) than for males, where appreciable inhibition was only observed at the highest tested concentration of 300/225 ppm. Increased serum amylase activity appeared to mimic plasma ChE inhibition in females, ie. a dose-response relationship with appreciably elevated activities being observed at the equivalent diazinon concentration. Amylase activity among males was elevated at all concentrations though not with an exact dose relationship, however, significance ($p \leq 0.05$) was only occasionally achieved among both males and females. A possible explanation to account for this lack of significance despite the 2-3 fold activity increase was the rather large intragroup variability (as shown by a large standard error among treatment groups); typically a phenomenon observed when group size is small (here $n=4$). There were no corresponding pancreatic lesions associated with these increased amylase activities.

Following literature reports of possible retinal degeneration following administration of organophosphates, eyes and optic nerves from this study were re-assessed 2 years after the study had been completed, by a different pathologist from an independent laboratory. The conclusions drawn after re-examining sections from control and 300/225 ppm groups confirmed the earlier finding, namely that there were no significant ocular or optic nerve lesions at the terminal kill.

In conclusion, diazinon administered to dogs in their food caused significant bodyweight loss in males and inappetence in both sexes at 150 ppm (equal to 4.7 mg/kg bw/day in males). The

NOEL for the study was established at 0.1 ppm (equal to 0.0037 mg/kg bw/day in females) based on significant plasma ChE inhibition and elevated serum amylase activity in females at the next higher concentration of 0.5 ppm (Rudzki et al, 1991 & Mann, 1993).

Diazinon administered PO by stomach tube to Rhesus monkeys at doses of 0, 0.05, 0.5, or 5 mg/kg bw/day (as a.i.) for 6 days/week resulted in clinical signs and reduced bodyweight. The clinical signs appeared to be treatment-related and were predominantly observed at 0.5 and 5 mg/kg bw/day where tremors, soft faeces and a degree of hypersensitivity to touch and sound were transiently observed throughout treatment. The number and gender of monkeys having these signs was not reported except for hyperesthesia that was observed in 1 monkey at 5 mg/kg bw/day and 0.05 mg/kg bw/day respectively. Reduced bodyweight gain was observed in all groups throughout treatment but the reduced mean bodyweight after 106 weeks only achieved significance (14%) for the 5 mg/kg bw/day group.

The median plasma ChE activity was substantially depressed at 0.5 mg/kg bw/day (av. 55%), whereas the median ChE activity in erythrocytes became substantially inhibited (av. 72%) at the highest dose of 5 mg/kg bw/day. No data for brain ChE activity was supplied but the summary indicated that a significant reduction was observed in 1 monkey at the highest dose and in 2 monkeys that died during the study following exposure at 0.05 and 0.5 mg/kg bw/day respectively.

The reporting of many important parameters in this study were so limited that no independent assessment of treatment-related changes other than for ChE inhibition was possible. However, since plasma ChE inhibition is normally the most sensitive marker of OP exposure, a NOEL based on ChE inhibition can be established at 0.05 mg/kg bw/day due to the reduction of ChE activity in plasma at 0.5 mg/kg bw/day (Cockrell et al, 1966)

Reproductive Toxicity

Female mice were exposed to diazinon throughout gestation at doses of 0, 0.18 or 9.0 mg/kg bw/day. Delayed bodyweight gains were seen in high-dose offspring, and this delay in development might have been treatment-related, but the level of reporting was not adequate for regulatory purposes. No other clear dose-related effects on pup development, endurance or coordination were seen at any dose. A reduction in running speed in a number of trials, and the presence of some chromatin-containing cells in the brain tissues of some high-dose pups were noted, but the toxicological relevance of these findings was not clear (Spyker & Avery, 1977).

Groups of six mated adult female F₂ hybrid mice given diazinon at doses of 0 (control), 0.18 or 9.0 mg/kg bw/day until parturition on day 22. There were two concurrent control groups. Within 6 h of birth, pups were randomised within treatment groups to give each treated or control dam four male and four female pups from a like-treated dam. Offspring were weighed daily and examined for morbidity and mortality until weaning on day 28. All animals were killed at 101 days of age. Statistically significant increases in plasma corticosterone levels (males and females), bodyweight (females), liver weight (females), total liver corticosterone reduction (males and females), and liver reduction of side-chain corticosterone (males) were observed at 0.18 mg/kg bw/day only. At 9.0 mg/kg bw/day, a reduction in adrenal weight was observed in females only. In the absence of any dose-response relationship, the above findings were not considered to be treatment-related. This study was not adequate for regulatory purposes (Spyker Cranmer et al, 1978).

In a 2-generation reproduction study, technical diazinon (94.9% purity) was given to Sprague-Dawley rats at dietary concentrations of 0, 10, 100, or 500 ppm. Clinical signs were limited to tremors in a few animals (F0 and F1) at the high dose. There was a low incidence of parental mortality (F0) at 500 ppm, possibly related to difficulty in delivering offspring, which may have been related to treatment. There were no treatment-related gross or histopathological findings associated with treatment at any dose. Decreases in bodyweight and/or bodyweight gains were seen in high-dose F0 (P1) females and F1 (P2) males and females. Pup bodyweights at 500 ppm were markedly reduced in both generations at delivery and during lactation, as was pup survival. The decrease in pup survival may have been due to starvation, as milk was absent from the stomachs of a number of F1 pups sacrificed on day 4. Reproductive parameters were generally unaffected by treatment at concentrations of 100 ppm and below, but there were reductions in fertility and mating indices in the F1 (P2) generation at the high-dose level. The NOEL for this study was 100 ppm (approximately 5 mg/kg bw/day) based on reduced parental and pup bodyweights, parental mortality and clinical signs, and reduced pup survival at 500 ppm (approximately 25 mg/kg bw/day) (Ginkis, 1989).

Technical diazinon (97.36% purity) was given to Fischer 344 rats (13 males/26 females first generation; 15 males/30 females second generation) continuously in the diet through two successive generations at dose levels of 0 (control), 0.1, 1.0, or 10.0 mg/kg bw/day. No parental animals died or were sacrificed in a moribund condition during the study, and there were no compound-related clinical signs evident in treated animals. Group mean bodyweights were not affected by treatment in either generation during the growth phase of the study nor during gestation or lactation, and group mean food consumption was also similar in control and treated groups. There were no significant treatment-related effects on reproduction data in either generation. Pregnancy rate, mean gestation duration, and gestation and lactation indices were generally comparable between control and treated groups. High-dose females had slight decreases in pregnancy rate and lactation index (number of females with litters surviving to weaning/the number of females that delivered viable offspring) compared with controls during the first generation, and there was a slight reduction in the survival index at the high dose in the first generation, mainly due to a decrease in survival in the day 1-4 period. The relationship between these findings and treatment was not clear. Gross and histopathological examinations did not reveal any effects that were considered to be treatment-related. The NOEL for this study was 10 mg/kg bw/day. The adequacy of this study for regulatory purposes was limited, as no treatment-related toxicity was observed at any dose (Weatherholz, 1982).

The reproductive toxicity potential of the diazinon wettable powder formulation 50W (containing 50% diazinon) was tested in albino rats for three generations. In the F0 generation, animals received a diet containing 0 or 4 ppm of the active ingredient. In the second and third generations, animals received diets containing 0, 4, or 8 mg/kg bw/day diazinon. No adverse, treatment-related effects were observed in parental animals or offspring during the study. The NOEL for reproductive toxicity was 8 ppm (approximately 0.8 mg/kg bw/day) diazinon, but as no toxicity was demonstrated at the high dose, the adequacy of this study for regulatory purposes is limited (Johnston, 1965).

When male rats were given diazinon by oral gavage at doses of 1.5 and 3.0 mg/kg bw/day for 65 consecutive days, there were statistically significant, dose-related decreases in sex organ weight, sperm cell count, percentage of live sperm cells, sperm motility, and serum testosterone concentration, and an increase in the total sperm head deformity incidence. These effects persisted after a 21-day recovery period that followed the treatment period. When treated males were mated

with untreated females, there was a decrease in the male fertility, even after removal of the males from the test diets for 60 days (Abd El-Aziz et al, 1994).

Developmental Toxicity

Diazinon technical was given to groups of pregnant Sprague-Dawley derived rats by oral gavage at doses on 0, 15, 50, or 100 mg/kg bw/day on days 6 to 15 of gestation. Decreases in food consumption and bodyweight were observed at the high dose. No treatment-related effects were observed on reproductive parameters or on the incidence of malformations. No major developmental effects were seen at any dose. The NOEL for this study was 50 mg/kg bw/day, based on maternal bodyweight loss at 100 mg/kg bw/day. The NOEL for developmental toxicity was 100 mg/kg bw/day (Fritz, 1974).

When diazinon was given by gavage to groups of female Wistar-Imamichi rats at doses of 0 (controls), 0.53, 1.45, or 4.0 mg/kg bw/day on days 7 to 17 of gestation, the NOEL was 1.45 mg/kg bw/day, based on the reduction in maternal food consumption and the increased incidence of delayed ossification of sternebra in fetuses at 4.0 mg/kg bw/day. No other signs of developmental toxicity were observed and no major malformations were seen. Brain ChE activity was not assessed in the main study (Tauchi, 1979).

Groups of pregnant Charles River SD rats were given technical diazinon by oral gavage at doses of 0, 10, 20, or 100 mg/kg bw/day from days 6 through 15 of gestation. No unscheduled deaths or treatment-related clinical signs were reported at any dose. At 100 mg/kg bw/day, decreases in mean food consumption, mean bodyweights (5-10%; days 10, 14, and 20), and mean bodyweight gains were observed. There were no treatment-related effects on reproductive indices. At the high dose, slight increases in both the pre-implantation and post-implantation losses and in the mean number of resorptions, and a consequent small decrease in the mean number of live fetuses, were not significantly different to controls. An increase in the incidence of rudimentary T-14 ribs at the high dose was considered to be treatment-related, and attributed to the maternotoxicity seen at this dose. No other treatment-related variations or malformations were observed at 100 mg/kg bw/day, or at 10 and 20 mg/kg bw/day. The NOEL for this study was 20 mg/kg bw/day, based on the maternotoxicity (decreased food consumption, bodyweight and bodyweight gains), and fetotoxicity (increased incidence of rudimentary ribs) at 100 mg/kg bw/day. The NOEL for teratogenicity was 100 mg/kg bw/day (Infurna, 1985).

Diazinon was given to pregnant Syrian Golden hamsters (0.125 or 0.25 mg/kg bw/day) or New Zealand White rabbits (7 or 30 mg/kg bw/day) during gestation. High maternal mortality occurred in rabbits at the highest dose (30 mg/kg bw/day) and cholinergic signs were seen in both species, but no signs of developmental toxicity or fetal malformations were reported at any dose. The level of reporting in this paper was not adequate for the establishment of a developmental toxicity NOEL for regulatory purposes (Robens, 1969).

Diazinon was given by oral gavage to groups of pregnant New Zealand White rabbits on gestation days 6 through 18 at doses of 0 (CMC control), 7, 25, or 100 mg/kg bw/day. Frank maternotoxicity was observed at the high dose, with significant mortality (40%), an increase in the incidence of a range of clinical signs of intoxication, and decreases in bodyweight gains. There were no significant effects on reproductive parameters at any dose, and no major treatment-related malformations were observed. At 100 mg/kg bw/day there was a slight increase in a number of skeletal variations, including delayed ossification. These effects were not significantly increased compared with controls, but were consistent with delayed development in

the presence of frank maternotoxicity. The NOEL for this study was 25 mg/kg bw/day, based on maternotoxicity and delayed fetal development at 100 mg/kg bw/day. The NOEL for teratogenicity was 100 mg/kg bw/day (Harris, 1981).

Pregnant New Zealand White rabbits were given diazinon (96.02% purity) at doses of 0, 2.5, 10, or 40 mg/kg bw/day on days 6 through 18 of gestation. At 40 mg/kg bw/day, maternal body weight gain and food consumption were decreased and animals displayed signs of intoxication including unsteadiness, abnormal movement or posture, body and/or facial tremors, salivation, and piloerection. No consistent, treatment-related affects were seen on litter size, or on pre- or post-implantation losses. There was a statistically significant decrease in the mean fetal weight at 10 and 40 mg/kg bw/day, but there was no dose-response relationship associated with this finding. There was no statistically significant increase in the incidence of visceral or skeletal anomalies or malformations at any dose. The NOEL for this study was 10 mg/kg bw/day, based on the clinical signs, reduced bodyweight gains and reduced food consumption in maternal animals at 40 mg/kg bw/day (Edwards et al, 1987).

Diazinon administered to pregnant Beagle dogs at doses up to 5 mg/kg bw/day caused no significant increases in the incidence of malformations, or on reproductive performance. The dogs were described as nervous, but the level of reporting in this study was inadequate to determine if the high dose produced frank maternotoxicity. This study was inadequate for the establishment of an NOEL for regulatory purposes (Earl et al, 1973).

In pregnant swine given diazinon at up to 10 mg/kg bw/day, there was significant maternal mortality at the high dose unaccompanied by any increase in the incidence of malformations. Several instances of abnormalities were reported at 5 mg/kg bw/day, but because of the small number of animals in this study, and the isolated nature of the findings, it was not possible to attribute the abnormalities to diazinon administration. This study was inadequate for the establishment of an NOEL for regulatory purposes (Earl et al, 1973).

Treating pregnant sows on gestation day 85 with a single topical application of diazinon (19.9% formulation) at doses of 20, 60, or 100 mg/kg bw (active ingredient) did not result in any treatment-related mortality or clinical signs of intoxication. Plasma ChE activity was inhibited at all doses on day 7 and at 60 and 100 mg/kg bw on day 2 after treatment. This effect was reversible. No adverse effects were observed on other biochemical or haematological parameters, or on reproductive parameters (Cameron, 1995).

Genotoxicity

A tabulated summary of the results for the mutagenicity studies performed with diazinon are shown in Section 9.

The mutagenicity of diazinon was examined using a battery of tests including *in vitro* and *in vivo* gene mutation, DNA damage and repair, and various chromosomal aberration assays. Most (ie. 22) of the 26 studies cited were negative indicating by weight of evidence that diazinon is not genotoxic. Of 4 studies reporting genotoxicity, the 2 published studies separately reported an increased frequency of sister-chromatid exchange in Chinese hamster lung cells (V79) and human lymphoid cells (LAZ-007) in the presence of metabolic activation (Matsuoka, 1979; Sobti et al, 1982). Neither of these observations has been confirmed in the same cell line and/or at the same concentrations. However, in other cell lines, ie. human lymphocytes (CCL 156) or PHA-stimulated human lymphocytes (or V79 cells in the presence of a slightly reduced diazinon

concentration, ie. 80 compared with 100 µg/mL), diazinon at sublethal concentrations was unable to increase the frequency of sister-chromatid exchange in either the presence or absence of metabolic activation.

Jones and Wilson (1988) reported an increased number of histidine revertants in *S. typhimurium*, strain TA 1535, in the absence of metabolic activation. However, 3 other studies were unable to confirm these results in the same strain (Marshall et al, 1976; Shirasu et al, 1976; Geleick & Arni, 1990). Similarly, a study by Henderson et al, (1988) that described a significant increase in the mutation frequency at the thymidine kinase locus in mouse lymphoma cell line L5178Y in the presence of metabolic activation could not be confirmed by Beilstein et al, (1986), using the same concentrations of diazinon.

Neurotoxicity

Diazinon administered orally to White Leghorn fowls (both sexes) at 1, 2.15, 3.59, or 10 mg/kg bw on two occasions, 21 days apart did not cause any delayed neurotoxic symptoms in surviving fowls at 1, 2.15 or 3.59 mg/kg bw or any nerve degeneration in fowls at 2.15 or 3.59 mg/kg bw (Krinke et al, 1973). Similar results were obtained when diazinon was administered to 'Production Red Heavy Breed' hens at 28.09 mg/kg bw by gastric intubation. Apart from the expected reduced activity and ataxia, no delayed neurotoxicity or histopathological lesions were observed (Jenkins & Jones, 1988).

Diazinon administered by oral gavage to LSL-Lohmann hens at 10, 30, or 100 mg/kg bw caused an impaired gait. The mean time to onset of the impaired gait did not appear to be related to dose, ie. 1, 1.8 and 1.1 days at 10, 30 and 100 mg/kg bw respectively, however, the duration did appear to be related to dose, ie. 1, 1.3 and 5.1 days, respectively. Diarrhoea was observed in most hens at 30 and 100 mg/kg bw with the duration being related to dose, ie. 1.4 and 5 days respectively, whereas recumbency was observed only in all surviving hens (7/7) at 100 mg/kg bw. Bodyweight loss occurred in the 100 mg/kg bw diazinon group where the group mean was significantly reduced on days 7, 10, 14 and 21 (all by 11-12%) after dosing. ChE inhibition in plasma was almost complete at all doses after 24 and 48 h, whereas marked inhibition of ChE in the spinal cord and brain over the same duration was only observed at 30 and 100 mg/kg bw. Very little inhibition of ChE activity in erythrocytes was observed at any dose after either 24 or 48 h. However, as anticipated there was a substantial reduction in the neurotoxic esterase activity in the brain and spinal cord at 24 and 48 h for the tri-orthocresyl phosphate-treated group (positive control) but none was observed among any of the diazinon-treated groups. No histopathological lesions were observed for any of the diazinon-treated groups (Classen, 1996).

Diazinon administered to groups of 12-hour fasted Sprague-Dawley rats once by gavage at 2.5, 150, 300, or 600 mg/kg bw caused reduced activity, tremors, chromodacryorrhoea and diarrhoea 9-12 h after dosing together with reduced food consumption and bodyweight gain for a week at doses ≥ 300 mg/kg bw. Function observation battery tests performed on day 1, 8 and 15 revealed significant effects only on day 1, when the acute OP poisoning effects (clinical signs) were maximal. No histopathology related to treatment was observed, however, significant plasma ChE inhibition was observed 9-11 h after dosing in both sexes at the the lowest tested dose of 2.5 mg/kg bw (Chow & Richter, 1994).

Sprague-Dawley rats fed diazinon in the diet at 0.3, 30, 300, or 3000 ppm for 13 weeks lost bodyweight and had characteristic clinical signs of OP poisoning at the highest concentration tested. FOB studies revealed reduced fore and hindlimb grip strength throughout treatment

among all rats at 3000 ppm with additional findings, ie. reduced hindlimb foot splay and rectal temperature, among females. However, the NOEL for the study was established at 0.3 ppm (equal to 0.017 mg/kg bw/day in males and 0.019 mg/kg bw/day in females) based on significant plasma and erythrocyte ChE inhibition at 30 ppm in both sexes (Pettersen & Morrissey, 1994)

Porphyrin Biosynthesis Studies

Prompted by the observation that chlorinated hydrocarbons cause experimental porphoria similar to the human disease, porphoria cutanea tarda, and a published report implicating diazinon exposure as a possible cause, a research group investigated and published 4 articles on the effect of diazinon on the porphyrin biosynthesis in rats. These are described below.

Diazinon administered either in the diet (equivalent to 47 mg/kg bw/day) or topically (approx. 114 or 228 mg/kg bw/day) to female Dark Agouti rats for 12 weeks resulted in an increased concentration of porphyrin in the faeces but not in urine. Relative to an untreated control group, the porphyrin concentration in faeces was 2.4-fold greater after 8 weeks ($p < 0.025$) and 4.9-fold after 12 weeks ($p < 0.005$) of daily dermal application at 114 mg/kg bw/day. Though somewhat surprisingly, excretion at the higher topical concentration of 228 mg/kg bw/day was not significantly elevated after 8 weeks but was after 12 weeks (3.8 fold, $p < 0.005$). No significant elevation in porphyrin excretion in urine was evident after 4, 8, or 12 weeks of treatment, irrespective of dose or administration route. Electrophoresis of the pooled fecal porphyrins from treated rats revealed a number of decarboxylated intermediates in the conversion of uroporphyrinogen to protoporphyrinogen; a feature consistent with a disturbed porphyrin biosynthesis pathway and diagnostic of porphyria. However, the absence of a concomitant elevation of porphyrin concentration in urine was difficult to explain (as was the lack of any dose relationship) (Bleakley et al, 1979).

Impurities present in technical diazinon were separated by HPLC and incubated with *in vitro* cultures of chicken embryo liver cells to investigate their potential to induce porphyrin accumulation. Diazinon, isodiazinon and sulfo-TEPP were found to increase the porphyrin concentration in these cultures, ie. 900, 1700 and 540 pmoles/mg/24 h respectively in the presence of insulin or 730, 1600 and 220 pmoles/mg/24 h in the absence of insulin. Solvent controls had porphyrin concentrations of 18 and 15 pmoles/mg/24 h in the presence or absence of insulin respectively. It was speculated that the presence of isodiazinon in technical diazinon preparations may be a major contributor in the disturbance of the porphyrin biosynthesis pathway observed following topical administration in rats (Nichol et al, 1982).

To investigate the contention that the presence of isodiazinon in technical preparations of diazinon is a major cause of the porphyrin biosynthesis disturbance, purified diazinon approx. 148 mg/kg bw/day in xylene was topically applied (on a dorsal area) daily to female Dark Agouti rats for 100 days. Other groups were treated similarly with isodiazinon at approx. 15 mg/kg bw/day, a 1:9 mixture with purified diazinon or with xylene (vehicle control). A (provided) graph revealed that the excretion of porphyrin in faeces was elevated after about 60 days and continued to rise for the duration of the treatment with the 1:9 isodiazinon mixture (no other data were shown). An activity assay of the porphyrin biosynthesis enzymes in the liver indicated that only the ferrochelatase activity was reduced by approximately half relative to the control group. However, neither purified diazinon nor isodiazinon alone caused any marked changes in activity. In view of the differing characteristics of diazinon-induced porphyrin accumulation both *in vivo* and *in vitro*, ie. the absence of a concomitant increase in urinary excretion of porphyrin and the accumulation of coproporphyrin and protoporphyrin instead of

uroporphyrin, its use as a model to establish a causal relationship between diazinon exposure and porphyrin cutanea tardis in humans seems speculative. Given that it is generally accepted that porphyrin cutanea tardis in humans is associated with an intrinsically low concentration of the enzyme uroporphyrin decarboxylase (because of a pre-existing genetic defect), the investigators postulated an alternate hypothesis that involved an increased demand for haem for the cytochrome P450 system and some undefined inhibition of an enzyme in the porphyrin biosynthesis cascade resulting in porphyrin accumulation in the presence of a diazinon degradation product, isodiazinon. This hypothesis was tested in the following published report by Nichol et al, 1983 (Collins et al, 1982).

Isodiazinon at 15 mg/kg bw/day, purified diazinon at 148 mg/kg bw/day), a 1:9 mixture of isodiazinon in purified diazinon or xylene (as vehicle control) were separately applied each day onto a dorsal region of female Dark Agouti rats for 100 days. As in previous studies of this series, excretion of porphyrin in faeces was increased above control group levels by about day 70 in the isodiazinon and isodiazinon mixture groups and continued to rise for the remainder of treatment. An assessment of the activity for enzymes involved in the porphyrin biosynthesis, namely ALA synthetase, ALA deaminase, uroporphyrinogen synthetase, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase, protoporphyrinogen oxidase and ferrochelatase together with cytochrome P-450, succinate dehydrogenase, glutamate dehydrogenase and kyneuramine hydroxylase, revealed that ferrochelatase activity was significantly reduced ($p<0.01$) by 13% and 42% after isodiazinon and the isodiazinon:diazinon mixture treatment respectively. Coproporphyrinogen oxidase was also significantly reduced ($p<0.01$) for the diazinon:isodiazinon mixture (by 28%) but not for isodiazinon alone. TLC analysis of the faecal porphyrins after diazinon:isodiazinon treatment indicated that although the total amount of protoporphyrin and coproporphyrin in faeces had increased, the ratio to one another remained unchanged; a result inconsistent with the reduced ferrochelatase and coproporphyrinogen oxidase activity (Nichol et al, 1983).

Immunotoxicity

In order to detect humoral changes in the adult immune system as a result of *in utero* exposure, diazinon in peanut meal at doses of 0, 0.18, or 9 mg/kg bw/day was fed daily to groups of presumed pregnant mice throughout gestation. Although the number of pups/litter (at parturition) for dams at 0.18 and 9 mg/kg bw/day was not significantly different from controls (ie. 7.2, 6.3 and 7.8 at 0, 0.18 or 9 mg/kg bw/day respectively), the mortality for the 9 mg/kg bw/day pups was significantly increased ($p<0.05$) so that at weaning 12% had died compared with 6% in controls and 2% at 0.18 mg/kg bw/day. This increased mortality was attributed to an increased susceptibility to respiratory infections since necropsy confirmed pulmonary congestion and mucosal infiltration consistent with acute bronchitis. However, longevity for post-weaning mice was not significantly different at day 800 (ie. 33%, 21% and 31% at 0, 0.18 or 9 mg/kg bw/day respectively). A confounding factor for the survival of the high-dose pups to weaning was the significantly ($p<0.05$) reduced bodyweight gain (exact difference not reported); a difference that diminished after weaning.

Prenatal exposure to diazinon had no effect on serum IgG2b, IgM or IgA levels. However, IgG1 and IgG2a concentrations were significantly ($p<0.05$) different from controls, though not in any dose-related relationship or with respect to time after exposure. Thus, although changes in immunoglobulin subclass concentration were apparent at the 3 time intervals there were no data to indicate that any impairment of immunocompetence had occurred. Longevity or incidence of

disease among groups was comparable, so the biological significance of these immunoglobulin changes is speculative (Barnett et al, 1980).

Human Studies

Oral Administration

A study, intended to monitor the effects of progressively increasing the oral dosage of diazinon in 3 volunteers from 0.05 to 5.0 mg/kg bw/day over 13 weeks, was terminated after only 5 days when significant ChE inhibition in plasma (mean, 38%) was detected at the lowest dose of 0.05 mg/kg bw/day. After a 23-day recovery period, dosing at 0.05 mg/kg bw/day for 5 days was repeated and the extent of the significant plasma ChE inhibition observed in the first exposure confirmed (ie. 33%). There were no clinical signs or changes in bodyweight during treatment or recovery. Similarly, there was no skin sensitisation or any changes to any of the measured haematological (ie. Hb, erythrocyte count, total and differential leucocyte count and prothrombin time), clinical chemical (ie. BUN, AP and AST) or urinary parameters (ie. albumin, protein and microscopic elements) other than for plasma ChE activity. Significant plasma ChE inhibition ($p < 0.05$) persisted for 6 days after treatment in the first cycle, and substantial though non-significant inhibition (ie. 22%) was observed for 6 days after treatment in the second. No changes in erythrocyte ChE activity were observed during treatment. Therefore, based on significant inhibition of plasma ChE activity after a 5-day treatment at 0.05 mg/kg bw/day, a NOEL for this study could not be established (Sze & Calandra, 1965).

A daily oral administration of 0.0245-0.03 mg/kg bw/day diazinon for 33-34 days as a divided dose resulted in no clinical signs or changes in bodyweight, haematology or urinalysis in 4 volunteers. However, although ChE activity in erythrocytes was apparently unaffected by the 34- or 36-day treatment, ChE activity in plasma in 2 volunteers would appear to have been completely inhibited after a single administration, albeit from a pre-test activity of 5% and 13% respectively. A second administration after a 5-day recovery resulted in activities greater than pre-test levels with no apparent reduction by subsequent daily administration. Based on the observation that the inter-assay ChE variability was quite marked whereas intra-assay appeared relatively consistent, a comparison of results derived on the same day of assay suggests that significant inhibition (~40%) of ChE in plasma occurs. Thus, in the presence of a 60% reduction in acid phosphatase activity and 40% reduction in plasma ChE activity, a NOEL could not be established because only one dose was tested in this study (Payot, 1966).

Ciba-Geigy Corp., Ardsley, New York, USA. Study duration: not stated. Report date: 22 Nov, 1966. (Validated and Pre-GLP)

Diazinon administered to groups of 3 adult male volunteers in capsules for 37 days at 0.020 mg/kg bw/day or 43 days at 0.025 mg/kg bw/day caused no clinical signs or significant changes in bodyweight, haematology (ie. Hb, erythrocyte count, total and differential leucocyte count and prothrombin time), urinalysis (ie. pH and microscopic elements) or clinical chemistry (ie. BUN, AP and ALT) parameters except for plasma ChE activity. Treatment at 0.020 mg/kg bw/day resulted in a non-significant inhibition of ChE in plasma (ie. 8%) and erythrocytes. However, at 0.025 mg/kg bw/day a significant change in the mean plasma ChE inhibition relative to the combined mean pretest values for the treated volunteers indicated a clear treatment-attributable effect. By contrast, mean erythrocyte ChE activity was not significantly inhibited at any time during treatment at 0.025 mg/kg bw/day. Recovery of plasma ChE activity was evident after cessation of treatment so that full activity returned by approximately day 61.

Based on the significant inhibition of plasma ChE activity at 0.025 mg/kg bw/day, the NOEL for this study was established at 0.020 mg/kg bw/day (Lazanas et al, 1966).

Percutaneous Absorption

In Vivo

A study designed to measure the percutaneous absorption rate of [2'-¹⁴C] diazinon in humans after application onto the forearm or abdomen was unable to account for 95% of the dose when applied in either acetone or lanolin (wool grease) and left unoccluded for 24 h. Surface stripping with adhesive tape and washing of the application site recovered between 0.01-0.04% and 0.35-1.4% of the dose respectively. Urinary excretion over 7 days accounted for only 2.2% and 1.8% of the radioactivity when applied to the forearm or abdomen in acetone and 1.6% in lanolin when applied to the abdomen. In a concurrent study, 56% of the radiolabelled diazinon injected intravenously to Rhesus monkeys was excreted in urine within 7 days. Assuming that the pharmacodynamics in humans is the same as in Rhesus monkeys, the percutaneous absorption rate corrected for incomplete or other route excretion was estimated to be 3.8% and 3.2% for acetone and 2.9% for the forearm, abdomen (both acetone) and abdomen (lanolin) groups respectively (Wester et al, 1993).

In Vitro

In the same report, the *in vitro* percutaneous absorption rate assessed using 2 human cadaver skin samples in flow-through cells were found to be 8.5% and 19.7% respectively (of the total applied). However, the total radioactivity recovered after 24 h from the open flow-through cells was 62.4% and 59.1% respectively. It was speculated that the 40% (approx.) of the radioactivity not recovered was lost into the atmosphere by evaporation (Wester et al, 1993).

Skin Sensitisation

Pesticides were patch tested in 652 subjects to establish the optimal test concentration, and the frequency of irritant and allergic reactions. Allergic reactions to fungicides were found in 46 subjects, with captan folpet and difolatan the most common. Irritant and allergic reactions to other pesticides (insecticides and herbicides) were rare. Diazinon did not produce either irritant or allergic reactions. It was noted that pesticide sensitivity was more common in individuals who worked, or who had worked in agriculture (Lisi et al, 1987).

Occupational Exposure

Two Egyptian workers experienced acute toxicity after using a 60% diazinon EC formulation, which had been packaged in tin-plated sheet steel (previously packaged in aluminium containers). Both workers were experienced sprayers, having used diazinon for more than 18 months, and were accustomed to applying between 1200 to 1500 L of diluted diazinon (0.1%) once weekly by backpack spray without using either mask or gloves. They noted when preparing this batch of spray that crystals had formed in the storage container.

In the first case study, a 33-year-old male developed nausea and vomiting, followed by progressive weakness and muscle twitching in his arms and legs. He was treated with atropine sulphate and discharged from hospital the next day. Relative to a control group of unexposed

males his plasma ChE activity was inhibited >20% on day 8 after poisoning, with recovery at day 15. Erythrocyte ChE activity was inhibited >20% on day 18 and had returned to control levels by day 28.

In the second case study, a 50-year-old male developed nausea and vomiting after a full day's spraying. This progressed to burning eyes, blurred vision and difficulty breathing, as well as a severe headache persisting for 3 days. He did not seek any medical advice, but recovered by 3 days after exposure. Plasma ChE activity was inhibited in excess of 20% on day 10 after the incident, with recovery on day 17. Erythrocyte ChE activity was inhibited >20% on day 17 after the incident with recovery on day 20.

A sample of the crystallised material found in the batch was examined using GC-MS. The major component was 2-isopropyl-4-methyl-6-hydroxypyrimidine, in two tautomeric forms. Small amounts of other products were also found, including 4-ethoxy-6-methyl-2-(1-methyl-ethyl)-pyrimidine, 4-thioethoxy-6-methyl-2-(1-methyl-ethyl)-pyrimidine, 4,4'-thiobis[6-methyl-2-(1-methyl-ethyl)-pyrimidine], 4,4'-dithiobis[6-methyl-2-(1-methyl-ethyl)-pyrimidine], O,O,O-triethylphosphorothioate; and O,O,S-triethylphosphorothioate. SulfoTEPP (S,S-TEPP) and monothiono-TEPP (O,S-TEPP) were also found in small quantities. O,O-TEPP, however, was not found. It is likely that the presence of these toxic metabolites resulted in the clinical signs observed (Soliman et al, 1982).

In an attempt to simulate diazinon use in rice paddies, 5 male volunteers mixed a dry granular formulation of diazinon (10%; no other formulation details provided) in a plastic bucket with either their right or left bare hands, whilst standing with bare feet in a solution of diazinon at 1.7 ppm for 30 min. No clinical effects or ChE inhibition in erythrocytes were observed, however, relative to pre-exposure activities plasma ChE was inhibited from between 17 to 27%, 4 h after of exposure. Recovery was gradual so that after 4 days the degree of ChE inhibition ranged between 9 and 14% of pre-exposure values (Loosli, 1983).

A 51-year-old male sprayed 3 cows with a commercial mixture containing malathion and diazinon in a closed shed. Several hours later he was found unconscious and treated with atropine prior to admission into hospital. He was unresponsive to all stimuli except pain (which produced withdrawal). Neurological examination revealed increased muscle tone and neuromuscular excitability. Pupils were small and unreactive, and corneal reflexes and Doll's eye movements were absent. There was a sinus tachycardia, and chest X-ray showed a mild increase in heart size with increased pulmonary vasculature. Plasma ChE activity was inhibited by 75% relative to the normal values. On the day of admission, the patient suffered a cardiorespiratory arrest and was resuscitated. On the second day he was areflexic and unresponsive to all stimuli. On the 4th day he suffered a second cardiac arrest and died.

On postmortem examination, there was diffuse subarachnoid intraventricular and cerebral cortical haemorrhage with autolysis at the base of the brain. Microscopic examination revealed haemorrhagic necrosis without inflammatory or glial response. There was moderate left ventricular hypertrophy, with no dilation or hypertrophy of the other cardiac chambers. Microscopic examination of intercostal muscle revealed mild pathologic changes, with subsarcolemmal granular basophilic inclusions, and scattered necrotic fibres. Lesions were randomly scattered throughout the muscle tissue; these types of lesion were not seen in control samples. The neuromuscular ChE activity in the intercostal samples obtained from the patient with pesticide poisoning were approximately half those seen in control patients. Necrosis had

been seen previously in muscle tissue from patients dying of acute OP poisoning (Wecker et al, 1985).

Eighteen workers at a mushroom farm were exposed to diazinon when the only entrance to a darkened room in which they were working was (accidentally) sprayed. Within 15 minutes, 17 workers developed cholinergic signs, including headache, blurred vision, dizziness, fatigue, nausea and vomiting. Four workers went to hospital, where they were treated with atropine and admitted. At the time their plasma and erythrocyte ChE activity was at the low end of the normal range. Two of these workers developed nausea and vomiting after returning to work 2 days later. Eight other workers sought advice within 48 h of exposure, and their ChE activity was determined. All of these workers had a follow-up ChE test 15 days later. In all cases, plasma and erythrocyte ChE activity increased between these tests. If the levels found at 15 days after exposure were taken to be the normal levels for these workers, the mean plasma ChE inhibition seen was 29% and mean erythrocyte ChE inhibition was 27%. This may be an underestimate of the degree of inhibition, as in many cases ChE activity has not returned to normal within 15 days of exposure, depending on the degree of inhibition initially seen. Following this work, it was recommended that where there are cholinergic signs, a history of exposure, no baseline values for the individual and plasma ChE levels are at the low end of normal values the worker should be kept from work and retested by the same laboratory 3 to 5 days later. If there is an increase in ChE activity between the tests, a further test should be done 3 to 5 days later. A rise in activity between these tests would be confirmatory that pesticide exposure and ChE inhibition had occurred (Coye et al, 1987).

Neurobehavioural effects of short-term, low-level exposure to diazinon among 99 pest controllers were assessed before and after work with a computer-assisted neurobehavioural test battery. Each subject completed a brief neurological screening examination, a symptom questionnaire, and tests of concentration, eye-hand co-ordination, pattern recognition, visual memory, and finger tapping. A diazinon metabolite, diethylthiophosphate, was measured in pre- and post-shift urine samples collected from 46 applicators applying granulated diazinon onto residential properties with lawn spreaders, and with 56 non applicators. Post-shift median diethylthiophosphate concentration for applicators and non-applicators was 24 and 3 ppb, respectively. Full shift, whole-body exposure to diazinon was quantified for 19 subjects using personal air monitoring and passive badges. Median diazinon exposure for applicators and non-applicators was 2.1 and 0.03 mg, respectively. Mean duration of pesticide application was 39 days before testing.

No adverse diethylthiophosphate-related changes in pre- and post-shift neurobehavioural function were found with multiple linear regression models after adjusting for age, sex, education, and ethanol intake, although Symbol-Digit pairing speed was slower among applicators as a group. The prevalence of 18 symptoms possibly related to diazinon exposure was not elevated among applicators. Thus, in conclusion, there was no evidence that short-term, low-level diazinon exposure in a controlled pest control program, where care had been taken to minimise exposure by using protective clothing and direct supervision, caused any behavioural effects (Maizlish et al, 1987).

Workers involved in the application of diazinon granules (14%) in residential areas using four types of application equipment were monitored for exposure by detection of diethylthiophosphate (relative to creatinine) in 24-h pooled urine. Estimated diazinon exposures ranged from 0.1 to 11 mg/day for workers, with the highest levels being found among workers using the belly grinder type applicator. Urine diethylthiophosphate levels ranged from less than 0.1 to 360 µg/g creatinine (Weisskopf et al, 1988).

A 68-year-old sheepfarmer used diazinon to treat sheep without wearing protective clothing. He presented at hospital the next day with periumbilical and upper abdominal pain. He was transferred to a regional hospital the next day, and was diagnosed with acute haemorrhagic pancreatitis. He was transferred to a major hospital two days later and placed in intensive care. There was progressive deterioration of his condition, with multi-organ failure and he died two days after admission.

On post-mortem examination, there were a number of incidental findings, including left ventricular dilatation, extensive moderate atherosclerosis in the aorta, with an early infrarenal abdominal aortic aneurysm. The lungs showed moderate emphysema and pulmonary oedema, and the pleura contained 200 mL of serous fluid. The gallbladder contained numerous friable stones. There was 300 mL of blood stained fluid in the peritoneal cavity. The mid-portion of the pancreas was necrotic and adherent to the stomach. There was extensive greenish-black discoloration of the peritoneal surface, and ischaemic necrosis of the small bowel and transverse colon.

On histopathological examination, the lungs showed pulmonary oedema and focal presence of saponificated lipid material in the arterial vessels. There was early tubular hyperplasia in the kidney with vascular scarring. The pancreas showed extensive necrosis, which was also seen in the retroperitoneal tissue. There was also centrilobular necrosis in the liver. The blood, urine, liver and gall bladder were sent for toxicological examination. The blood contained pethidine and lignocaine, and the urine contain paracetamol, metronidazole and metoclopramide in quantities consistent with therapeutic use. Diazinon was not detected in the organs or tissues at this time. Blood ChE activity was 26 units (normal is 80 to 150 units), which is consistent with OP exposure.

The cause of death was determined to be severe acute haemorrhagic pancreatitis caused by exposure to diazinon, probably by the dermal route (NSW State Coroner's Office - Report on death of a sheep farmer related to Topclip Blue Shield Dip (diazinon 200 g/L), 1995).

Poisoning Incidents

Twenty-five cases of diazinon poisoning, admitted to the Sassoon Hospital in Poona, India in 1961-2, were reviewed. There were 14 males and 11 females, with ages ranging from 18 months to 60 years, though the majority of cases were aged from 15 to 24 years. Each had ingested between approximately 14 and 57 g of diazinon (presumably formulation); the exact quantities ingested were not generally known. Only two patients died following hospitalisation; the remaining 23 appeared to recover uneventfully.

Symptoms experienced by the patients included: vomiting (18/25); abdominal cramps (10/25); stupor (8/25); restlessness (6/25); giddiness (3/25) and sweating (3/25). Additionally there were single instances of fever, diarrhoea, hiccup and coma. Signs on clinical examination included: pulmonary oedema, detected by rales heard on auscultation (15/25); hypertension (12/25); tachypnoea (10/25); tachycardia (9/25); albuminuria (7/25); azotaemia (6/25); nystagmus (2/25); hypotonia (2/25) and cyanosis (1/25). There was no report on any clinical chemistry or haematological examination of any of the patients.

Treatment generally included gastric lavage, atropine, oxygen and antibiotics as appropriate. No details of dosing were provided. Post-mortem examination of the two patients dying following

poisoning were performed. There were petechial haemorrhages in the brain, kidney, pericardium, respiratory tract and liver. Congestion was seen in the brain, and the kidney was hyperaemic, particularly in the medullary region. There was no report of any changes in the pancreas in this examination (Mutalik et al, 1962).

Twenty-five cases of diazinon poisoning occurring in the 12 months between October 1964 and Oct 1965 in Ahmedabad, India were reviewed. There were 17 males and 8 females admitted to the hospital with 21 of the poisoned patients being between 10 and 30 years old. Most patients ingested less than 21 mL of diazinon; 11 ingested between 21 and 28 mL. The most common clinical sign was vomiting (21/25), accompanied by unconsciousness, giddiness and excessive sweating observed in 7/25 cases. Other common signs were tachycardia (15/25), constricted pupils (14/25) and pulmonary oedema (12/25). Treatment typically involved gastric lavage, atropine treatment and airway maintenance. However, 3 patients died despite treatment; no post-mortem findings for these patients were reported (Kabrawala et al, 1965).

A 19-year-old male who ingested approximately 4 ounces (113 g) of Tik-20 (diazinon; formulation not otherwise specified) was admitted to hospital approximately 1-2 h after ingestion. He presented with vomiting and diarrhoea and was cyanotic. He was responsive to noise and pain and had tremor and spasticity of the limbs. He presented with pinpoint, unresponsive pupils. There were excessive secretions present in the trachea and respiratory passages, and moist rales in the lungs. He was treated with gastric lavage and IV atropine sulfate. Oxygen and antibiotic therapy were also started. A tracheostomy was required to maintain an adequate airway, and digoxin and noradrenaline were administered to counter cardiovascular abnormalities. Pupils gradually returned to normal, while tendon reflexes remained sluggish.

On the second day of hospitalisation, the patient reported retrosternal pain; examination revealed evidence of pericarditis. The pericardial friction rub persisted for two days, with no other evidence of cardiac involvement. One day after the tracheostomy was closed, the patient developed respiratory distress and an increased temperature; left lower lung lobe consolidation had developed.

ChE activity determined in whole blood was significantly decreased (based on time taken for a reaction to occur) for two weeks after poisoning; levels had returned to near-normal within four weeks. The patient recovered without incident (Banerjee, 1967).

Sixty cases of diazinon poisoning occurring between 1963 and 1965 in Ahmedabad, India, were reviewed. (This review probably includes the cases cited by Kabrawala et al, 1965; see above.) There were 42 males and 18 females involved, aged between 11 and 60 years and most (55/60) had suicidal intentions. The common clinical signs were vomiting, giddiness, constricted pupils and signs of bronchoconstriction with pulmonary congestion. Urinalysis showed mild albuminuria in 4 cases and haematuria in 10 cases. Treatment with atropine was successful in most cases; 5 patients died despite atropine therapy. In all cases where death occurred, the patients had ingested at least 15 mL of diazinon and treatment had been delayed by more than 8 h after ingestion. Where patients presented within 3 h of ingestion, atropine therapy was successful. Post-mortem examination of the patients that died revealed congestion of viscera with or without pulmonary oedema and subendocardial haemorrhaging (Gupta & Patel, 1968).

A 65-year-old female was found at home after attempting suicide by cutting both radial veins. She was admitted to hospital and died some hours later. Postmortem and pathological examination failed to reveal the cause of death, however the stomach was found to contain a

green, oily fluid. The lungs were oedematous. Diazinon was found in extracts from the stomach and small intestine contents. The concentration of diazinon (quantified by GC) in the brain (30 µg/100g) was higher than that in the liver (8 µg/100g), kidney (4 µg/100g) or lung (1.5 µg/100g), but all were markedly lower than that in the stomach (756 µg/100g) and small intestine contents (262 µg/100g) (Heyndrickx et al, 1974).

Five children from a family became ill 30 min after eating breakfast (oatmeal, sugar and evaporated milk). The children had profuse sweating, nausea, vomiting and abdominal cramps, with the youngest having muscle weakness, muscle contractions and cramps. They were treated with atropine, and recovered without incident. Their mother indicated that she regularly sprayed the shelves and cupboards and painted the baseboards with a 25% diazinon formulation. This had occurred without removing packaged food (including cardboard boxes of oatmeal), dishes or glasses. An unopened box of oatmeal was given to a related family; the 3 children in this family also presented with acute signs of OP poisoning after eating an oatmeal breakfast. Plasma and erythrocyte ChE activity measured 17 or 23 days after poisoning did not appear to be significantly decreased relative to levels observed 52 or 58 days after poisoning, although there was a slight increase in all family members. Urinalysis revealed diethyl phosphate in the urine at the measuring times indicated above. There was not a major change in the excretion of this metabolite between the two time intervals. This may have reflected ongoing diazinon exposure. Diethylphosphorothioate was not found at either time intervals; it is possible that the level of this metabolite was below the limit of detection (0.02 ppm) (Reichert et al, 1977).

Five cases of successfully treated cases involving intentional ingestion of 25% diazinon were reported. Patients ingested between 60 and 180 mL of the pesticide formulation (between 15 and 45 mg diazinon). Four patients were seen at a hospital within 30 min; the male who ingested 60 mL did not present at the hospital until 5 h after ingestion. In general, clinical signs included vomiting, profuse sweating, pinpoint pupils, hyperreflexia and muscle twitching. One patient had abdominal pains that persisted for several days; it was unclear as to whether serum amylase activity was determined. In general patients were treated with gastric lavage, atropine, pralidoxime chloride and oxygen supplementation. One patient required assisted ventilation; he had ingested approximately 25 mg diazinon and had not vomited prior to hospital admission. Four of the patients recovered without incident within 4 to 10 days after treatment; the fifth patient developed chemical pneumonitis and required extensive hospital treatment (Klemmer et al, 1978).

A 54-year-old female was found dead at home. On the kitchen sink, there were 2 vials containing chlordiazepoxide and furosemide, and a half empty 600 mL bottle of a 10% diazinon solution. A resident in the house indicated that the pesticide bottle had been stored for many years and had not previously been opened. On gross postmortem examination there were atherosclerotic plaques in the left anterior descending coronary artery. The lungs were heavy and congested. There were petechial haemorrhages through the stomach and gastric mucosa and the white and grey matter of the brain. Samples of fat tissue, bile, blood, brain, stomach contents, kidney and liver were collected for toxicological analysis.

Diazinon concentration was highest in the blood at 28 mg/dL, with high levels also found in the stomach contents (22 mg/dL) and bile (10 mg/dL). Diazinon was also found in omental fat (1.5 mg/100g), and the liver (0.4 mg/100g), brain (0.2 mg/100g) and kidney (0.01 mg/100g). Plasma ChE activity was also measured; this was found to be 0 U/mL (normal 40-80 U/mL). Based on the case history and lack of other findings, the cause of death was determined to be diazinon poisoning (Poklis et al, 1980).

A 16-year-old female was admitted to hospital 1 h after consuming 10 mL of Tik-20 (diazinon). On admission, signs included nausea, headache and upper abdominal pain. On clinical examination, there was an elevated heart rate, small non-reactive pupils and epigastric tenderness. Clinical chemistry examination found normal blood glucose, blood urea, creatinine and calcium levels, but serum amylase was increased to more than 4 times the normal level (680 Somogyi units). Urinalysis was normal. She was treated by gastric lavage with potassium permanganate. Atropine was administered until an obvious atropine effect had occurred. She was also treated with 2-PAM. After 2 h, she had improved clinically, with the only abnormality being mild abdominal pain. Eight hours after admission, serum amylase was within the normal range (110 Somogyi units), and she was later discharged. The author presumed from observation and other experimental information in dogs, that functional ductal obstruction, pancreatic interstitial oedema and acinar cell vacuolation with resulting hyperamylasemia were caused from the anticholinesterase effects of diazinon. The rapid resolution of signs in this case indicates that the pancreatic damage was transient in nature, possibly related to the prompt treatment. In the addendum, the authors noted that there had been 4 new additional cases of acute pancreatitis following diazinon poisoning (Dagli et al, 1981).

A 58-year-old female was admitted to a hospital 1 h after ingesting 30 mL of an unknown poison (later identified as diazinon). Her pupils were small (1 mm) but equal and reactive. The patient was comatose and responsive to pain but not to verbal stimuli. Plasma ChE activity was 138 mIU/mL (normal 1800 - 4800 mIU/mL). Initial treatment was gastric lavage, supportive therapy, 2 g of pralidoxine and 3 mg atropine IV. Plasma ChE activity remained around 100 mIU/mL until day 11, with atropine therapy required until day 7. Clinical improvement was noted on days 8 to 9, which was associated with a slight rise in plasma ChE activity. Plasma ChE activity increased relatively rapidly from day 13, returning to normal by about day 20 (Hassan et al, 1981).

A 30-year-old female was admitted 1 h after consuming a bottle of diazinon (Tik-20). On admission, she was drowsy, with bradycardia and an increased respiratory rate. Blood pressure was normal. Muscle fasciculations and pinpoint pupils were observed. She was treated with gastric lavage, atropine, 2-PAM and IV fluids. The signs of OP poisoning resolved over the next 36 h of treatment (41 mg atropine administered in total). On day 3 after admission, the patient reported being unable to hold objects in her hands. On neurological examination the patient was unable to walk, although she had normal strength in all extremities. There was lateral nystagmus and gross incoordination on the finger-nose test and heel-knee test. Sensory test (pinprick, touch and temperature) were all normal, and the biceps, triceps, knee and ankle jerks were brisk. A diagnosis of bilateral cerebellar signs was made, and the patient was treated with prednisolone; coordination improved after 48 h of treatment. Recovery was complete in one week, and the patient was discharged (Bichile et al, 1983).

A 26-year-old male was admitted to hospital 2 h after consuming 50 mL of Tik-20 (diazinon). He was drowsy, with bradycardia, pinpoint pupils, sweating, cyanosed and tachypnoeic. Chest examination indicated pulmonary oedema. He was intubated and given 5 mg atropine IV every 5 min until the pupils were fully dilated (40 mg atropine within 30 min). Ten minutes after the final atropine dose, the patient had ventricular tachycardia, which degenerated to ventricular fibrillation. He was converted to a normal sinus rhythm using an electric shock, maintained on a lignocaine drip, and recovered uneventfully. It was proposed that the hypoxia, pulmonary oedema and bradycardia induced by the muscarinic effect of diazinon lowered the threshold for

ventricular tachyarrhythmia. This threshold was further lowered by the rapid atropinisation (Hase et al, 1984).

A 26-year-old male presented approximately 1 h after ingesting a mixture of diazinon in water (unknown concentration). At presentation he was conscious and appeared to be oriented, however, he vomited, and had diarrhoea and bradycardia. Atropine treatment improved clinical signs, and he was admitted and treated with IV fluids and atropine. Shortly after admission, monitoring revealed a dark and cloudy urine and reduced frequency. On the second day of treatment, there were amorphous crystals in the urine that was not able to be identified by the hospital laboratory. IV fluids were increased, and treatment with pralidoxime commenced. Amorphous crystalluria persisted until day 9 of hospitalisation. Serum creatinine and urea nitrogen levels remained unchanged, indicating normal renal function. Direct renal toxicity is not generally associated with OP poisoning, however, there have been a few reports of acute renal failure in which an immune complex nephropathy was postulated. It is possible that these findings are related to a nephrotoxic substance present in the formulation, rather than being directly related to the active constituent (Wedin et al, 1984).

A 20-year-old woman was admitted to hospital after complaining of blurred vision and collapsing. She was comatose, unresponsive to commands and had pinpoint pupils. She was intubated, and initially treated with naloxone and glucose without effect. On admission, pinpoint pupil, decerebrate positioning and vertical nystagmus were noted. Later, bradycardia, hypotension and deep coma developed, which was treated with atropine. After 3 h she was conscious and responsive, displaying full conjugate voluntary gaze with episodes of atypical ocular bobbing (ocular bobbing with preserved movement on the horizontal plane). Occasional instances of decerebrate posturing were seen, with hyperreflexia but without limb weakness. A case history of diazinon ingestion (unknown quantity) was subsequently obtained. Plasma ChE activity was depressed for 4 days. After this time the neurologic examinations returned to normal and the bronchial secretions gradually decreased. It was proposed that the ocular bobbing was produced by a disturbance in the cholinergic transmission at the brain-stem level, probably in the tectal and pretectal areas (Hata et al, 1986).

A 58-year-old man was admitted to hospital after applying approximately 5 mL of diazinon to his genital area to treat pubic lice. He had complained of thirst and profuse sweating prior to asking to be taken to hospital. He regularly took a range of medication, including phenytoin, phenobarbital, hydrochlorothiazide, spironolactone, clonidine and insulin. On arrival, the patient had a seizure, and was unconscious with salivation and shallow respiration. His clothing smelt of insecticide. There was left periorbital oedema and bilateral chemosis. The pupils were small and minimally reactive to light. The patient did not respond to deep pain, and corneal and doll's eye signs were absent. Extremities were flaccid with no muscle fasciculations and deep tendon reflexes were absent. The patient was intubated and activated charcoal and magnesium citrate were administered via nasogastric tube (no case history was available at this time). Atropine and pralidoxime were administered IV, and his clothing was removed. He was also given α -methyldopa, phenytoin and potassium chloride. He gradually recovered consciousness after approximately 15 minutes. When vital signs stabilised and secretions decreased, the patient was extubated. He recovered without incident and was discharged 6 days after admission. The plasma ChE activity at the time of admission was 1 IU/mL (normal 7 to 19 IU/mL). The author noted the high absorption of OP pesticides from the scrotum, and indicated that this may have contributed to the severity of the symptoms (Halle & Sloas, 1987).

Two female horticultural workers were exposed to diazinon when an open bottle in a store room spilled onto the back of one of the workers. As she changed her clothes, her companion mopped up the spilt pesticide with rags. Approximately 3 h later the worker who cleaned up the spill became giddy and had diarrhoea and vomiting. She was admitted into hospital and was frothy at the mouth, cyanotic, tachypnoeic and drowsy. The history of pesticide exposure was not explained, and despite a clear chest X-ray was treated for pulmonary disease with furosemide, aminophylline and morphine, intubated and maintained on a respirator. Blood tests indicated serious disruption of the clinical chemistry, with raised LDH, ALT and CPK levels, as well as hyperglycaemia, hypokalemia and an increased leucocyte count. She improved, and was removed from the respirator. Later in the evening she vomited and had epigastric pain. Serum amylase levels were increased approximately 10-fold over normal levels. She was treated for acute pancreatitis and recovered, with enzyme levels returning to normal over the next 3 days.

The second worker developed nausea, vomiting, diarrhoea, abdominal pain and dizziness approximately 7 h after exposure. She was admitted to hospital 4 h after first showing signs, and was treated for pesticide poisoning with atropine. Plasma ChE activity was decreased by approximately 75% relative to average (normal) values, increasing over the next 4 days to be within 25% of average (normal) values. On clinical chemistry evaluation, there was hyperglycaemia, hypokalemia and an increased leucocyte count. Serum amylase levels were not determined, and it was therefore not possible to determine if an underlying pancreatitis was present.

Given the role of acetylcholine in stimulation of the acinar cells of the pancreas, the link between OP poisoning and pancreatitis is conceivable. It would appear that other risk factors for pancreatitis may also be involved, given that not all poisoning victims develop the symptoms of acute pancreatitis (Lee, 1989).

A 20-year-old male ingested an unknown volume of diazinon, and was admitted into hospital 6 h later after vomiting at home, and being treated with atropine (6 mg; route not specified). On admission the patient was conscious with no muscle fasciculations or twitching. His pupils were slightly constricted but reacted to light. He was maintained on atropine as required and 2-PAM at 1 g IV every 12 h. He recovered uneventfully until the second day after admission when he developed difficulty in swallowing and was unable to move any limbs. A neurological examination revealed deficits of function in the 6th and 7th cranial nerves with some effects on 9th and 10th cranial nerves. Distal muscle groups were more severely affected than proximal groups, and deep tendon reflexes were absent. There was no sensory loss. Some muscle fasciculation became evident, but no respiratory paralysis was present until approximately 20 h after the first signs of weakness were noted. The patient died of respiratory failure approximately 5 h after the onset of respiratory paralysis (Samal & Sahu, 1990).

A family moved into a new apartment that had been treated with a diazinon containing preparation. Soon after moving in, the mother experienced fatigue, sleep problems and irritability. The infant had vomiting and a runny nose, and a 4.5-year-old had vomiting. The cleaner also reported dizziness, headache and heaviness in the chest when she was in the house. There was a strong odour in the house, and clothes and bedding smelt of a pesticide. Blood and urine samples were obtained from household members for determination of plasma ChE activity and urinary diethyl phosphate concentration. Air samples and wall surface samples were obtained to determine diazinon levels in the house.

Plasma ChE activity measured before clean up of the house but after the family had been in residence for 4 months were reduced by between 6% and 12% in the mother and children (the levels in the father were normal, while the housekeeper was not tested). Urinary diethyl phosphate levels were increased in all family levels, with the highest being found in the father (at 1.7 mg/L), and lowest in the mother (0.45 mg/L). After the house was cleaned by an exterminator company that included washing the wall surfaces, plasma ChE activity of family members returned to normal while diethyl phosphate was no longer detected in urine.

The wall surfaces were substantially contaminated prior to the clean up; diazinon concentration ranged between 0.13 to 1.1 mg/m². The air levels were also high (2.5 µg/m³). After the cleaning procedure surface levels of diazinon decreased to 0.01-0.08 mg/m², and air levels were below the level of detection. The spraying practices of the company were subsequently investigated, given the high residual levels still present approximately 4 months after the spraying (Richter et al, 1992).

A study investigated seventeen children (59% males), ranging in age from one to eight years admitted to a hospital for accidental OP or carbamate poisoning. The ingested pesticide was identified in 12 of the patients (ie. parathion - 4, malathion - 3, diazinon - 2, and unspecified carbamates - 3). One of the diazinon-poisoned children was diagnosed with pancreatitis, while overall 5/17 was diagnosed with pancreatitis. Common signs of poisoning included vomiting and diarrhoea. Abdominal pain was also seen; this was severe in two children diagnosed with pancreatitis.

In the patients diagnosed with pancreatitis, there were increased levels of immunoreactive trypsin in comparison to controls; this was also associated with an increase in the serum amylase levels. Hyperglycaemia was also seen in these patients. Four patients had raised serum amylase levels with no abdominal pain or increase in serum trypsin levels. All patients recovered without incident.

The study demonstrated that acute pancreatitis is not rare in children who have ingested OP or carbamate pesticides. It also indicated that a raised serum amylase level can occur without pancreatic involvement; this may occur secondary to a range of disorders, and may be related to hypersalivation. Serum trypsin would appear to be a more specific and sensitive test for diagnosis. It was also proposed that the hyperglycaemia seen relatively frequently following OP poisoning may result from damage to the endocrine islets, and be associated with an excess of glucagon rather than a deficit of insulin (Weizman & Sofer, 1992).

A 12-week-old infant girl developed persistent hypertonicity of the extremities, and, at the age of 8 months, it was discovered that her home had been treated with an excessive and inappropriate application of diazinon 3 weeks prior to the onset of symptoms. At this time (6 months after application) the remaining diazinon residue on the floor was 230 ng/cm², in comparison to the 38 ng/cm² that is expected immediately after a normal application. Vacuum cleaner dust contained 1700 mg/kg diazinon, while air contained 2.8 ng/m³. Measurement of metabolites in urine, 60 ppb diethylphosphate and 20 ppm diethylthiophosphate, enabled the diazinon dose to be estimated at 0.02 mg/kg bw/day. However, there was no appreciable reduction of ChE activity in comparison with normal values for an infant of this age. When the infant was removed from the home, muscle tone returned to normal within 6 weeks and normal development proceeded without any apparent sequelae. At 20 months, the last reported observation, motor abilities and speech had developed normally (Wagner & Orwick, 1994).

A 42-year-old male farmer was admitted to hospital after accidental ingestion of 200 mL of a pesticide containing diazinon. On admission he had mild bradycardia, and increased salivation.

Plasma ChE activity was 269 IU (normal: above 4000 IU). He was treated with activated charcoal, atropine and abidoxime, along with cathartics and enemas. He was intubated for a few hours to prevent aspiration and maintain a patent airway, but did not require assisted ventilation. One day after admission, his serum creatinine began to rise, peaking on the 7th day. It returned to normal within 2 weeks. Urine output was between 2 and 5 L/day over this period. Urine sediment was normal, and urinary protein did not exceed 500 mg/day throughout the time. Kidneys were normal on ultrasound examination. The renal failure could not be attributed to any nephrotoxic substances or haemodynamic effects, ie. the pathogenesis was not defined. Previous OP poisoning experiments in rats had indicated that the predominant effect was an increased urinary flow with low osmolality, suggesting there may be a direct effect on tubular function that was unrelated to the degree of ChE inhibition (Abend et al, 1994).

A 25-year-old woman was admitted to hospital following a suicide attempt, in which she ingested acetaminophen and unknown amounts of a laboratory agent (found to be diazinon). Her initial condition did not reveal any signs related to ChE inhibition, and she was stabilised without incident. The patient was admitted to the psychiatric ward for treatment. Twenty days later she requested ECT, which she had previously received without incident. For the therapy, she was anaesthetised with methohexital, *d*-tubocurarine, atropine and succinylcholine. Following ECT, she was slow to resume spontaneous ventilation, requiring assisted ventilation for 12 minutes. She remained weak for the next 25 min, but was able to breathe spontaneously. Blood samples revealed plasma ChE activity of 1.1 IU/mL (normal 5.9-12.2 IU/mL). It was planned to track plasma ChE activity with serial blood tests, however the patient left the hospital the next day. The plasma ChE inhibition in this case appears particularly prolonged, especially given the lack of significant signs at the time of poisoning (Jaksa & Palahniuk, 1995).

DISCUSSION

Acute Toxicity

The toxicological profile of diazinon is typical of most organophosphorus ChE-inhibiting pesticides, with clinical symptoms being similar in experimental animals and humans. Although female animals (rats, dogs) tend to be more sensitive to diazinon-induced ChE inhibition, it is noteworthy that the inherent instability of diazinon causes an appreciable increase in toxicity in both sexes. Nichol et al (1982) reported that at receipt a 90% pure preparation of technical diazinon resulted in a median lethal dose (LD₅₀) of 170 mg/kg bw in rats whereas after 12 months the same preparation stored under nitrogen at room temperature gave an LD₅₀ of only 30 mg/kg bw. This increased toxicity with 'ageing' was first described by Margot & Gysin in 1957 and has been confirmed on several occasions subsequently (eg. Gaines, 1969 and Soliman et al, 1982).

Investigation into the cause of the increased toxicity has implicated two possible pathways for the formation of very toxic degradation products. Margot & Geysin (1957) have suggested that the presence of oxygen and a trace of water in technical diazinon promotes the formation of diethylphosphorothionate and diethylphosphate. Provided only a trace of water is present then these intermediates, under the catalytic influence of other uncharacterised by-products, combine with themselves or one another to form highly toxic tetraethyl-pyrophosphate (O,O-TEPP or TEPP), monothiono-tetraethyl pyrophosphate (O,S-TEPP) and dithiono-tetraethyl pyrophosphate (S,S-TEPP or sulfoTEPP) (see Figure 2 at section 3.1.3 for the synthesis pathway). Median lethal

dose studies, performed in 1989 but not reported until 1995, have confirmed the markedly increased toxicity of each of these 3 compounds in female rats, ie. 0.66 mg/kg bw for O,O-TEPP, 0.46 mg/kg bw for O,S-TEPP and 3.48 mg/kg bw for S,S-TEPP (Kuhn, 1995a, b, c). However, in the presence of relatively large volumes of water, formation of the biologically inactive compound diethylphosphate (a metabolite often monitored in human urine to assess exposure for several different OPs) is the predominant product.

An alternative pathway for the formation of these toxic impurities was suggested by Meier et al, (1979) whereby a trace of water, present during diazinon synthesis, could react with diethyl thiophosphorylchloride (see Figure 2 at section 3.1.3 for the synthesis pathway). Diethyl thiophosphorylchloride, a precursor for diazinon is also used in the synthesis of several other OPs, namely parathion, chlorpyrifos, phosalone, coumaphos, disulfoton, demeton and terbufos. Hence, batches of the various TGACs of these OPs normally contain variable quantities of these toxic impurities depending on the amount of water introduced during synthesis. However, unlike diazinon and demeton, the concentration of the impurities in these other OPs has never been shown to increase after manufacture. Moreover, the presence of these impurities in stored demeton is much less of a problem relative to diazinon because of the high intrinsic acute toxicity of the parent compound and hence the greater dilution in formulations and/or the greater care taken in handling a known high-toxicity product.

From a regulatory perspective, the main difference between these two possible pathways is that whereas the latter can be readily monitored and quantified (after synthesis), the former is more difficult because the rate of formation after synthesis and storage will be dependent on several factors that have not yet been properly investigated and may be very difficult to control. Since water, oxygen and presumably temperature are major factors promoting the formation of O,O-TEPP, O,S-TEPP and S,S-TEPP, it follows that the inherent ability of the storage vessel to prevent the access of water and air, and the frequency of lid or cap opening are some of the parameters that might need to be considered. Another factor may be the nature of the container itself. Soliman et al (1982) reported that the toxicity of diazinon increased rapidly when it was stored in tinned-steel containers instead of in inert-lined aluminium ones. In an attempt to minimise the formation of O,O-TEPP, O,S-TEPP and S,S-TEPP, formulators have introduced a stabiliser (epoxidised soybean oil) immediately after manufacture (Spindler, 1969; Sterling, 1972). Whilst most of the toxicology studies submitted to assess hazard have used diazinon containing a stabiliser, concern remains that this additive may not be sufficient to reliably prevent the formation of these toxic products.

Given that diazinon is readily available in products for use in the home (eg. as dog washes and insect killers) and the farm, (eg. sheep dips and stock sprays), steps need to be taken to minimise the potential risk to the end user. This is especially important, given that it would normally be counter intuitive to users that any product (like diazinon) might become more toxic (and probably more efficacious) on storage. A spate of dog and cattle deaths, which were reported in the Australian print and visual media and apparently linked with the use of a dog wash and a buffalo fly control formulation respectively which had passed their label use-by date, highlight the need for surveillance. Moreover a small scale nation-wide survey in 1993, co-ordinated by NRA indicated that of 157 unopened liquid diazinon formulations that had been randomly purchased from retail outlets throughout Australia and checked for the presence of O,S-TEPP and S,S-TEPP, 35 (or 22%) failed to meet the $\pm 10\%$ label claim for diazinon, 26 (or 16.5%) contained some S,S-TEPP and 13 (or 8.3%) of these 26 exceeded a benchmark impurity limit of 2 g/kg ai (for S,S-TEPP). All 7 (or 4.4%) of the batches that were found to contain O,S-TEPP also exceeded the benchmark impurity limit of 0.2 g/kg ai established for O,S-TEPP. Five of the 7

batches with O,S-TEPP also contained S,S-TEPP in excess of the benchmark. Hence, a total of 15 batches (or 9.5%) exceeded either one or both impurity limits. Any further degradation which may occur after purchase, eg. due to exposure to air and water vapour during repeated opening of the container or by washing the cap, has yet to be investigated (McDonald, 1993, 1994; Allender & Britt, 1994).

Cholinesterase Inhibition

As ChE inhibition is a primary target for diazinon toxicity, a summary of the NOEL findings for ChE inhibition in a range of repeat-dose studies is shown in the Table below. NOELs are presented for the plasma, erythrocyte and brain ChE activity.

Summary of NOELs (mg/kg bw/day) for Cholinesterase Activity Inhibition Following Diazinon Administration

Species	Duration	Route	Plasma	Erythrocyte	Brain	E:P ratio
Rat	3 mo	Diet	0.01	0.1	1.5	10
	3 mo		0.1	>0.4	>0.4	-
	3 mo		0.03	0.04	0.06	1.3
	3 mo		0.017	0.017	1.9	1
	2 yr		NE (<0.025)	0.1	1.5	-
	2 yr		0.004	0.06	0.06	15
Dog	3 mo	Diet	0.0034	0.02	0.02	5.9
	1 yr		0.0037	0.02	0.02	5.4
Monkey	2 yr	Gavage	0.05	5	ND	100

NE=Not established; ND=Not determined; E:P ratio=NOEL for erythrocyte ChE activity/NOEL for plasma ChE activity.

Inspection of the summary Table above indicates that the inhibition of pseudo- or butyryl-ChE in plasma (as judged from the E:P inhibition ratio) generally occurs at appreciably lower doses relative to the inhibition in true- or acetyl-ChE in erythrocytes and brain. The Figure below shows the relationship between the NOEL and LOEL for plasma ChE inhibition for all short-term, subchronic and chronic studies. It is apparent that no LOEL for any of the animal studies is much less than 0.02 mg/kg bw/day, the NOEL for humans.

Toxicokinetics and Metabolism

Following oral administration to rats, diazinon was almost completely absorbed from the GI tract with only about 3% of the administered dose being measurable in the faeces. Furthermore a substantial proportion of the dose found in the faeces was derived from biliary excretion. From the GI-absorbed diazinon the systemic bioavailability was 35.5% indicating that the hepatic first-pass extraction was quite pronounced. The short half life of 2.86 h in plasma was consistent with a rapid elimination of diazinon from the circulation. Excretion studies indicated that most of the absorbed diazinon (96-97%) was present in urine as metabolites within 24 h. The major degradative pathway includes the oxidase/hydrolase-mediated cleavage of the ester bond leading to the pyrimidinol derivative 2-isopropyl-6-methyl-4(1H)-pyrimidone, which was further oxidised to more polar metabolites.

Percutaneous Absorption

Two studies which reported percutaneous absorption of diazinon (without occlusion) essentially support a contention that a significant proportion of any topically applied dose is lost to the atmosphere by evaporation. In one of these studies where the diazinon metabolite excretion was monitored following topical application (50 µL over approx. 10 sq. cm) in human volunteers, only about 5% of the dose, irrespective of whether it was applied in acetone or lanolin was able to be recovered in urine and at the application site after 7 days (Wester et al, 1993). Another study, performed with rats in metabolic cages, found that nearly 18% of the dose (at 1 mg/kg bw and 10.3% at 10 mg/kg bw) applied (in an unknown volume of tetrahydrofuran to an area of skin measuring 3 sq. cm) was present in the atmosphere as 'volatiles' after 144 h (Williams & Marco, 1984). A third study, where the aim was to characterise radiolabelled metabolites formed after dermal application in sheep, reported that the volatility of the preparation precluded an accurate assessment of the applied dose (Capps, 1990 with an amendment by Carlin, 1994).

Owing to its volatility (ie. vapour pressure at 25⁰ is 2100 mPa) dichlorvos is the most commonly used OP in pest strips, however, as reported in this review there are also pest strips which contain diazinon. Hence, collectively these data suggest that the principal source of diazinon loss was via evaporation from the skin surface (and not expiration), since metabolite recovery in urine and faeces after 24 h following oral administration accounted for >95% of the administered pyrimidine-ring labelled diazinon dose.

Although there are no definitive studies which measure the percutaneous absorption rate of the degradation products (O,O-TEPP, O,S-TEPP or S,S-TEPP), it can be inferred from the numerous dog and cattle deaths which have occurred after the use of out-of-date diazinon formulations that significant amounts of these highly toxic degradation products are readily absorbed. Data suggesting that percutaneous absorption rates for the degradation products are greater than for unchanged diazinon comes from a published report by Gaines (1969). Gaines compared the oral and dermal toxicities (LD₅₀) of an unstabilised batch of technical diazinon at receipt and at some unspecified time thereafter. The results indicated that whereas the oral toxicity in male rats had increased 2.3-fold (ie. from 250 mg/kg bw to 108 mg/kg bw) the increase in dermal toxicity was 4.5-fold, (ie. from 900 mg/kg bw to 200 mg/kg bw).

In a legal action in the state of NSW in the 1990's, four members of a shearing team took action against their employer (Allambie Pastoral Company), alleging that they were suffering from the effects of organophosphate poisoning sustained in the course of employment as shearers. In a judgement of the Supreme Court of NSW (8th October 1997) which dealt with

three of the plaintiffs, the judge found in their favour and accepted that, during shearing, they had become wet with a tar containing diazinon, despite the precautionary label on the product ("Topclip") warning about avoiding skin contact and thereby, absorption by the dermal route. He also "formed an impression that [a toxic] breakdown product [of diazinon] was a likely culprit in this instance", leading to "the consequence of permanent deficits in the plaintiffs as a result of the poisoning" [Supreme Court of NSW. *McKenzie v Harper & Ors T/as Allambie Pastoral Co Johnson v Harper & Ors T/as Allambie Pastoral Co Tiedemann v Harper & Ors T/as Allambie Pastoral Co Matter Nos R 400073/93; Matter Nos R 400074/93; Matter Nos R 400075/93* (8 October 1997)].

Pancreatitis

Published and unpublished reports describe an increased secretion of amylase and formation of pancreatic lesions in dogs following exposure to diazinon. Mechanistic studies suggested that whilst diazinon was the OP under investigation, other organophosphorus pesticides could also be anticipated to elicit a similar response. It would appear that the formation of such pancreatic lesions in humans are also probable as judged from both *in vitro* data and several poisoning incidents in which an elevated serum amylase was observed (when measured). A retrospective review of medical records for the preceding 3 years by Lee et al (1998) revealed that, in accord with the mechanistic studies in dogs which showed an increased intraductal pressure, secretory rate and induction of histopathological sequelae in the pancreas resulting from the inhibition of tissue-fixed butyrylcholinesterase activity, acute oral organophosphate poisonings in humans appeared to mimic such findings. Moreover, there appeared to be a good correlation between the observed hyperamylasemia and the severity of the poisoning.

From the available data it seems likely that acute exposure to either a high concentration of a low-toxicity or a low concentration of a high-toxicity organophosphorus compound is required to elicit pancreatic lesions. Thus, the death of a Australian sheepfarmer in 1994 as a result of severe acute haemorrhagic pancreatitis following exposure to diazinon solely by the dermal route would appear to be inconsistent with the chemical's moderate acute dermal toxicity and its low rate of percutaneous absorption. For these reasons, it has been suggested that the pancreatitis and death must have occurred as a result of repeated, or chronic exposure to diazinon. However, the markedly reduced ChE activity (by 68-83% relative to the average population ChE activity) measured in the blood at death was consistent with a pronounced acute exposure to an organophosphorus compound, six days earlier. Hence, it is possible that chronic exposure to diazinon *per se* may not have been the cause of the pancreatitis but rather, the acute dermal exposure to highly toxic impurities found in some diazinon products may have caused the acute pancreatitis and subsequent death. Support for this contention comes from the following information.

The 1993 NRA sponsored nationwide small-scale survey (157 samples) revealed that 9.5% of off-the-shelf, unopened products contained O,S- and S,S-TEPP in excess of benchmark limits. The toxicity of these impurities is very high, with the oral LD₅₀ in female rats being 0.46 and 3.5 mg/kg bw for O,S- and S,S-TEPP respectively cf. 1160 mg/kg bw for diazinon. Whilst there are no definitive studies to assess the percutaneous absorption rates of these impurities, there are data which suggest that they may be dermally absorbed to a greater extent than diazinon (see further discussion under *Percutaneous Absorption*).

Whilst there are circumstantial data to implicate the toxic diazinon impurities as the cause of the pancreatitis and subsequent death of the sheepfarmer, strong supportive evidence would have come from measuring the concentrations of these impurities in the sheep dip used; there is no indication that this was done. However, it is noteworthy that the NRA survey of 1993 revealed that of four tested batches of the same brand of dip as used by the sheepfarmer, three were found to contain S,S-TEPP (range 0.54-0.81 g/kg ai) albeit within the benchmark impurity limit of 2 g/kg ai while a fourth was found to contain both O,S-TEPP (3.53 g/kg a.i.) and S,S-TEPP (1.24 g/kg a.i.); the benchmark limit for O,S-TEPP was set at 0.2 g/kg a.i.. The presence of any O,O-TEPP in the dips could not be determined owing to the absence of a suitable reference standard.

Genotoxicity and Carcinogenicity

The weight of evidence indicates that diazinon is not a mutagen, based on several mutagenicity studies performed using various end points both *in vitro* and *in vivo*. Similarly, there was no evidence of carcinogenicity from studies in rats and mice.

Reproduction and Development

No teratogenic effects were observed in rat reproduction studies or in any of the developmental studies with rats, rabbits or pigs. Although there was a published study which reported that male rats treated with a diazinon product at 1.5 or 3.0 mg/kg bw/day for 65 days had dose-related decreases in sex organ weight, sperm cell count, percentage of live sperm, sperm motility, and serum testosterone, together with an increase in the total sperm head deformity incidence, this finding could not be confirmed in a 2-generation study using technical diazinon doses of up to 10 mg/kg bw/day.

Porphyrin Biosynthesis

In 1992, the Standing Committee on Toxicity (SCOT) considered a possible relationship between exposure to diazinon in wool fat during shearing by farm workers and an abnormally high incidence of porphyria cutanea tarda in humans in parts of Western New South Wales, Australia (and also Brazil). The following is a summary of their findings:

Scrutiny of the supplied bibliography indicated that porphyria cutanea tarda occurred as a result of a congenital deficiency of the haem synthesis cycle enzyme uroporphyrinogen decarboxylase. In the rats treated cutaneously each day with 20 mg of technical grade diazinon (85%) for 10 to 12 weeks there was an increase in daily faecal porphyrin levels but not urinary porphyrin levels. There was moreover no evidence of inhibition of uroporphyrinogen decarboxylase and diazinon dosed by the oral route did not result in increased porphyrin production. It was subsequently shown that an impurity, isodiazinon rather than diazinon, was probably the porphyrogenic agent in the technical grade active ingredient and that the accumulating porphyrins were mainly coproporphyrin and protoporphyrin not uroporphyrin. Evidence suggesting for about 40% inhibition of ferrochelatase in rat liver following 100 days of cutaneous treatment with 20 µg of a 9:1 mixture of diazinon and isodiazinon in xylene and that these compounds appear to act synergistically. Neither of them on their own are particularly effective in inducing porphyria.

Consultation with Dr F De Matteis of the MRC Toxicology Unit and Professor GH Elder of the Welsh National School of Medicine (co-author of the Nichol et al, 1983 study) who are both acknowledged experts on porphyria revealed that a mechanism of a porphyrogenic action for

diazinon metabolites is not clear and that the apparently higher incidence of porphyria cutanea tarda reported in inland Australia is probably associated with persons having congenital low levels of liver uroporphyrinogen decarboxylase activity. Increased synthesis of hepatic cytochrome P450 caused by the presence of various environmental inducing compounds including pesticides could "stress" the haem biosynthesis pathway in such persons, result in abnormal accumulation of uroporphyrin followed by porphyria cutanea tarda. The authors of the report allow for such an explanation of the possible porphyrogenic action of technical grade diazinon.

Human Toxicity

Apart from characteristic clinical signs of poisoning resulting from accidental or deliberate ingestion, it would appear that diazinon has an additional attribute not associated with all OPs, namely induction of "Intermediate Syndrome" (see Samal & Sahu, 1990). This effect can be distinguished from the characteristic muscarinic, nicotinic and CNS effects observed very soon after exposure by a delayed (24-96 h) onset of muscular weakness affecting neck, proximal limb and respiratory muscles. There have been no reported cases of delayed neuropathy (OPIDN) following accidental or deliberate diazinon poisoning; a result possibly anticipated given the negative findings observed in animal studies.

NOEL considerations

To establish the lowest NOEL for diazinon, a summary of the NOELs determined in those studies deemed adequate for regulatory purposes are shown in the Table below.

Study Type	NOEL (mg/kg bw/day)	LOEL and Toxic Effect
F344 rat: 2-year dietary	Not established (<0.025)	Plasma ChE inhibition observed at 0.025 mg/kg bw/day in females. LOEL for erythrocyte ChE inhibition was 1.5 mg/kg bw/day and for brain, 22.5 mg/kg bw/day.
Sprague-Dawley rat: 2-year dietary	0.004	Plasma ChE inhibition observed at 0.06 mg/kg bw/day in males and 0.07 mg/kg bw/day in females. LOEL for erythrocyte and brain ChE inhibition was 5 mg/kg bw/day.
Beagle dog: 1-year dietary	0.0037	Plasma ChE inhibition and elevated serum amylase observed in females at 0.02 mg/kg bw/day. LOEL for erythrocyte and brain ChE inhibition was 4.5 mg/kg bw/day.
Sprague-Dawley rat: 3-gen reproduction	10 [100 ppm]	Maternal body weight loss at 150 ppm.
	3 [30 ppm]	Reduced pup survival and an inability to gain weight during lactation at the next higher dose (100 ppm).
Sprague-Dawley rat: 2-gen reproduction	M, 0.65; F, 0.74 [10 ppm]	Reduced food consumption, body weight gain and body weight in both sexes at next higher dose of 40 ppm.
	3 [40 ppm]	Reduced pup weight, viability and lactation indices at the highest dose (120 ppm).
Sprague-Dawley rat: Gavage teratology	20	Maternal body weight loss and reduced food consumption at 100 mg/kg bw/day.
	20	Skeletal variants observed at the highest dose tested (100 mg/kg bw/day).
NZW rabbit: Gavage teratology	25	Maternal survival, body weight loss and cholinomimetic signs at 100 mg/kg bw/day.
	25	Delayed fetal ossification observed at 100 mg/kg bw/day.

NZW rabbit: Gavage teratology	10	Maternal body weight loss, reduced food consumption and cholinomimetic signs at 40 mg/kg bw/day.
	40	No embryo/fetotoxicity effects observed at the highest dose tested (40 mg/kg bw/day).
Human: 37-43 days, Capsules PO (3/group)	0.02	Plasma ChE inhibition observed at 0.02 mg/kg bw/day in males. LOEL for plasma ChE inhibition was 0.025 mg/kg bw/day. No ChE inhibition in erythrocytes observed at 0.025 mg/kg bw/day.

Determination of Public Health Standards

Acceptable Daily Intake

The current acceptable daily intake (ADI) is 0.001 mg/kg bw/day. This ADI was derived from a NOEL of 0.1 mg/kg bw/day, based on plasma ChE inhibition observed in a 3-month rat study.

This review identified a validated 37-43 day human study performed by IBT laboratories that has a lower NOEL (0.02 mg/kg bw/day) based on the same endpoint, namely plasma ChE inhibition. Applying a 20-fold safety factor to this NOEL results in retention of the ADI at 0.001 mg/kg bw/day. The ACPH provided advice on this issue in April 1999 (see below).

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

The draft toxicological evaluation of diazinon recommended a change to the existing ADI, and this issue was considered at the 17th meeting of the ACPH. The Committee supported the retention of the existing ADI for diazinon of 0.001 mg/kg bw/day on the basis of plasma ChE depression in humans and application of a 20-fold safety factor. The additional 2-fold safety factor was applied due to the closeness of the NOEL and LOEL (0.025 mg/kg bw/day) and the limited nature of the study.

The Committee expressed concern that appreciable quantities of two highly toxic diazinon degradation products, namely O,S-TEPP and S,S-TEPP had been detected in unopened off-the shelf products that were within the expiry date. In view of such information the Committee questioned whether a pesticide known to readily form toxic degradation products should be available for use in and around the home and recommended the withdrawal of home garden and companion animal diazinon products on the basis of potential hazard to the user. Furthermore, the Committee recommended that diazinon products currently available for agricultural use continue to be registered only where there was proven storage stability under a range of Australian climatic conditions. In particular, registrants should be required to demonstrate through appropriate stability testing that their diazinon product(s) are adequately stabilised so that they comply with FAO specifications (ie. 0.2 and 2.5 mg/kg max. for O,S-TEPP and S,S-TEPP respectively) when stored according to label requirements for two years. Moreover, they supported a proposal to include a warning statement on diazinon product labels to draw users attention to the possibility of an increased toxic hazard with use of the product after the expiry date.

Public exposure

In Australia, most diazinon products are registered to control insects in livestock, domestic pets or in the home garden. Hence, a major source for public exposure is via ingestion of diazinon residues in food, especially meat. Diazinon has MRLs established in a wide range of foods that includes meat; the current Australian MRL list is outlined below.

Australian Maximum Residue Limits for Diazinon

Codex Code	Food	MRL (mg/kg)
GC 0080	Cereal grains	0.1
FC 0001	Citrus fruits	0.7
MO 0105	Edible offal (mammalian)	0.7
PE 0112	Eggs	0.05*

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	Fruits [except citrus fruits, Kiwifruit, olives, peach]	0.5
FI 0341	Kiwifruit	0.5
MM 0095	Meat (mammalian)(in the fat)	0.7
ML 0106	Milks (in the fat)	0.5
OC 0305	Olive oil, crude	2
FT 0305	Olives	2
FS 0247	Peach	0.7
PO 0111	Poultry, edible offal of	0.05*
PM 0110	Poultry meat	0.05*
GS 0659	Sugar cane	0.5
VO 0447	Sweet corn (corn-on-the-cob)	0.7
TN 0085	Tree nuts	0.1
OC 0172	Vegetable oils crude [except olive oil, crude)	0.1
	Vegetables	0.7

* at or about the limit of detection.

Dietary Exposure Considerations

An estimate of daily diazinon intake from food was derived from the Australian Market Basket Survey. This survey, performed under the auspices of the Australia New Zealand Food Authority, monitors pesticide and contaminant concentrations in food and publishes the results every 2 years. It is generally considered that using actual residue concentrations is more representative of the actual intake rather than assuming all pesticides are present at the MRL. However, inspection of the published values for various pesticides in successive editions reveals that some become present or absent in some food items. This variability is most likely the result of changes in pesticide use patterns, or possibly (though less likely) in the sampling protocol used in the survey. The result of this changing incidence and concentration is that the estimated intake for the six age-sex categories also changes. For example, in the last four published Market Basket Surveys, ie. 1990, 1992, 1994, and 1996, the estimated diazinon intake for toddlers aged 2 years (approximately 12 kg bw) varied from 0.025 µg/kg bw/day in 1990 to 0.002 µg/kg bw/day in 1992, 0.0228 µg/kg bw/day in 1994, and 0.0147 µg/kg bw/day in 1996. Since this age category usually has the highest average daily intake (except for 1992), the maximal intake (using the highest concentration measured in 1990) accounted for approximately 1.2% of the ADI, thereby giving an additional 40-fold safety factor between the current ADI and the maximum estimated dietary intake.

Market Basket Survey

The 1994 Market Basket Survey found detectable levels of diazinon in a range of meats and vegetables. The highest residue concentration of 0.08 mg/kg was detected in beef mince, and apart from mushrooms, lettuce, onions and eggs that had concentrations of 0.05, 0.04, 0.02 and 0.02 mg/kg respectively, all others (wheat, bread, cabbage, carrots, cabbage, and celery) had a concentration of 0.1 mg/kg. The Market Basket Survey estimated the daily intake of a range of pesticides based on the average energy intake. In the groups studied, the highest exposure for diazinon was in toddlers aged 2; estimated at 0.0228 µg/kg bw/day.

Acute Reference Dose

The acute reference dose (ARfD) is an estimate of the amount of a chemical (residue) in food or water that can be ingested over a short period of time, usually during a meal or in one day, without an appreciable health risk. An acute reference dose of 0.005 mg/kg was set following the initial public comment phase of this review, based on inhibition of RBC ChE activity in a 13 week human study with an LOEL of 5.0 mg/kg BW.

Safety Directions

The current safety directions are as follows:

Diazinon

BL 95 g/L or less with dibutyl phalate 720 g/L or less, with surfactants	
Product is poisonous if swallowed	120, 130, 133
Will irritate the eyes and skin	161, 162, 164
Repeated minor exposure may have a cumulative poisoning effect	190
Obtain an emergency supply of atropine tablets 0.6 mg	373
Avoid contact with eyes and skin.	210, 211
Do not inhale vapour or spray mist	220, 222, 223
When opening the container and preparing spray and using the prepared spray	279, 280, 281, 282
wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat	290, 292a
and PVC or rubber apron and elbow-length PVC gloves	293, 294
and water resistant footwear	298b
If clothing becomes contaminated with product remove clothing immediately.	330, 332
If product on skin, immediately wash area with soap and water	340, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350
After each day's use, wash gloves, and contaminated clothing.	360, 361, 366
DU 20 g/kg or less except as specified below	
Repeated minor exposure may have a cumulative poisoning effect	190
Avoid contact with eyes and skin	210, 211
Do not inhale dust	220, 221
When using the product wear cotton overalls buttoned to the neck and wrist and washable hat	279, 283, 290, 292
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash contaminated clothing	360, 366
DU 20 g/kg or less 300 g pack	
Avoid contact with eyes and skin	210, 211
Wash hands after use	351
Ear tags 200 g/kg or less	
Product is poisonous if swallowed	120, 130, 133
Avoid contact with eyes and skin	210, 211
Repeated minor exposure may have a cumulative poisoning	190

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effect	
Obtain an emergency supply of atropine tablets 0.6 mg	373
When using the product wear rubber gloves	279, 283, 290, 312
Wash hands after use	351
After each day's use, wash gloves	360, 361
EC ULV 200 - 800 g/L	
Product is poisonous if absorbed by skin contact or swallowed	120, 130, 131, 133
Repeated minor exposure may have a cumulative poisoning effect	190
Avoid skin contact with eyes and skin	210, 211
Do not inhale spray mist	220, 223
Obtain an emergency supply of atropine tablets 0.6 mg	373
When preparing spray and using the prepared spray	279, 281, 282
wear cotton overalls buttoned to the neck and wrist and a washable hat	290, 292
and elbow-length PVC gloves	294
and face shield or goggles	299
If product on skin, immediately wash area with soap and water	340, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and face shield and goggles and contaminated clothing	360, 361, 365, 366
EC 10 g/L or less	
Harmful if swallowed	129, 133
May irritate the eyes and skin	160, 162, 164
Repeated minor exposure may have a cumulative poisoning effect	190
Obtain an emergency supply of atropine tablets 0.6 mg	373
Avoid contact with eyes and skin	210, 211
When preparing spray and using the prepared spray	279, 281, 282
wear elbow-length PVC gloves	290, 294
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and contaminated clothing	360, 361, 366
EC 30 – 80 g/L in liquid hydrocarbons (other than xylene) 660 g/L or less, with surfactants	
Product is poisonous if swallowed	120, 130, 133
Will irritate the eyes and skin	161, 162, 164
Repeated minor exposure may have a cumulative poisoning effect	190
Obtain an emergency supply of atropine tablets 0.6 mg	373
Avoid contact with eyes and skin	210, 211
Do not inhale vapour or spray mist	220, 222, 223
When opening the container and preparing spray	279, 280, 281
wear cotton overalls buttoned to the neck and wrist(or equivalent clothing) and a washable hat	290, 292a
and elbow-length PVC gloves and water resistant footwear	294, 298b
When using the prepared spray	279, 282

wear protective waterproof clothing [or cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and PVC or rubber apron] and elbow-length PVC gloves and water resistant footwear	290, 291, [or 292a, 293], 294, 298b
If clothing becomes contaminated with product remove clothing immediately	330, 332
If product on skin, immediately wash area with soap and water	340, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and contaminated clothing	360, 361, 366
EC 50 g/L or less more than 10 g/L	
Product is poisonous if swallowed	120, 130, 133
Avoid contact with eyes and skin	210, 211
Do not inhale vapour	220, 222
Wash hands after use	351
EC 200 g/L or less in xylene	
Product is poisonous if swallowed	120, 130, 133
Will irritate the eyes and skin	161, 162, 164
Repeated minor exposure may have a cumulative poisoning effect	190
Obtain an emergency supply of atropine tablets 0.6 mg	373
Avoid contact with eyes and skin	210, 211
Do not inhale vapour or spray mist	220, 222, 223
When opening the container and preparing spray	279, 280, 281
wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat	290, 292a
and elbow-length (nominate other specific material) gloves and water resistant footwear	295, 298b
When using the prepared spray	279, 282
wear protective waterproof clothing [or cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and PVC rubber apron]	290, 291, [or 292a, 293]
and elbow-length (nominate other specify material) PVC gloves	295
If clothing becomes contaminated with product remove clothing immediately	330, 332
If product on skin, immediately wash area with soap and water	340, 342
If product in eyes, wash it out immediately with water	340, 343
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and contaminated clothing	360, 361, 366
EC 200 g/L or less, in liquid hydrocarbons 600 g/L or less, with surfactant 150 g/L or less, when packed as one part of a two-part product containing amitraz EC 125 g/L or less in the other part	
Product is poisonous if swallowed	120, 130, 133
Will irritate the eyes and skin	161, 162, 164
Repeated minor exposure may have a cumulative poisoning	190

effect	
Obtain an emergency supply of atropine tablets 0.6 mg	373
Avoid contact with eyes and skin	210, 211
Do not inhale vapour or spray mist	220, 222, 223
When opening the container and preparing the spray	279, 280, 281
wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat	290, 292a
and elbow-length PVC gloves and water resistant footwear	294, 298b
When using the prepared spray	279, 282
wear protective waterproof clothing [or cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and PVC or rubber apron]	290, 291, [or 292a, 293],
and elbow-length PVC gloves and water resistant footwear	294, 298b
If clothing becomes contaminated with product remove clothing immediately	330, 332
If product on skin, immediately wash area with soap and water	340, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and contaminated clothing	360, 361, 366
EC 215 g/L or less in liquid hydrocarbons (other than xylene) 650 g/L or less, with surfactants	
Product is poisonous if swallowed	120, 130, 133
Will irritate the eyes and skin	161, 162, 164
Repeated minor exposure may have a cumulative poisoning effect	190
Obtain an emergency supply of atropine tablets 0.6 mg	373
Avoid contact with eyes and skin	210, 211
Do not inhale vapour or spray mist	220, 222, 223
When opening the container and preparing the spray	279, 280, 281
wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat	290, 292a
and elbow-length PVC gloves and water resistant footwear	294, 298b
When using the prepared spray	279, 282
wear protective waterproof clothing [or cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat and PVC or rubber apron]	290, 291, [or 292a, 293]
and elbow-length PVC gloves and water resistant footwear	294, 298b
When using the prepared spray	279, 282
wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat	290, 292a
and elbow-length PVC gloves	294
If clothing becomes contaminated with product remove clothing immediately	330, 332
If product on skin, immediately wash area with soap and water	340, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	360, 361, 366

EC 250 g/L or less more than 50 g/L	
Avoid contact with eyes and skin	210, 211
Do not inhale spray mist	220, 223
When preparing spray and using the prepared spray	279, 281, 282
wear elbow-length PVC gloves	290, 294
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and contaminated clothing	360, 361, 366
EC SA 3 g/L or less, in liquid hydrocarbons (other than xylene) 660 g/L or less, with surfactants	
Product is poisonous if swallowed	120, 130, 133
Will irritate the eyes and skin	161, 162, 164
Repeated minor exposure may have a cumulative poisoning effect	190
Obtain an emergency supply of atropine tablets 0.6 mg	373
Avoid contact with eyes and skin	210, 211
Do not inhale vapour or spray mist	220, 222, 223
When opening the container and preparing the spray and using the prepared spray	279, 280, 281, 282
wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat	290, 292a
and elbow-length PVC gloves and water resistant footwear	294, 298b
If clothing becomes contaminated with product remove clothing immediately	330, 332
If product on skin, immediately wash area with soap and water	340, 342
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and contaminated clothing	360, 361, 366
HV AC ME 465 g/L or less	
Product may irritate the eyes and skin	120, 160, 162, 164
Avoid contact with eyes and skin	210, 211
Wash hands after use	351
HV EC 200 g/L or less	
Product is poisonous if absorbed by skin contact or swallowed	120, 130, 131, 133
Product will irritate the eyes and skin	120, 161, 162, 164
Avoid contact with eyes and skin	210, 211
Wash hands after use	351
HG EC 200 g/L or less	
Product is poisonous if absorbed by skin contact or swallowed	120, 130, 131, 133
Product will irritate the eyes, nose and throat and skin	120, 161, 162, 163, 164
Avoid contact with eyes and skin	210, 211
Wash hands after use	351
ME 240 g/L or less	
Avoid contact with skin	210, 164
When opening the container and preparing the spray and using the prepared spray	279, 280, 281, 282

wear elbow-length PVC gloves	290, 294
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves	360, 361
PD 15 g/L or less and pyrethrin 1 g/kg or less	
Harmful if swallowed	129, 133
Repeated minor exposure may have a cumulative poisoning effect	190
Obtain an emergency supply of atropine tablets 0.6 mg	373
Avoid contact with eyes and skin	210, 211
Do not inhale dust	220, 221
When using the product	279, 283
wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and a washable hat	290, 292a
and elbow-length PVC gloves	294
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350
After each day's use, wash gloves and contaminated clothing	360, 361, 366
SR Pet Collar	
Do not open inner envelope/pouch until ready for use	380
Do not allow children to play with collar	382
Wash hands after handling the collar	383

These Safety Directions are considered to be acceptable for public health considerations, with the following amendments:-

1. Amend entry "EC 215 g/L or less in liquid hydrocarbons (other than xylene) 650 g/L or less, with surfactants" to "EC 215 g/L or less in liquid hydrocarbons (other than xylene) **750** g/L or less, with surfactants"
2. Amend entry "ME 240 g/L or less" to "ME **300** g/L or less"
3. Amend entry "EC ULV 200 - 800 g/L" by inserting 161, 162 and 164 viz. "will irritate the eyes and skin"
4. Amend entry "EC 50 g/L or less, more than 10 g/L" by inserting 160, 162 and 164 viz. "may irritate the eyes and skin"

First Aid Instructions

Currently a, h. No changes to the current first aid directions are recommended

The T-value is currently 7. No change is recommended.

MAIN TOXICOLOGY REPORT

1. INTRODUCTION

1.1 Regulatory History of Health Considerations in Australia

Diazinon is an insecticidal organophosphorus compound of the phosphorothioate class. It was first synthesised in the laboratories of JR Geigy (renamed Ciba-Geigy AG and more recently Novartis), Basle in 1951 and was first used in Australia in 1956 for the control of the sheep blowfly. Large scale use is now mainly confined to the treatment of mammalian ectoparasites (ie. in cattle, sheep, goats, pigs, horses, dogs and cats), although there are some small-scale uses for insect control in pineapple, mushroom and onion cultivation. It is registered for home garden and domestic use as well as insect control in turf and buildings (industrial, farm, domestic and abattoirs). There are over 100 products of diazinon with over 1250 approved (registered) uses in Australia.

The toxicology of diazinon has been evaluated by the Department on a number of occasions. This resulted from factors such as identification by the IBT Taskforce Program as having IBT generated data in its toxicological data package which required verification or replacement. Also the Technical Grade Active Constituent (TGAC) approval scheme (1985-1992) resulted in the review of the toxicology profile of some 400 agricultural chemicals, which included diazinon. Additionally four applications for approval of a source of manufacture for diazinon have been evaluated since 1987.

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 Family Services. 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). In the case of maximum residue limits (MRLs), these were formerly established by the Pesticide and Agricultural Chemicals Committee (PACC) of the NHMRC, however, in 1992, the Department of Health became directly responsible for establishing MRLs, a function subsequently transferred to the NRA in June 1994.

Maximum residue limits (MRLs) for diazinon were set, in Australia, in compliance with the 1965 and 1967 JMPR evaluations. Based on an NOEL of 0.02 mg/kg bw/day in a subchronic dog study, the ADI was originally set at 0.0002 mg/kg bw/day in 1965. In 1966, this was increased to 0.002 mg/kg bw/day based on an IBT short-term study in humans which found the NOEL based on inhibition of plasma ChE to be 0.02 mg/kg bw/day. To reflect intraspecies variability a safety factor of 10 was used to calculate an Acceptable Daily Intake (ADI).

The regulatory history of public health considerations of diazinon in Australia is summarised below.

History of Public Health Consideration of Diazinon in Australia

Jun 1962	PACC: MRL recommended for all products.
Dec 1964	PACC: Amend MRL for fruit, vegetables, grains.
Dec 1970	PACC: MRL recommended for fat of meat of cattle and sheep.
Mar 1971	PACC: Amend MRLs for peaches, citrus fruits, cole crops, fat of meat of cattle and sheep, all

	other fruits and vegetables.
Jul 1971	PACC: New entry: Section 3 – pastures, forage crops.
Feb 1974	PACC: MRL recommended for leafy vegetables. Delete MRL for cole crops. Delete Section 3. New entry: Appendix 2, part 1 – insecticide on pastures, forage crops.
May 1974	PACC: MRL recommended for fat of meat of pigs and raw cereals.
Aug 1974	PACC: MRL recommended for milk and milk products. [ADI of 0.0025 (FAO)]
Nov 1975	PACC: MRL recommended for sugar cane. [ADI of 0.002 (FAO)]
Nov 1976	PACC: MRL recommended for nuts.
Feb 1977	PACC: MRL recommended for olives (unprocessed), olive oil, sweetcorn, nuts, vegetable oil (except olive oil).
Dec 1978	PACC: MRL recommended for kiwifruit. Use in Australia requires Australian residue data.
May 1979	PACC: MRL recommended for kiwifruit. MRL for water recommended.
Nov 1980	PACC: MRLs in meat of goats. Extended definition of meat in Appendix 4 to include rabbit and venison.
Feb 1980	PACC: Current MRL of 0.1 for diazinon in raw cereals would be sufficient to cover compound used for the control of bloodworm in rice crops.
Feb 1983	PACC: Microencapsulated insecticide containing diazinon for control of various insect pests on turf. Appendix 2, Part 2 – New Entry Polyurea-polimide polymer B microencapsulating agent. Current MRL for raw cereals covers use. Residue data not required.
May 1984	PACC: Clarification of use patterns. Application requesting establishment of an ERL for pineapples was withdrawn – pending the availability of residue data to support the application Current MRLs adequate with 14 day WHP
May 1985	PACC: Delete entries for kiwifruit (whole fruit) and kiwifruit (edible portion). Confirmed the entry for diazinon in kiwifruit 0.5.
Feb 1986	PACC: Agreed that tick collars were covered by the Schedule 6 entry for diazinon.
May 1986	PACC: Teratology study to be requested. Received replacement studies for invalid IBT studies. A NEL of 0.2mg/kg/day was agreed resulting in an ADI of 0.002 mg/kg bw/day.
May 1986	DPSC: Recommended that diazinon be retained in Schedule 6.
Sept 1986	PACC: Delete Appendix 2, Part 1 – insecticide on pastures and forage crops. MRL recommended for poultry meat, eggs.
Feb 1987	DPSC: Change in scheduling was not warranted. Sulfotepp impurity was found, but level was low. Committee recommended new entry. Appendix L – Part 1 Diazinon, Max. SulfoTEPP 2g/kg
Feb 1987	PACC: No amendment to the ADI & NEL were necessary
Apr 1987	SCOT: NSW request for evaluations on diazinon replacements for organophosphates.
Jun 1988	PACC: Tox update (TGAC)
Feb 1988	DPSC: TGAC approval – new data did not warrant alterations to existing scheduling, first aid instructions or safety directions and did not raise objection to the approval.
May 1989	DPSC: Additional studies reviewed – members concluded that the additional studies had no bearing on the current Schedule 5 or 6 classification of diazinon
May 1989	PACC: No change to the present ADI of 0.001 mg/kg bw/day.
Dec 1990	PACC: MRL add 0.7 meats (mammalian) (in the fat) 0.7 edible offal (mammalian) delete all reference to entries for fat of meat of cattle, pigs, goats and sheep WHP for livestock would apply to horses.
Jun 1991	PACC: No change to MRL Standard. TGAC approval granted.
Jun 1991	DPSC: Microencapsulated diazinon for use in a domestic veterinary pesticide. Agreed that microencapsulated diazinon was suitable for use in domestic veterinary pesticides
Aug 1992	PACC: Ciba Geigy sought approval of TGAC – Committee referred this to the Scientific Director.
Oct 1992	SCOT, PACC & DPSC were informed of the SCOTs concerns regarding the formation of TEPP and sulfoTEPP in stored diazinon. ‘Pesticide Contamination in Lanolin’ – referred to Worksafe Aust.
Aug 1995	NDPS Committee was not convinced that a sufficient increase in safety had been demonstrated for a product which, if rescheduled, would result in a significant change in the use pattern, e.g. in the domestic situation. Human safety data required by sponsor

PACC - Pesticide and Agricultural Chemicals Committee; DPSC - Drugs and Poisons Scheduling Committee; SCOT - Standing Committee on Toxicity; NDPS - National Drugs and Poisons Scheduling Committee.

Acceptable Daily Intake

The current acceptable daily intake (ADI) is 0.001 mg/kg bw/day. This ADI was derived from a NOEL of 0.1 mg/kg bw/day, based on plasma ChE inhibition observed in a 3-month rat study.

MRLs

The current MRLs for diazinon are outlined in the Table below.

The current Australian MRL Standard listing for diazinon.

Codex Code	Food	MRL (mg/kg)
GC 0080	Cereal grains	0.1
FC 0001	Citrus fruits	0.7
MO 0105	Edible offal (mammalian)	0.7
PE 0112	Eggs	*0.05
	Fruits [except citrus fruits, kiwifruit, olives, peach]	0.5
FI 0341	Kiwifruit	0.5
MM 0095	Meat (mammalian)(in the fat)	0.7
ML 0106	Milks (in the fat)	0.5
OC 0305	Olive oil, crude	2
FT 0305	Olives	2
FS 0247	Peach	0.7
PO 0111	Poultry, edible offal of	*0.05
PM 0110	Poultry meat	*0.05
GS 0659	Sugar cane	0.5
VO 0447	Sweet corn (corn-on-the-cob)	0.7
TN 0085	Tree nuts	0.1
OC 0172	Vegetable oils crude [except olive oil, crude)	0.1
	Vegetables	0.7

* at or about the limit of detection.

1.2 International Toxicology Assessments

JMPR

Diazinon has been reviewed by the Joint FAO/WHO Meeting of Pesticide Residues (JMPR) in 1963, 1965, 1966, 1967, 1968, 1970, and 1993. In 1965, the JMPR established an ADI for diazinon of 0.0002 mg/kg bw/day based on plasma ChE inhibition in a 43-week dog study. In 1966, the ADI was amended to 0.002 mg/kg bw/day based on a NOEL of 0.02 mg/kg bw/day for inhibition of plasma ChE activity in a human 37-43 day study (3/group; Lazanas et al, 1966). In support of this ADI, the NOEL for the same endpoint in other species were as follows;

Rat: 0.1 mg/kg bw/day (90-day study)
Dog: 0.02 mg/kg bw/day (30-day study)
Monkey: 0.05 mg/kg bw/day (2-year study).

The most recent review in 1993 that incorporates the change in JMPR policy to use inhibition of brain ChE (or erythrocyte ChE inhibition as a surrogate) as the toxicologically-relevant endpoint has the same ADI based on the NO(A)EL in a different human study (4/group; Payot, 1966). In that study significant plasma ChE inhibition at 0.025 mg/kg bw/day was observed but with no corresponding significant erythrocyte ChE inhibition. In other species, NOELs were;

Rat: 5 ppm, equal to 0.4 mg/kg bw/day (90-day study)
1.5 ppm, equal to 0.07 mg/kg bw/day (99-week study)
10 ppm, equivalent to 0.5 mg/kg bw/day (reproduction study)
20 mg/kg bw/day (maternotoxicity in a teratogenicity study)

Rabbit: 25 mg/kg bw/day (maternotoxicity in a teratogenicity study)

Dog: 0.5 ppm, equal to 0.02 mg/kg bw/day (1-year study)

Human: 0.025 mg/kg bw/day (34-36 day study)

IARC

Diazinon has not been evaluated by the International Agency for Research on Cancer (IARC).

1.3 Identification

Nomenclature:

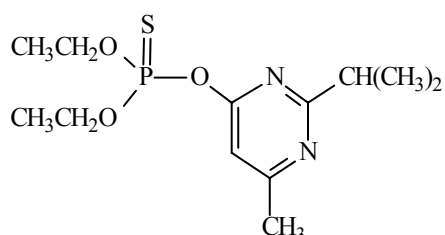
O,O-diethyl-*O*-(2-isopropyl-6-methylpyrimidin-4-yl)-phosphorothioate
(IUPAC);
O,O-diethyl-*O*-(6-methyl-2-(1-methylethyl)-4-pyrimidinyl)-
phosphorothioate. (CA)

Empirical Formula: C₁₂H₂₁N₂O₃PS

Common Name: Diazinon

CAS no.: 333-41-5

Chemical Structure:



Molecular Weight: 304.35

1.4 Physico-Chemical Properties

Colour: colourless

Odour: slight

Physical State:
(at 25°C) liquid

Boiling Point: 84°C

Octanol/Water:
partition coeff.
Kow log P 3.30

Vapour Pressure: 12 mPa at 25°C

Density: 1.116 - 1.118 g/mL at 20°C

Due to its high purity (95%) the physicochemical properties of technical diazinon were stated to be the same as pure diazinon.

1.5 Products

There are a large variety of diazinon products registered in Australia, from emulsifiable concentrates (EC), ultra low volume EC, dusts, microencapsulated aqueous concentrates and slow release pet collars.

2. TOXICOKINETICS AND METABOLISM

2.1 Absorption, distribution, metabolism and excretion.

Excretion and metabolite profiles for rats, dogs, goats, sheep and cows are compared and presented as Tables in section 2.2. A generalised metabolic pathway for diazinon in mammals, generated using data described in the following studies, is shown in Figure 1 (end of section 2.2).

Mouse

Tomokuni K, Hasegawa T, Hirai Y & Koga N (1985) The tissue distribution of diazinon and the inhibition of blood cholinesterase activities in rats and mice receiving a single intraperitoneal dose of diazinon. Department of Community Health Science, Saga Medical School, Saga, Japan. Toxicology 37: 91-98

Diazinon (Kyushu Sankyo Co., 97% pure) in olive oil, injected intraperitoneally to male ddy mice (5/group, Kyudo, Kumamoto, Japan) at 20 or 100 mg/kg bw, was found to achieve a maximal concentration (as determined by GC) in blood after about 1 h. Although maximal concentrations in blood were about 100 ng/mL (estimated from the supplied graph) after a dose of 20 mg/kg bw and 750 ng/mL (estimated) after 100 mg/kg bw, the concentration in brain, liver and kidney (the only tissues tested) were approximately 2.5-, 20- and 45-fold higher than blood at C_{max} and declined at a rate corresponding to that in blood.

Relative to controls, plasma ChE activity had maximally decreased by 90% after 1 h at 100 mg/kg bw and 65% after 3 h (the next sampling time) at 20 mg/kg bw whereas erythrocyte ChE activity was maximally reduced by 85% and 80% respectively after 3 h. At 1 h, brain ChE activity was reduced by 30% at 20 mg/kg bw and 90% at 100 mg/kg bw but this activity rapidly recovered to about 45% inhibition by the next sampling time (3 h), indicating a possible spurious result at the earlier time point. Little ChE activity recovery was evident over the 24-h sampling duration except in plasma at 20 mg/kg bw where only about 45% inhibition was observed at 24 h.

Rat

Wu HX, Evreux-Gros C & Descotes J (1996) Diazinon toxicokinetics, tissue distribution and anticholinesterase activity in the rat. Department of Pharmacology, Faculty of Medicine, INSERM U80, Lyon, France. Biomedical Environ Sci 9: 359-369

Diazinon (99.8% pure; Ciba-Geigy, Basel, Switzerland) emulsified in 5% Tween 20/saline was administered either intravenously (10 mg/kg bw) or orally (80 mg/kg bw) by intragastric tubing to male Wistar rats (16/treatment, IFFA-Credo, France). Blood (0.3 mL) was collected from 8 rats/treatment after 0, 10, 20, 30 and 60 min and from the remainder after 2, 4, 6 and 8 h. Diazinon quantified in plasma by GC fitted with an electron capture detector (sensitivity 25 ng/mL) enabled a number of toxicokinetic parameters to be calculated, ie. Volume of distribution

at steady state (Vd_{ss}), Clearance (Cl), Area under the curve (AUC (0- ∞)), time to maximal concentration in plasma (T_{max}) and half life ($T_{1/2}$); these are shown in the Table below.

Dosing route	Dose (mg/kg bw)	Vd_{ss} (L/kg)	Cl (L/h/kg)	AUC (mg.h/L)	T_{max} (h)	$T_{1/2}$ (h)
Oral	80	22.93	4.60	6.44	2	2.86
IV	10	20.01	4.69	2.27	-	4.7

The hepatic extraction ratio determined by comparing the concentration in the blood from the left carotid artery with that taken from the hepatic vein at 60, 90 and 120 min after an intravenous dose of 5 or 10 mg/kg bw was 54.8% and 47.7% respectively. The oral bioavailability was relatively low at 35.5% indicating that hepatic first-pass extraction had a pronounced effect. Ultrafiltration revealed that 10.9% of unchanged diazinon over a concentration range of 0.4-30 $\mu\text{g/mL}$ was not bound to plasma proteins.

Consistent with a large volume of distribution, a large hepatic first-pass effect and a rapid elimination, more diazinon was found in the kidneys (0.95 $\mu\text{g/g}$), liver (0.47 $\mu\text{g/g}$) and brain (0.45 $\mu\text{g/g}$) than in plasma (0.17 $\mu\text{g/mL}$) at 2 h after intravenous dosing. The concentration in these tissues then declined over the next 6 h at a rate similar to that in blood.

ChE activity in erythrocytes declined to a greater extent than that observed in plasma. Ten minutes after IV administration, erythrocyte ChE activity was significantly reduced by 44% (estimated from graph) and maximally, 75%, after 2 h. In plasma, activity was reduced by 12% after 10 min and by 45% after 2 h. Oral dosing resulted in maximal inhibition of about 56% for erythrocyte ChE and 20% for plasma ChE after 2 h. Little ChE recovery was evident after either oral or IV dosing during the remaining 6 h of observation.

Capps T (1989) Characterization and identification of diazinon metabolites in rats. Report no. ABR-88164. Labs: Wil Research Laboratories Inc., Ashland, OH, USA (Biological phase & Analytical phase I) & Ciba-Geigy Corp, Greensboro, NC, USA (Analytical phase II). Sponsor: Ciba-Geigy Corp, Greensboro, NC, USA. Study duration: Oct 1988 - Feb 1989. Report date: Feb 1989. (GLP - US EPA statement provided)

Diazinon (96.7% pure, lot no. S87-1185) with tracer [$2\text{'-}^{14}\text{C}$]diazinon (>98.7% pure, final specific activity 30.3 $\mu\text{Ci/mg}$ (low dose, lot no. RAF-V-74) or 9.7 $\mu\text{Ci/mg}$ [(high dose, lot no. CL-XIV-23 same as for Group 4)] in polyethylene glycol were administered at 0, 10, or 100 mg/kg bw to Crl:CD Br rats (5/sex/group or 1/sex for controls) by gavage. A fourth group of rats (Group 4) was conditioned by daily treatment with 10 mg/kg bw of unlabelled diazinon for 14 days and then treated with 10 mg/kg bw of labelled diazinon (as for group 2) on day 15. Rats were housed in metabolism cages for 7 days after dosing, after which they were sacrificed and blood and tissues collected for residue analysis and metabolite identification. Rats were monitored twice daily for mortality and clinical signs. Bodyweights were measured prior to dosing and before sacrifice; in Group 4 also on days 8 and 15 (before the radioactive dose) of treatment. Food consumption for Group 4 rats was recorded daily. Tissues analysed were liver, kidney, lung, brain, muscle, testes, heart, spleen, uterus, ovaries, bone, blood, plasma and fat. Metabolites in the urine and faeces were isolated and identified using TLC, HPLC and GC/MS.

There were no treatment-related clinical signs and bodyweight was not affected by treatment. Radioactivity excreted in urine and faeces was 98.4, 99.4 and 98.5% for the low, high and the

pre-conditioned low-dose treatment respectively. For each of these groups an average of 95.9%, 96.6% and 95.7% of the radioactivity was found in urine and the difference, namely 2.5%, 2.8% and 2.8%, was found in the faeces. Excretion of most of the administered radioactivity (>90%) generally occurred during the first 24 h after exposure although the high-dose females showed slower elimination; at 24 h, 60% of the radioactivity had been excreted and after 48 h this had increased to >90%. Radioactivity levels detected in the tissues of rats in both 10 mg/kg bw groups were low with less than 0.01 µg eq/g in most tissues except heart, lung and spleen (all 0.01 µg eq/g); RBC (0.049-0.053 µg eq/g); fat (0.029 µg eq/g in low dose females); ovaries (0.029 µg eq/g). Radioactivity in the fat of high-dose females averaged 0.94 µg eq/g whereas for males it was 0.053 µg eq/g. Other tissues having substantial amounts of radioactivity were RBC (0.38 µg eq/g), ovaries (0.16 µg eq/g), heart (0.1 µg eq/g), spleen (0.1 µg eq/g), lung (0.09 µg eq/g), kidney (0.08 µg eq/g), brain (0.09 µg eq/g), bone (0.04 µg eq/g), testes (0.02 µg eq/g) and plasma (0.02 µg eq/g).

Three metabolites identified in the urine collectively accounted for about 65% of the radioactivity excreted in urine. These were 2-isopropyl-6-methyl-4(1H)-pyrimidone (G27550, 38.2%), 2- α -hydroxyisopropyl-6-methyl-4(1H)-pyrimidone (GS31144, 17.3%) and 2- β -hydroxyisopropyl-6-methyl-4(1H)-pyrimidone (isomer of GS31144 (M3), 9.7%). Small quantities of metabolites with intact ester bonds, ie. diazinon (0.11%), diazoxon (0.08%) and O,O-diethyl O-[2-(α -hydroxyisopropyl)-4-methyl-6-pyrimidinyl]phosphoro-thioate (CGA14128, 0.14%), were also detected. The majority of the remaining radioactivity was associated with polar metabolites (14.9%), presumably sulfates and glucuronides although incubation of urine with β -glucuronidase and aryl sulfatase gave equivocal results. The two major urinary metabolites, G27550 (0.08%) and GS31144 (0.01%) were also the main metabolites identified in the faeces.

Mücke W, Alt KO & Esser HO (1970) Degradation of ¹⁴C-labelled diazinon in the rat. Agricultural Chemicals Research Department, JR Geigy, Basle, Switzerland. J Agr Food Chem 18: 208-212

The metabolism of diazinon (purity not specified), [¹⁴C]-labelled in the pyrimidine ring (2' position, specific activity 4.0 µCi/mg; 4' position, 2.6 µCi/mg) or in an ethoxy group (3.2 µCi/mg) was investigated following gavage (or intravenous to ascertain the formation sequence of metabolites) administration to Wistar WU rats (source not given). Following daily oral gavage administration of [2'-¹⁴C], [4'-¹⁴C] or [ethoxy-¹⁴C]diazinon (4 mg/kg bw, based on a stated mean bw of 200 g) in water-ethanol (8:2, v/v) for 4 days, urine, faeces and expired air were collected from rats (4 M & 2 F for [2'-¹⁴C]; 1 M for [ethoxy-¹⁴C]; not reported for [4'-¹⁴C]) housed in metabolic cages. Metabolites in excreta were isolated using TLC and silica gel column chromatography then characterised by IR, UV, NMR and mass spectrometry. In a second series of experiments to determine tissue distribution, [2'-¹⁴C]diazinon was administered daily by gavage at 0.5 mg/kg bw/day to male rats (number not given) for 10 days. Rats were then sacrificed at 0.25, 1, 2, 5, and 8 days after cessation of treatment. Total radioactivity in excreta, CO₂ and tissues was measured using liquid scintillation spectrophotometry, while radioactive zones on TLC were detected with a scanner.

The half-life of diazinon based on excretion in urine, faeces and expired air over 168 h was estimated to be 12 h irrespective of gender after administration of the 2'- label and 7 h in males after the ethoxy-label; recovery of radioactivity (expressed as % of administered dose) was 98.3% in males and 94.6% in females for the 2'- label and 90.2% for males treated with the ethoxy label. Most of the 2'-radioactivity was excreted in urine (M 80.0%, F 68.9%) and faeces (M 16.0%; F 23.5%) whereas for the ethoxy label, 5.6% of the dose was excreted in expired air, 65.4 % in

urine and 17.5% in faeces. Radioactivity in expired air for the 2'-label was below the level of detection. The metabolic stability of the pyrimidine heterocycle was apparently confirmed with the 4'-labelled diazinon, though no data were shown.

Apart from fat and oesophagus/stomach, no radioactivity was detected 1 day after cessation of treatment in any of the other organs examined, namely small intestine, caecum, colon, liver, spleen, pancreas, kidney, lung, testis and muscle. The total radioactivity (expressed as % of administered dose) detected in all these tissues 6 h after dosing was only 2.92%. The slowest rate of decline was observed in fat where the 0.23% present after 6 h was reduced to 0.18% 1 day later. The most rapid decline was observed in oesophagus/stomach where 0.25% was detected after 6 h and only 0.02% after 1 day.

TLC resolved 4 radioactive fractions in the excreta with 3 being individual compounds and the fourth being a mixture of polar compounds. The individual metabolites were characterised as being 2-isopropyl-6-methyl-4(1H)-pyrimidone (G27550), 2- α -hydroxyisopropyl-6-methyl-4(1H)-pyrimidone (GS31144) and 2- β -hydroxyisopropyl-6-methyl-4(1H)-pyrimidone (isomer of GS31144 (M3)). The median lethal doses of G27550 and GS31144 were shown to be substantially less than diazinon (250 mg/kg bw), ie. 2700 & >5000 mg/kg bw respectively. The relationship of these metabolites (ie. the degradation sequence) was determined by intravenous injection of each separate [2'- ^{14} C]-labelled compound. Metabolite G27550 gave rise to the other three metabolite fractions in urine and faeces, whereas metabolite GS31144 was excreted either unchanged or as fraction 4 metabolites. Metabolite M3 was excreted unchanged.

Tomokuni K, Hasegawa T, Hirai Y & Koga N (1985) The tissue distribution of diazinon and the inhibition of blood cholinesterase activities in rats and mice receiving a single intraperitoneal dose of diazinon. Department of Community Health Science, Saga Medical School, Saga, Japan. Toxicol 37: 91-98
and

Tomokuni K & Hasegawa T (1985) Diazinon concentrations and blood cholinesterase activities in rats exposed to diazinon. Department of Community Health Science, Saga Medical School, Saga, Japan. Toxicol Lett 25: 7-10

Diazinon (Kyushu Sankyo Co., 97% pure) in olive oil injected intraperitoneally to male Wistar rats (5/group, Kyudo, Kumamoto, Japan) at 20 or 100 mg/kg bw was found to achieve a maximal concentration (determined by GC) in blood after 1-2 h. Maximal concentrations in blood were 80 ng/mL (estimated from supplied graph) after a dose of 20 mg/kg bw and 760 ng/mL after 100 mg/kg bw. After 8 h at 20 mg/kg bw no diazinon could be detected in the blood whereas 50 ng/mL was present at a dose of 100 mg/kg bw; the diazinon half life in blood was therefore about 3 h.

Eight hours after dosing at 100 mg/kg bw, the diazinon concentration in the liver was approximately 14-fold lower than in the blood (165 ng/mL) whereas the brain and kidneys were about 3- and 37-fold respectively higher.

Erythrocyte and plasma ChE activity measured only at 100 mg/kg bw were reduced by 77% and 65% respectively after 3 h. There was little evidence of any erythrocyte activity recovery up to 24 h after dosing whereas at 24 h the activity in plasma was 40%.

Garcia-Repetto R, Martinez D & Repetto M (1995) Coefficient of distribution of some organophosphorous pesticides in rat tissue. Instituto Nacional de Toxicologia, Sevilla, Spain. Vet Human Toxicol 37: 226-229

To estimate the extent of pesticide accumulation in rat liver, brain, muscle and adipose tissue, a ratio between the concentration in tissue relative to blood was calculated for various organophosphorus pesticides including diazinon. Each pesticide in olive oil was administered separately by gavage to male Wistar rats (3/group, IFA-Credo, France) at a dose equivalent to 1/3 that of an oral LD₅₀ (values not reported). Measured by GC after 4, 8, 12, 16 and 20 days, diazinon was present in tissues at a lower concentration than that in blood. Hence all distribution coefficients were less than 1 and only adipose tissue (range 0.35-0.62) had a coefficient greater than 0.36 at any time, indicating little diazinon tissue accumulation.

Dog

Iverson F, Grant DL & Lacroix J (1975) Diazinon metabolism in the dog. Pesticide Section, Chemical Safety Bureau, Health Protection Branch, Ottawa, Canada. Bull Environ Contam Toxicol 13: 611-618

To determine the elimination rate of diazinon and its metabolites from plasma, [ethoxy-¹⁴C]diazinon (specific activity 3.4 µCi/mg) in ethanol was administered intravenously to 2 female Beagle dogs (7 and 9 kg bw, source not given) at 0.2 mg/kg bw. To estimate radioactivity content and ChE activity in plasma, blood was drawn from an indwelling cannula in the femoral vein at various times ranging between 5 min and 7 h. These 2 dogs together with another 2 dogs who had received capsules containing 4 mg/kg bw of ring-labelled diazinon (specific activity 3.6 µCi/mg, label position not stated) PO were kept in metabolism cages. Urine was collected for 24 h after dosing and the radioactive fractions separated by GLC, TLC, silica gel and Dowex column chromatography.

The mean plasma half-life of radiolabelled diazinon and its metabolites was calculated to be 6 h (363 min) after IV dosing. ChE activity in plasma was significantly inhibited by approximately 39% relative to predose levels after only 10 min. Activity continued to decline thereafter, albeit at a much slower rate, so that the maximal inhibition of about 50% was detected 2.5 h after dosing.

Urine collected over 24 h from [ethoxy-¹⁴C]diazinon treated dogs contained 58% of the administered radioactivity dose. These radioactive metabolites in the urine were resolved into 2 discrete peaks after passage through a Dowex column and were subsequently characterised as being diethylphosphorothionate (42%) and diethylphosphate (16%). By contrast, the radioactivity in the urine of ring-labelled diazinon treated dogs accounted for 86% of the administered dose after 24 h. Silica gel column chromatography also resolved 2 peaks that accounted for 10% and 23% of the total dose respectively; the balance of the radioactivity (53%) eluting with the solvent front was associated with the most polar (water-soluble) fraction. The two resolved peaks corresponded to 2-isopropyl-6-methyl-4(1H)-pyrimidone (G27550, 10%) and 2- α -hydroxyisopropyl-6-methyl-4(1H)-pyrimidone (GS31144, 23%) and no unchanged diazinon was detected.

Millar KR (1963) Detection and distribution of ³²P labelled diazinon in dog tissues after oral administration. Wallaceville Animal Research Station, Department of Agriculture, Wellington, New Zealand. NZ Vet J 11: 141-144

[³²P]diazinon (specific activity 0.33 mCi/mg) administered orally in 2 capsules to an aged dog (gender, breed and source not given) at 225 mg/kg bw was not fatal but caused excessive salivation immediately and vomiting after 30 min. After a further bout of vomiting and the onset of unco-ordinated movement 2 h later, the dog was sacrificed 10 h after dosing. Of the 14 tissues removed after sacrifice, radioactivity was detected (in descending order) in perirenal fat (81 µg eq/g), pericardial fat (39 µg eq/g), adrenals (31.2 µg eq/g), hind leg muscle (20.6 µg eq/g), tongue (18.6 µg eq/g), omental fat (17.2 µg eq/g), spleen (17.5 µg eq/g), liver (16.4 µg eq/g), hind leg fat (13.6 µg eq/g), heart (11.9 µg eq/g), lung (3.4 µg eq/g), brain (3.3 µg eq/g), kidney (3.2 µg eq/g) and temporal muscle (2.6 µg eq/g). Stomach contents had 838 µg eq/g and blood, 86.6 µg eq/mL; urine collected prior to sacrifice had 694 µg eq/mL of diazinon.

TLC of the radioactivity in urine, stomach (contents), hind leg muscle, pericardial fat and perirenal fat revealed that fat and muscle contained only unchanged diazinon whereas the radioactivity in urine and the stomach contents was predominantly associated with diethylphosphorothionate and diethylphosphate.

Monkey

Wester RC, Sedik L, Melendres J, Logan F, Maibach HI & Russell I (1993) Percutaneous absorption of diazinon in humans. Department of Dermatology, University of California, California, USA and CSIRO-Division of Wool Technology, Belmont, Victoria, Australia. Food Chem Toxicol 31: 569-572

To determine the extent of diazinon excretion, 31.8 µg of [2'-¹⁴C]diazinon (2.1 µCi) in propylene glycol was injected intravenously into 4 female Rhesus monkeys and their urine and faeces collected for 7 days. The overall recovery of radioactivity measured by liquid scintillation spectrophotometry was only 78.4%, of which 55.8% was in urine and 22.6% in faeces. Most of the radioactivity (~40%) in urine was excreted on day 1 whereas in the faeces, radioactivity was excreted per day almost uniformly throughout the 7-day observation period. Since about 22% of the administered radioactivity was not recovered after 7 days and some was still being excreted in the faeces, it was suggested that enterohepatic recycling might be occurring.

Goat

Pickles M & Seim V (1988) Biological report for the metabolism of 2-pyrimidinyl-¹⁴C-diazinon in a lactating goat. Report no. BIOL-88004. Vero Beach Research Center, Ciba-Geigy Corp., Vero Beach, Florida, USA. Study duration: 18 -21 Apr 1988. Report date: 8 Aug 1988. (No GLP statement provided)

and

Simoneaux BJ (1988a) Disposition of ¹⁴C-diazinon in goats. Report no. ABR-88117. Analytical Phase - Ciba-Geigy Corp., Greensboro, NC, USA. Study duration: 27 Apr-16 Sep 1988. Report date: 10 Oct 1988. (GLP - US EPA statement provided)

and

Simoneaux BJ (1988b) Characterization of ¹⁴C-diazinon metabolites in goats. Report no. ABR-88118. Analytical Phase - Ciba-Geigy Corp., Greensboro, NC, USA. Study duration: 27 Apr-26 Sep 1988. Report date: 14 Oct 1988. (GLP - US EPA FIFRA statement provided)

and

Simoneaux BJ (1988c) Metabolite identification in hens and goats treated with ¹⁴C-diazinon. Report no. ABR-88135. Analytical Phase - Ciba-Geigy Corp., Greensboro, NC, USA. Study

duration: 19 May-26 Sept 1988. Report date: 24 Oct 1988. [Data in this report appears to be the same as that described in report no. ABR-88118.]

[2'-¹⁴C]diazinon (>98.8% pure, specific activity 9.7 µCi/mg (lot no. CL-XIV-23) was administered in capsules to 2 lactating goats (1 was of unknown breeding and the other was an Alpine cross. The control goat was a Nubian-Alpine cross and all were procured from the Ciba-Giegy Research Center). Each goat received 1 capsule (containing 150 mg) per day for 4 days, ie. approx. 4.3 mg/kg bw. Goats were housed in metabolism cages for the duration of dosing, after which they were sacrificed (23.27 h and 23.5 h respectively after the last dose); a gross necropsy was performed and blood and some tissues collected for residue analysis and metabolite identification. The goats were monitored daily for mortality and clinical signs while bodyweights were measured prior to dosing and before sacrifice. Food and water consumption were recorded daily. Urine, faeces and milk were collected daily and blood on day 2 and 4; predose samples were collected 4 days prior to dosing. The tissues analysed for residues and metabolite characterisation were liver, kidney, tenderloin, leg muscle, omental fat and perirenal fat. Metabolites in the urine, faeces, milk and tissues were isolated and identified using column chromatography with DEAE-Sephadex or XAD-4, TLC, HPLC and GC/MS. Radioactivity was quantified by liquid scintillation spectrophotometry after sample combustion.

There were no treatment-related clinical signs though bodyweight declined by 2% and 6% respectively and milk production by 7% and 2% respectively despite there being no appreciable decline in food or water consumption. Total radioactivity excreted in urine and faeces over the 4 days of dosing averaged 64.1% and 10.4% respectively. Radioactivity levels detected in the tissues were low with less than 1.2 µg eq/g being in all tissues (ie. tenderloin, leg muscle, omental fat and perirenal fat; 0.29, 0.29, 0.26 and 0.23 µg eq/g respectively) except liver (1.22 µg eq/g) and kidney (2.01 µg eq/g). Leg muscle and liver radioactivity accounted for 0.66% and 0.13% respectively of the dose. On day 2, the average radioactivity present in blood was 0.43 µg eq/mL (0.17% of the dose) whereas at sacrifice it was 0.36 µg eq/mL (0.15%). A similar amount of radioactivity was detected in milk where the range over 4 days was 0.33-0.46 µg eq/mL (or 0.07-0.08%) so that at sacrifice the total amount excreted in milk was 0.31% of the administered dose.

Radioactivity in the urine separated evenly (50%) into an aqueous and organic (butanol/ethyl acetate) phase. Two urinary metabolites found in the organic phase, 2-isopropyl-6-methyl-4(1H)-pyrimidone (G27550) and 2- α -hydroxyisopropyl-6-methyl-4(1H)-pyrimidone (GS31144), accounted for 4.5% and 12.5% respectively of the radioactivity administered. Much of the radioactivity associated with the aqueous phase polar metabolites was fractionated into 4 peaks after DEAE-Sephadex gel filtration and two of these reduced from 14.6% and 11.2% to 2.1% and 1.6% after β -glucuronidase and acid hydrolysis treatment, suggesting the presence of glucuronides. The other 2 peaks were unaffected by these treatments and aryl sulfatase had no effect on any fraction. Metabolites in faecal samples separated mainly into the organic phase (98%) and the metabolites G27550 and GS31144 accounted for 2.6% and 1.7% respectively of the radioactivity of total administered dose. Metabolite profiles in milk and tissues, except omental and perirenal fat, were essentially similar to that found in the urine. Fat appeared to favour the accumulation of unchanged diazinon and metabolites with an intact ester linkage (eg. CGA14128) so that more than 3/4 of the radioactivity found in these tissues was in this form. In milk there was proportionally more free GS-31144 and G27550 relative to urine because there were fewer diazinon conjugates.

Gross pathology examination did not reveal any treatment attributable changes in the cardiovascular, respiratory, urogenital, musculoskeletal, lymphatic or gastrointestinal systems, and the integument.

Sheep

Janes NF, Machin AF, Quick MP, Rogers H, Mundy DE & Cross AJ (1973) Toxic metabolites of diazinon in sheep. MAFF, Central Veterinary Laboratory, Surrey, England. J Agr Food Chem 21: 121-124

Diazinon administered at 1 g/kg bw by stomach tube to 2 sheep (gender, breed and source not given) produced "moderate symptoms" of poisoning (though these were not detailed). Unchanged diazinon and hydroxydiazinon (Figure 1, metabolite ii) were quantified in the blood collected at 3, 6, 24 and 48 h of 1 sheep. Metabolites were extracted and characterised by silica gel column chromatography, Florisil-alumina columns, TLC, GLC, infrared, NMR and mass spectroscopy. The maximal diazinon concentration in blood (5.4 µg/mL) was achieved 24 h after administration whereas the hydroxydiazinon concentration was greatest (1.9 µg/mL) at 48 h.

The distribution of diazinon monitored after 48 h (in 1 sheep) showed that the concentration (µg/g) of unchanged diazinon in the tissues (taken) was; liver (18), brain (15), muscle (14), kidney (12), fat (624), and blood (4.9 µg/mL). Apart from liver and fat, the tissue concentration of hydroxydiazinon ranged between 3-5% that of diazinon. For liver, the diazinon:hydroxydiazinon ratio was nearly 8:1 and for fat 68:1 indicating that diazinon is accumulated in fat and hydroxydiazinon in the liver.

Two other metabolites were characterised (Figure 1, metabolites vii and iv) and one of these was quantified (isohydroxydiazinon, metabolite vii) in urine and appeared to be excreted at an erratic rate over the 48 h monitoring period.

Cow

Robbins WE, Hopkins TL & Eddy GW (1957) Metabolism and excretion of phosphorus-32 labelled diazinon in a cow. Entomology Research Branch, Agricultural Research Service, US Department of Agriculture, Oregon, USA. J Agr Food Chem 5: 509-513

Diazinon (97% pure, Geigy Agricultural Chemists Research Laboratory, NJ, USA) mixed with [³²P]diazinon and administered orally by capsule to a lactating Hereford cow at a dose of 20 mg/kg bw (final specific activity 5.2 x 10⁸ cpm/g) was monitored in urine (0-36 h), blood (at 0.5, 1, 2, 3, 6, 9, 12, 15, 18, 24, 36, 48, 72, 96 and 120 h), faeces (0-36 h) and milk (6, 12, 18, 24, 36, 48, 72, 96, 120, 144 and 168 h). In blood, maximal radioactivity (3.3 µg eq/mL) was detected 9 h after dosing and was no longer able to be detected at 96 or 120 h. Analysis by paper chromatography revealed that only 18.1% of the radioactivity at C_{max} was associated with unchanged diazinon. Similar kinetics, albeit slightly delayed, were observed in milk where the maximal radioactivity occurred at 18 h (2.3 µg eq/mL) and 25% of the radioactivity in the 6-24 h samples was in the form of unchanged diazinon. The total diazinon excreted in milk represented 0.15% of total dose whereas the bulk of the radioactivity, 74%, was excreted in the urine within 36 h. Analysis of the pooled 0-36 h urine indicated that only 0.2% of the radioactivity was in the form of unchanged diazinon whereas diethylphosphorothioate and diethylphosphate accounted for 50.5% and 44.8% of the total respectively. Relatively small amounts of radioactivity (6.7%) were excreted in the faeces but these metabolites were not characterised.

Claborn HV, Mann HD, Younger RL & Radeleff RD (1963) Diazinon residues in the fat of sprayed cattle. Agricultural Research Service, U.S. Department of Agriculture. J Econ Entomol 56: 858-859

Yearling Hereford cows (5/group except for controls with 4) were sprayed once weekly with about 1 to 1.5 gallons of a 0, 0.05 or 0.1% suspension of a wettable diazinon powder formulation in water for 16 weeks. Omental fat was sampled, by omentectomy, for the presence of diazinon (by a colorimetric assay) in 2 cows in each group 6 days after the first, second and sixth sprayings, and from the other 2 cows in each group, 6 days after the 10th spraying. For the 11th spraying, only 2 cows at 0.05% were tested whereas 2 cows in each of the other two groups were sampled 1, 7 and 14 days after the last spraying.

Cows at 0.05% had undetectable amounts of diazinon (<0.05 ppm) after the first and second sprayings whereas at 0.1% the mean concentration ranged between 0.56-0.67 ppm. Six days after the 6th spraying the concentration was 0.2 ppm and 0.51 ppm for the 0.05% and 0.1% spray respectively. After spraying, the diazinon concentration gradually declined so that by day 14 it was below the level of detection.

Dermal Studies

Rat

Williams SC & Marco GJ (1984) Percutaneous absorption of 2A-¹⁴C-diazinon in rats. Report no. ABR-84011. Lab: Ciba-Geigy Corp, Greensboro, NC, USA. Study duration: Not stated. Report date: Jun 1984. (No GLP statement provided)

[2'-¹⁴C]diazinon (specific activity was 25.2 µCi/mg at low dose or 2.62 µCi/mg at high dose) in tetrahydrofuran was dermally applied (application volume not stated) without occlusion at 1 or 10 mg/kg bw to a shaved dorsal area measuring 3 sq cm of Harlan Sprague-Dawley rats. For the duration of the radioactivity recovery study, the rats were housed in metabolism cages and a group (4/sex) sacrificed at 2, 8, 24, 48, 72 and 144 h, to enable blood and tissue (brain, spleen, heart, muscle, lung, kidney, liver, stomach, gonads, small and large intestines, fat and the treated skin area) collection for radioactivity quantitation. The optimal duration for the radioactivity recovery study was determined in a preliminary experiment with 1 rat/sex, where 72 h after application, fat still contained a substantial amount of radioactivity. In order to monitor the depletion of radioactivity from fat, rats in the main study were not sacrificed until 144 h after exposure. Monitoring of expired carbon dioxide in the preliminary experiment indicated that the total radioactivity excreted in this form over 72 h was only 0.04% and 0.06% in males and females respectively at 10 mg/kg bw, hence carbon dioxide was not monitored in the definitive study. Radioactivity in urine, faeces, solubilised skin, cage wash, skin wash, tissues and air from the cage (vaporised diazinon) was measured by liquid scintillation spectrophotometry at 24, 48, 72 and 144 h after treatment. Tissues, carcass, faeces and blood were combusted in a sample oxidiser prior to liquid scintillation counting.

Recovery of radioactivity 144 h after application was 96.3-101.5% for males and females at both concentrations tested; most was present in the urine (69-81%) followed by the volatiles in air (average 17.7% at 1 mg/kg bw and 10.3% at 10 mg/kg bw). Radioactivity in faeces accounted for 7.45% and 5.3% at 1 and 10 mg/kg bw respectively, whereas approximately 0.3% of both doses was retained in tissues. Radioactivity detected within the skin at the application site accounted for

0.19% of the dose in males at 1 mg/kg bw but was only at threshold detection levels (0.05%) for males at 10 mg/kg bw and females at both doses. Diazinon remaining on the skin surface, the cage wash and the blood had levels below the limit of detection.

The investigators claim that the rate of dermal absorption (assumed from the difference between that applied and that remaining on and in the skin) measured after 2, 8 and 24 h was relatively rapid and dose dependent, with the absorption T50 (time for 50% of the applied dose to be absorbed or penetrate into the skin) estimated to be 11.8 h and 10.2 h for males at 1 and 10 mg/kg bw respectively, and 5.2 h and ~ 3 h (though incorrectly reported as 5.25 h) for females at 1 and 10 mg/kg bw respectively. However, these calculations for percutaneous absorption rate ignore loss as 'volatiles' and so all would appear to be overestimates. The extent of the overestimate is not possible to accurately calculate from the supplied data because although cumulative 'volatiles' accounted for 9.5% and 5.1% of the low and high dose respectively in males and 13.4% (though one rat had 28.4% loss which appears to be correct based on a concomitant reduction in radioactivity excreted in urine and faeces, and a total recovery of 95%; otherwise the mean of 3 rats was 8.3%) and 5.5% respectively in females after 24 h; the loss after 2 and 8 h was not reported. However, making an assumption that the loss (due to vaporisation) from the application site will obey similar kinetics as for the cumulative loss after 24, 48, 72 and 144 h, ie. exponential, then most of the cumulative loss will occur during the first 2 h followed somewhat less for the next 6 h etc. Thus, using least squares curve fitting, the calculated T50s will be approximately an hour longer than estimated by the investigators.

The combined rate of excretion in urine and faeces, T50 (time for 50% of the applied dose to be excreted), were calculated to be 28.1 h and 24.1 h for males at 1 and 10 mg/kg bw respectively, and 26.8 h and 20.3 h for females at 1 and 10 mg/kg bw respectively.

Inspection of the individual animal data revealed that the radioactivity present in the stomach accounted for up to 5.8% of the dose 2 h after dermal application at 10 mg/kg bw [ie. %Dose: males - 1.64, 0.28, 0.14, 0.11; females - 2.22, 3.25, 2.97, 5.79, after 2 h at 10 mg/kg bw]. These results suggest that a significant proportion of the dermally applied dose was ingested and despite the absence of a corresponding increase in stomach radioactivity for rats at 1 mg/kg bw after 2 h (the first time point), the possibility that these rats also ingested a proportion of the dose cannot be excluded because orally administered diazinon is rapidly absorbed from the stomach (see Wu et al, 1996). Therefore, the use of jeweller's stainless steel chain attached to the rear legs to prevent scratching of the application area and then licking their feet would appear to be inadequate to prevent some oral ingestion of the dermally applied dose. An Elizabethan-type collar would probably have been more effective.

This study has been compromised by the oral ingestion of least a portion of the dermally-applied dose and is therefore unsuitable to permit any meaningful absorption rates to be calculated.

Dog

Ferrandes B (1990) Bioavailability of Dimpylate 20% Spot On in the dog after single administration of 20 mg/kg by the cutaneous route. Report no. 25/89/56. Lab: S.E.R.P Laboratories, France. Sponsor: Virbac Laboratories, Carros Cedex, France. Study duration: Jan - Feb 1990. Report date: Feb 1990. (No GLP statement provided)

A product, Dotton Flea Control (or Dimpylate 20% Spot On), containing 20% (w/v) of diazinon, was applied to the unshaved dorsal neck region of 6 Beagle dogs (3/sex, 10-12 kg bw, E.C.D.L

Breeding Centre, Gambais, France) to determine the percutaneous absorption rate. A dose of 20 mg/kg bw (as the product in approx. 1 mL or approx. 4 mg/kg bw as a.i.) was applied to a dermal area exposed by brushing the hair apart. Diazinon concentration in plasma was determined at 0, 1, 3, 5, 8, 12, 24, 48, 168, 336 and 504 h after treatment using a GC-MS method.

Diazinon was detected in the plasma of all dogs 1 h after treatment and a mean maximum concentration (C_{max}) of 19 ng/mL (range 14.5-28 ng/mL) was achieved 8-12 h after dosing except for 1 dog in which the maximum occurred after 48 h. The concentration in plasma declined slowly thereafter, falling below 0.5 ng/mL after 7 days, and reaching undetectable levels (<0.25 ng/mL) at 14 days in 5 of the 6 dogs. Mainly because of the large time intervals between blood sampling, the kinetic profiles generally did not permit calculation of plasma diazinon half-lives, however for 2 dogs in which this was possible, values of 25 and 84 h respectively were calculated.

Sheep

Capps T (1990) Characterization and identification of major metabolites in tissues of sheep treated dermally with ¹⁴C-diazinon. Report no. ABR-90014. Labs: Vero Beach Research Center, Ciba-Geigy Corp., Vero Beach, Florida, USA. (Biological phase) & Ciba-Geigy Corp, Greensboro, NC, USA (Analytical phase). Sponsor: Ciba-Geigy Corp, Greensboro, NC, USA. Study duration: Jun 1989 - Feb 1990. Report date: Feb 1990. (GLP - US EPA FIFRA statement provided)

and

Carlin TJ (1994) Supplemental report for the characterization and identification of major metabolites in tissues of sheep treated dermally with ¹⁴C-diazinon. Report no. ABR-90014, Amendment I. Sponsor: Ciba-Geigy Corp, Greensboro, NC, USA. Report date: Dec 1994. (GLP - US EPA statement provided)

Radiolabelled [2'-¹⁴C]diazinon (specific activity 3.7 µCi/mg) in acetone was topically applied to 2 sheep daily for 3 days at an approximate dose of 40 mg/kg bw/day. The dermal application site, approximating 10% of the body surface area, was shaved prior to the first dosing and left uncovered during treatment. Sheep were sacrificed 6 h after the last dose and pooled urine (1 sheep only) and some tissues collected for residue analysis and metabolite identification. The tissues analysed for residues and metabolite characterisation were liver, kidney, heart (1 sheep only), leg muscle (1 sheep only) and back fat (1 sheep only). Metabolites in the urine and tissues were isolated and identified using column chromatography with TLC, HPLC and GC/MS. Radioactivity was quantified by liquid scintillation spectroscopy after sample combustion.

During the first application it was noted that the volatility of the preparation precluded an accurate assessment of the applied dose, hence only data for concentrations found in tissues was reported (to reveal possible accumulation). In tissues, the highest concentrations of radioactivity (quantified by HPLC) were found in the kidneys (9.4 µg eq/g) followed by fat (7.3 µg eq/g), heart (4.4 µg eq/g), liver (4.4 µg eq/g) and leg muscle (4.0 µg eq/g). The percentage of extractable radioactivity was in excess of 95% for all tissues and characterisation of the metabolites indicated that most of the radioactivity in fat, heart, and leg muscle was associated with unchanged diazinon, ie. fat, 85.2% from a total extractable of 86.8%; heart, 55.9% from 84.3% and leg muscle, 59.2% from 95.4%. The average levels found in kidneys and liver were 6.1% and 3.6% respectively from an extractable total of 61.2% and 76.7% respectively. Yet despite its presence in kidneys, no unchanged diazinon was detected in the urine. Two major metabolites, 2-isopropyl-6-methyl-4(1H)-pyrimidone (G27550) and 2-α-hydroxyisopropyl-6-methyl-4(1H)-

pyrimidone (GS31144) accounted for most of the characterised metabolites in the tissues. Thus, of the total radioactivity present in tissues, G27550 was present at 1.6% in fat, 16.4% in heart, 23.2% in leg muscle, 24.5% in kidneys (mean) and 41.3% in liver (mean), whereas GS31144 was detected at 12.0% in heart, 13.0% in leg muscle, 22.55% in kidneys and 17.9% in liver. No GS31144 was detected in the fat whereas urine had 10% of G27550 and 22.7% of GS31144.

Polar metabolites whose chromatographic characteristics changed after incubation with β -glucuronidase accounted for 8.6% of the total radioactivity in kidneys and 13.8% in the liver. The balance of the polar metabolites, 27.9% of the total in kidneys and 10.8% in liver, were not characterised. In urine, the majority (40%) of the polar metabolites were glucuronidated while only 18.6% were uncharacterised. No polar metabolites (conjugates) were detected in the other tissues tested.

In Vitro Studies

Machin AF, Rogers H, Cross AJ, Quick MP, Howells LC & Janes NF (1975) Metabolic aspects of the toxicology of diazinon I. Hepatic metabolism in the sheep, cow, pig, guinea-pig, rat, turkey, chicken and duck. Central Veterinary Laboratory, MAFF, Surrey, England. Pesticide Sci 6: 461-473

Microsomes prepared separately from the livers of sheep, cow, Large White Landrace Cross pig, Dunkin Hartley guinea pig, Wistar rat, Dimple White turkey, Dalton chicken and Khaki Campbell duck were incubated with 7-10 $\mu\text{g/mL}$ (incubation conc.) of 95% pure diazinon (source not given). Metabolites produced were characterised by a combination of GC, TLC and GC-MS, and then quantified using GC.

All the species studied converted diazinon to the same metabolites, however, at a variable rate. The main metabolites formed were hydroxydiazinon, diazoxon and hydroxydiazoxon, suggesting that a major pathway observed *in vivo*, namely cleavage of the ester bond, is not functional in this model.

In a follow-up experimental series, metabolites formed after incubation with liver slices were found to be the same as for the microsomal preparations, only the rates were increased. The toxicity of diazinon did not appear to be related to the amount of diazoxon produced because although the rate of oxon formation with rat microsomes was approximately the same as found in the avians, their respective LD_{50} s differ by about 2 orders of magnitude. It was suggested that this difference in LD_{50} might be explained by their differing rates in clearance or detoxification of the oxon.

Nakatsugawa T, Tolman NM & Dahm PA (1969) Oxidative degradation of diazinon by rat liver microsomes. Department of Zoology and Entomology, Iowa State University, Iowa, USA. Biochem Pharm 18: 685-688

Microsomes (5%) prepared from the livers of adult male rats (strain not stated) were incubated with 25 μM of TLC-purified [ethoxy- ^{14}C]diazinon for 1 h and the metabolites analyzed by Dowex 1-X8 ion-exchange chromatography. Metabolism was rapid with about 85% of the substrate (diazinon) being converted to water-soluble metabolites after 20 min and essentially complete after 1 h. Ion-exchange chromatography of the water-soluble metabolites resolved 2 major radioactive peaks, with the smaller (23%) being diethylphosphate and the larger (69%) being diethylphosphorothioate (as judged by retention times). It was reasoned that the presence of

diethylphosphate indicated the transient formation of diazoxon because diethylphosphorothioate is not further metabolised by microsomes.

Yang RSH, Hodgson E & Dauterman WC (1971) Metabolism in vitro of diazinon and diazoxon in rat liver. Department of Entomology and Limnology, Cornell University, New York, USA. J Agr Food Chem 19:10-13

Microsomes prepared from rat (strain not stated) livers (100 mg wet weight) and incubated with [ethoxy-¹⁴C]diazinon (0.2 µmol) or [ethoxy-¹⁴C]diazoxon (amount not stated) for an unspecified time interval generated a number of metabolites that were analyzed by paper and Dowex 1-X8 ion exchange chromatography. The main two metabolites found were diethylphosphate and diethylphosphorothioate whereas diazoxon was metabolised to diethylphosphate only. No evidence of any desethylation was detected with either diazoxon or diazinon.

2.2 Comparative Toxicokinetics and Metabolism

Absorption: Oral absorption has been investigated in a variety of species (rat, dog, goat, sheep and cow). Urinary excretion data indicates rapid and complete absorption from the gastrointestinal tract.

Excretion:Excretion:Excretion:Excretion:Excretion:Excretion: The following Table is a summary of the results obtained in excretion studies following oral administration of [¹⁴C]diazinon by gavage to rats and by capsule to dogs, goats and cows. Excretion of labelled metabolites after intravenous administration of [¹⁴C]diazinon to Rhesus monkeys and after oral administration of [³²P]diazinon to a lactating cow are also shown. Diazinon was radiolabelled with [¹⁴C] at the 2' or 4' position of the pyrimidine ring or within the ring-linked ethoxy group (E).

Excretion of diazinon in rats, dogs, monkeys, goats and cows following oral or intravenous administration

Species [strain]	Dose (mg/kg bw)	Label Site	Collection Interval (h)	Faeces (% dose)	Urine (% dose)	Total (% dose)	Reference
Rat ¶ [Wistar]	4	2'	0-168	20	75	95	Mücke et al (1970)
Rat [Wistar]	4	Ethoxy	0-168	17.5	65.4	82.9	
Rat [NS]	10	2'	0-24	2.5	95.9	98.4	Capps (1989)
	100			2.8	96.6	99.4	
	Precond‡ 10			2.8	95.7	98.5	
Dog [Beagle]	4	†	0-24	ND	85	-	Iverson et al (1975)
Monkey§ [Rhesus]	0.006*	2'	0-168	22.6	55.8	78.4	Wester et al (1993)
Goat [NS]	4.3**	2'	0-96	10.4	64.1	74.5	Simoneaux (1988a)
Cow [Hereford]	20	P	0-36	6.7	74	80.7	Robbins et al (1957)

¶ Male and female excretion mean; ‡ Conditioned at 10 mg/kg bw/day for 14 days prior to treatment; * Assuming 5 kg bw; ** 150 mg/day administered over 4 days; † Pyrimidine-ring labelled but position not specified; § Intravenous administration; NS=Not stated; ND=Not determined.

Metabolism:Metabolism:Metabolism:Metabolism:Metabolism:2Metabolism: Metabolite Profiles in Excreta of Rats, Dogs, Goats and SheepMetabolite Profiles in Excreta of Rats, Dogs, Goats and SheepMetabolite Profiles in Excreta of Rats, Dogs, Goats and SheepMetabolite Profiles in Excreta of Rats, Dogs, Goats and SheepMetabolite Profiles in Excreta of Rats, Dogs, Goats and Sheep

Metabolism of diazinon in rats, dogs, goats and sheep was investigated by radio-TLC analysis of urinary and faecal samples collected after administration of diazinon labelled with [¹⁴C] in the 2-position of the pyrimidine ring. Rat samples were from the animals used in excretion studies with PO dosing and values shown are the mean of treatments. Urinary metabolite characterisation from sheep following a dermal application to simulate dipping is also shown. Metabolites were analysed by techniques such as DEAE-Sephadex, HPLC, TLC, GC/MS or LC/MS with or without prior hydrolysis (ie. acid, aryl sulfatase, glucuronidase). Metabolites formed after oral administration of [³²P]diazinon to a lactating cow were investigated using paper chromatography. Quantitative results (expressed as percent of recovered radioactivity in sample) are summarised in the Table below, while the sequence of metabolite formation is shown in Figure 1.

Metabolism of diazinon in rats, dogs, goats and sheep (expressed as % identified)

Metabolite		Rat			Dog	Goat		Sheep**
Code	Name [†]	Urine [‡]	Faeces [‡]	Urine [§]	Urine [¶]	Urine [¶]	Faeces [¶]	Urine [‡]
G24480	Diazinon	0.11	T	nd	Nd	nd	T	nd
G24576	Diazoxon	0.08	T	nd	Nd	nd	0.2	nd
CGA14128	O,O-diethyl O-2-(α -hydroxyisopropyl)-4-methyl-	0.14	T	nd	Nd	nd	0.1	nd
G27550	2-isopropyl-6-methyl-	38.2	0.08	22.5	10	4.5	2.6	10.0
GS31144	2-(α -hydroxyisopropyl)-6-methyl-	17.3	0.01	22	23	12.5	1.7	22.7
(M3) isomer of GS31144	2-(β -hydroxyisopropyl)-6-methyl-	9.7	T	9	Nd	2.2	0.3	nd
Aq. Conjugates	Glucuronide	14.9	T			9.6	nd	40
	Uncharacterised			21	52	40.4	nd	18.6

T=trace (<0.01); nd=not detected; [†] See Fig 1 for chemical structures; [¶] Simoneaux, 1988 a, b, c (characterisation of metabolites from excretion study), Pickles & Seim, 1988 (see Table at section 2.6); [‡] Urine collected for 6 h after dermal application; ** Capps, 1990; [§] Mücke et al (1970); Capps, 1989; [¶] Iverson et al (1975).

Major pathway $\longrightarrow$

Minor pathway ----->

Glutathionyl GS



3. ACUTE TOXICITY

3.1 Technical Grade Active Constituent

3.1.1 Median Lethal Dose Studies.1 Technical Diazinon

A summary of submitted and published findings of acute median lethal dose studies with technical diazinon is shown in the Table below.

Median Lethal Dose Studies

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
Oral							
Mouse [Tif:NAG]	M/F	5/sex	2% CMC	NS	100, 167, 215, or 359	187 (M/ F)	Bathe (1972a)
Mouse [Slc:ICR]	M/F	10/sex	Corn oil	96.02	105, 137, 178, 231, 300, or 390	177 (M) 178 (F)	Ishige et al (1986)
Mouse [NS]	M	NS	Corn oil	85	NS	82 (M)	Bruce et al (1955)
Rat [NS]						100-150 (M)	
Rat [Sherman]	M/F	NS	Peanut oil	NS	NS	108 (M) 76 (F)	Gaines (1960)
						250 (M) 285 (F)	Gaines (1969)
Rat [Wistar]	M	10	Cottonseed oil	91.4§	NS	271 (Unstabilised†) (M) 466 (normal diet) (M) 215 (low protein diet) (M)	Boyd & Carsky (1969)

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference [GLP]
Oral (Cont.)							
Rat [STD-Wistar]	M/F	10/sex	Corn oil	97.7	418, 503, 603, or 723 (M)	521 (M)	Yoshida et al (1978)
Rat [SD]	M/F	5/sex	Corn oil	95.7	178, 237, 316, 422, or 562	300 (M/F)	Piccirillo (1978)
Rat [Tif:RAIf]	M/F	5/sex	2% CMC	NS	600, 775, 1000 or 1670	850 (M/F)	Bathe (1972b)
Rat [Tif:RAIf]	M/F	5/sex	2% CMC	97.1	200, 300, 400, 600, or 1000	422 (M/F)	Bathe (1980)
Rat [SD]	M/F	5/sex	Corn oil	NS	250, 400, 640, or 1024*¶	775 (M) 499 (F)	Nissimov & Nyska (1984a) [GLP]
Rat [Tif:RAIf]	M/F	5/sex	Water	96.7	200, 1000, or 2000*	731 (M) 614 (F)	Schoch (1985a)
Rat [Tif:RAIf]	M/F	5/sex	Water	96.1	200, 1000, or 2000*	1031 (M) 870 (F)	Schoch (1985b)
Rat [Tif:RAIf]	M/F	5/sex	0.5% CMC & 0.1% polysorbate 80	99.6	200, 1000, or 2000*	870 (M) 878 (F)	Hartmann & Schneider (1987a)
Rat [SD]	M/F	5/sex	None	87.9	800, 1200, or 2020 ¶	1350 (M) 1160 (F)	Kuhn (1989a) [GLP]
Rabbit [English silver]	M/F	3/sex	10% PEG 400	NS	359, 464, 600, or 1000	520 (M/F)	Sachsse (1972a)
Dog [Beagle]	M/F	2/sex	Gelatine capsule	NS	215, 464, 1000, or 5000	>5000 (M/F) (1/4 deaths)	Sachsse (1972b)

NS=Not stated; CMC=carboxymethylcellulose; PEG=polyethylene glycol; * OECD guideline no. 401; ¶ US EPA guideline 81-1; † See section 3.1, Spindler, 1969 & Sterling, 1972; § Refers to stabilised product

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference [GLP]
Dermal {non-abraded}							
Mouse [Swiss Webster]	M	5	Acetone	99	795-4974 (individual doses not specified) (24 h, without occlusion)	2750 (M)	Skinner & Kilgore (1982)
Rat [Sherman]	M/F	NS	Xylene	NS	NS (Without occlusion and the Applied material was not removed)	900 (M) 455 (F)	Gaines (1960)
Rat [Sherman]	M	NS	Xylene	NS	NS (Without occlusion and the Applied material was not removed)	200 (Unstabilised†) (M)	Gaines (1969)
Rat [Tif:RAIf]	M/F	3/sex	None	NS	2150 (24 h, with occlusion)	>2150 (M/F) (0/6 deaths)	Bathe (1972c)
Rat [STD-Wistar]	M/F	10/sex	Methanol	97.7	(M) 982, 1178, 1413, 1696, 2035, or 2443. (F) 565, 654, 785, 942, 1131 or 1357 (24 h, without occlusion)	1666 (M) 876 (F)	Yoshida et al, (1978)
Rabbit [NZW]	M/F	3/sex	None	NS	1000, 2780, 3590, or 6000 (24 h, with occlusion)	3500 (M/F)	Sachsse (1972c)
Rabbit [NZW]	M/F	2/sex	None	NS	630, 1000, 1600, or 2500 (24 h, with occlusion)	960 (M/F) for abraded and non-abraded skin	Tompkins & Asselmeier (1980)
Rabbit [NS]	M/F	5/sex	None	NS	2000 (24 h, with occlusion)*¶	>2000 (M/F) (0/10 deaths)	Nissimov & Nyska (1984b) [GLP]
Rabbit [NZW]	M/F	5/sex	None	87.9	2020 (24 h, with occlusion)¶	>2020 (M & F) (only 2/5 F died)	Kuhn (1989b) [GLP]

NS=Not stated; CMC=carboxymethylcellulose; * OECD guideline no. 402; ¶ US EPA guideline 81-2; † See section 3.1.2, Spindler, 1969 & Sterling, 1972.

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
Intraperitoneal							
Mouse [Tif:MAG]	M/F	5/sex	2% CMC	NS	35.9, 100, 167, or 215	159 (M/F)	Bathe (1972d)
Rat [Tif:RAIf]	M/F	5/sex	2% CMC	NS	215, 317, 600, or 1000	260 (M/F)	Bathe (1972e)

NS=Not stated; CMC=carboxymethylcellulose

Species [strain]	Sex	Mode	Group Size	Vehicle	Purity (%)	Doses Tested (mg/m ³)	LC ₅₀ (mg/m ³)	Reference [GLP]
Inhalation {4 h}								
Mouse [dd]	M	Whole body	10	2% Xylene & 2% Sorpol	99.6	334, 401, 481, 578, 694, 833, 1000, 1200, or 1440 (No droplet sizes given)	630 (M)	Ueda & Aoki (1960)
Mouse [Tif:MAG]	M/F	Whole body	9/sex	None	NS	463, 1215, 2863, or 5254 (38-65% droplets <3 µm)	1600 (M/F)	Sachsse (1972d)
Rat [Tif:RAIf]	M/F	Whole body	9/sex	None	NS	1708, 3418, or 3680 (43-45% droplets <3 µm)	3500 (M/F)	Sachsse (1972e)
Rat [SD]	M/F	Nose only	5 F/group except 5/sex at 5300 mg/m ³	None	NS	3590, 4300, or 5300 (39-42% droplets <5 µm)	>5300 (M) (0/5 deaths) 4873 (F)	Cummins (1985)
Rat [Wistar]	M/F	Whole body	5/sex	None	NS	1970, 3320, 3390, 4130, 4680, or 6530 ¶ (73-91% droplets <5.5 µm)	4370 (M/F)	Hardy & Jackson (1984) [GLP]
Rat [Wistar]	M/F	Whole body	5/sex	None	96.02	2120, 2720, 3330, 3610, or 4180 (67-75% droplets <5.5 µm)	3100 (M/F)	Jackson et al (1987) [GLP]

Species [strain]	Sex	Mode	Group Size	Vehicle	Purity (%)	Doses Tested (mg/m ³)	LC ₅₀ (mg/m ³)	Reference
Inhalation {4 h} (Cont.)								
Rat [SD]	M/F	Whole body	5/sex	None	87.9	2327 mg/m ³ for 4 h¶ (84% droplets <5 µm)	>2327 (M/F) (0/10 deaths)	Holbert (1989) [GLP]
Rat [SD]	M/F	Nose only	5/sex	None	87.9	5437 mg/m ³ for 4 h¶ (50% droplets <3.3 µm)	>5437 (M/F) (0/10 deaths)	Holbert (1994) [GLP]
Guinea Pig [Pirbright White]	M/F	Whole body	9/sex	None	NS	3248 or 5231 (30-35% droplets <3 µm)	5500 (M/F)	Sachsse (1972f)

NS=Not stated; ¶ US EPA guideline 81-3.

Clinical signs of acute oral, dermal, IP and inhalation toxicity were similar for all species and were those typically seen in organophosphate intoxication, namely diarrhoea, salivation, ataxia, pupil constriction dyspnoea, ruffled fur, abnormal posture, tonic-clonic muscle spasms, trismus and hypoactivity. No changes in gross pathology were observed.

3.1.2 Eye and Dermal Irritancy & Sensitisation Studies

A summary of the findings of eye and dermal acute irritancy and sensitisation studies are shown in the Table below.

Irritation studies

Route	Species	Sex	Group size	Method	Result	Reference
Ocular	Rabbit [English silver]	M/F	3/sex	0.1mL/conjunctival sac, rinsed (F), unrinsed (M)	Slight	Sachsse (1972h)
	Rabbit [NS]	M/F	5 M & 1 F	0.1mL/conjunctival sac, unrinsed†	Slight	Nissimov (1984a)
	Rabbit [NS]	F	8	0.1mL/conjunctival sac, rinsed (5), unrinsed (3)	Slight	Hayashi & Yoshida (1979a)
	Rabbit [NZW]	M/F	3 M & 6 F	0.1mL/conjunctival sac, unrinsed (3 M & 3 F), rinsed (3 F)	Slight	Kuhn (1989c)
Dermal	Rabbit [English Silver]	M/F	3/sex	0.5 mL/abraded and non-abraded/24 h occlusive	Non-irritant	Sachsse (1972g)
	Rabbit [NS]	F	6	0.1 mL/abraded and non-abraded/4 h occlusive	Slight	Hayashi & Yoshida (1979b)
	Rabbit [NS]	M/F	2 M & 4 F	0.5 mL/non-abraded/4 h occlusive§	Non-irritant	Nissimov (1984b)
	Rabbit [NZW]	M/F	3/sex	0.5 mL/non-abraded/4 h occlusive§	Slight	Kuhn (1989d)

¶ US EPA guidelines 81-4; § US EPA guidelines 81-5

Sensitisation study

Skin	Guinea pig [DH]	M/F	10/sex	Induction applications†	Sensitiser	Cummins (1987)
Skin	Guinea pig [H]	M	10	Induction applications†	Non-sensitiser	Kuhn (1989e)

† US EPA guideline 81-6; H=Hartley; DH=Dunkin Hartley

Sachsse (1972h), Pre-GLP: Three female English Silver rabbits (source not given) who had 0.1 mL of technical diazinon instilled into one of their conjunctival sacs then rinsed out with

10 mL of water after 30 sec, had no ocular reactions 1, 2, 3, 4 and 7 days following application. However, 1 of 3 male rabbits in whom the diazinon was not rinsed out had a conjunctival reaction (though the exact details were not given) after 1 day but not thereafter. Thus, diazinon is considered to be a slight eye irritant.

Hayashi & Yoshida (1979a), Pre-GLP: A tenth of a millilitre of technical diazinon (purity 98.6%, batch no. not given) was instilled into the conjunctival sac of one eye of 8 female rabbits (strain and source not specified). Five rabbits in one group had their treated eyes washed with 300 mL of water after 5 min whereas the treated eyes of 3 rabbits in a second group remained unwashed. The untreated eyes of all rabbits served as controls. Evidence of ocular irritation was assessed and recorded 1, 24, 48, and 72 h, and 7 days after instillation. Grade 1 redness of the conjunctivae was found in 4/5 with washed eyes and 3/3 with unwashed eyes after 1 h. This redness was still evident at 24 h in 1/5 rabbits with washed eyes and 2/3 with unwashed eyes, but not thereafter in any rabbit. No other ocular reactions were observed. Thus, diazinon is a slight eye irritant.

Nissimov (1984a), OECD US GLP statement provided: A local albino rabbit strain supplied by A Loebenstein Laboratory Animals, Yoqnean (5 M & 1 F), were given a single ocular application of diazinon technical (Makhteshim Chemical Works, Israel, batch no. 660192-226, purity not stated). A volume of 0.1 mL was instilled into the conjunctival sac of one eye per rabbit. Assessment of ocular irritation/damage were made at 1, 24, 52 and 73 h. No corneal or iridial changes occurred but chemosis was observed in 2 of 4 rabbits with reddened conjunctivae (grade 1) at 1 h. All eyes were normal at 24 h. Thus, diazinon is classified a slight eye irritant.

Kuhn (1989c), US GLP statement provided: This study was conducted according to US EPA Guidelines 81-4. Nine New Zealand White rabbits (approx. 12-24 weeks of age), supplied by Ray Nichols Rabbitry, Texas, had technical diazinon (MG 8 FL-880045; batch code 790701, ML5755; purity 87.9%) instilled into one eye. A tenth of a millilitre of test material was placed into the conjunctival sac of the right eye of all rabbits (6 F, 3 M) and then rinsed out of the eyes of 3 (F), 30 sec later. The remaining 6 rabbits (3 M, 3 F) had their eyes rinsed with water after the 24 h recording. Assessments of ocular irritation/damage were made at 1, 24, 48 and 72 h. For rabbits whose eyes were rinsed soon after instillation, reddened conjunctivae (grade 1), chemosis (grade 1) and discharge (grade 1) were observed at 1 h in 3/3, 3/3 and 2/3 rabbits respectively and in 2/3, 0/3 and 1/3 at 24 h, and not thereafter. For those with unrinsed eyes, reddened conjunctivae (grade 1 in 5/6 and grade 2 in 1/6), chemosis (grade 1 in 3/6 and grade 2 in 3/6) and discharge (grade 3 in 4/6) were observed at 1 h and with somewhat reduced incidence and severity after 24 h (ie. grade 1 redness in 6/6, no chemosis and grade 1 discharge in 3/6) and 48 h (ie. grade 1 redness in 3/6 and no chemosis or discharge), and none at 72 h. The maximum irritation score for rinsed eyes was 5.3 and 9.0 for those without rinsing. Based on these results, both treatments caused slight irritation. According to the EEC directive, redness and conjunctival oedema mean values for all rabbits up to 72 h should be less than 2.5 and 2 respectively to permit the classification to be slight; actual redness and oedema scores were 0.5 and 0 respectively.

Sachsse (1972g), Pre-GLP: Undiluted technical grade diazinon (purity and batch not stated) was non-irritant to the skin of English Silver rabbits (3/sex, source not specified) when 0.5 mL was applied to normal and scarified skin (left and right side flank) under gauze, plastic film and adhesive tape for 24 h. Observation of the treated site at the time of removal (ie. 24 h) revealed that only 1 female rabbit had grade 1 erythema with on both intact and scarified skin. At the next (last) observation (72 h after application), no rabbits had any erythema or oedema.

Hayashi & Yoshida (1979b), Pre-GLP: Technical grade diazinon (purity 98.6%, batch not stated) diluted 1:10 with acetone was a slight skin irritant in female rabbits (6/group, strain and source not specified) when 0.1 mL was applied to abraded or unabraded back or flank skin under gauze, tape and bandage for 4 h. Observation of the treated site at the time of removal (ie. 4 h) revealed that 4/6 with intact skin and 6/6 with abraded skin had grade 1 erythema. At the next observation (24 h after application), the incidence had decreased to 1/6 for intact and 2/6 for abraded skin. At the final observation (48 h) all rabbits appeared to have recovered.

Nissimov (1984b), OECD US GLP statement provided: Tests in a local strain of albino rabbits (2 M, 4 F; A Loebenstein Laboratory Animals, Yoqnean) indicated that diazinon (Makhteshim Chemical Works, Israel, batch no. 660192-226, purity not stated) was virtually non-irritant to the skin. A diazinon (0.5 mL) impregnated piece of gauze was applied to a clipped dorsal area and held in place with a porous dressing strip and an adhesive bandage. After patch removal at 4 h, there were no systemic effects or skin irritation effects observed. Similarly, at 1, 24, 48, or 72 h thereafter, no changes were observed.

Kuhn (1989d), US GLP statement provided: This study was conducted according to US EPA Guidelines 81-5. New Zealand White rabbits (3/sex; approx. 12-24 weeks of age), supplied by Ray Nichols Rabbitry, Texas, had 0.5 mL of technical diazinon (MG 8 FL-880045; batch code 790701, ML5755; purity 87.9%) in a 6.25 cm² gauze patch applied to an area of clipped unabraded skin. The patch was secured with a piece of surgical adhesive tape and the torso of each animal wrapped in an elastic corset. The patches were removed after 4 h and the residual test material removed by gentle swabbing. Application sites were examined for irritation at 1 h and then 1, 2, 3, 7, 10 and 14 days following patch removal. Grade 2 erythema was observed in 6/6 rabbits at 1 h, 4/6 at 24 h, 3/6 at 48 h and 2/6 at 72 h, whereas grade 1 erythema was observed in 2/6 rabbits at 24 h, 3/6 at 48 h, 3/6 at 72 h, 3/6 at 168 h and 2/6 at 240 h. Most rabbits (5/6) having grade 2 erythema also had grade 1 oedema at the same time. Oedema associated with grade 1 erythema was observed in 1 rabbit at 48 h and in 2 at 72 h; no oedema was observed after 72 h. The irritation score of 2.8 from a maximum of 8 at 1 h indicates that this diazinon formulation is classified as a slight dermal irritant in rabbits. According to the EEC directive, erythema and oedema mean values for all rabbits up to 72 h should be less than 2 to permit the classification to be slight; erythema score was 1.45 and oedema score was 0.72 for this study.

Cummins (1987), OECD GLP statement provided: Technical grade diazinon (Lot 86032, purity 96.16%) was tested in a Magnusson and Kligman skin sensitisation test using virgin albino Dunkin-Hartley strain guinea-pigs (10/sex/group) from Porcellus Animal Breeding Ltd, Sussex, England. For the primary induction, 3 pairs of injections (0.1 mL) were given intradermally on day 1; Freund's Complete Adjuvant (FCA), diluted diazinon (10% v/v in dist. water), and in combination with FCA (1:1 v/v). A secondary induction with topically applied undiluted diazinon (0.6 mL) on day 8 was preceded by inunction with 10% sodium lauryl sulfate (to maximise dermal absorption) 24 h earlier. Skin contact was for 48 h under occlusion. Challenge with 30 µL of diazinon occurred on day 22 with the application being covered by an occlusive dressing for 24 h. These concentrations used in the main study were chosen on the basis of preliminary testing (where 18/20 either died or were killed *in extremis* after an intradermal injection of 50% v/v diazinon).

Although 2 guinea pigs (1M + 1F) died in the main study, only 1 (M) was attributable to treatment after displaying frank signs of OP toxicity (ie. ataxia, inactivity, pallor, salivation and bodyweight loss) prior to death. Necropsy was unable to determine a cause of death for the other diazinon-treated guinea pig in whom no signs of toxicity were observed before death. No clinical

signs were observed in survivors and bodyweight changes were within an expected range. Occluded topical application of diazinon for 48 h caused dermal exfoliation in all diazinon-treated guinea pigs. Although dermal challenge with dist. water caused 2/10 females to have patchy, slight erythema ($\pm$) after 24 and 48 h, diazinon challenge caused 4/9 females to have slight, confluent erythema (grade 1) after 24 h and after 48 h, 2/9 had $\pm$ erythema, 4/9 had grade 1 erythema and 1/9 had moderate confluent erythema (grade 2). For males, 1/9 had $\pm$ erythema and 2/9 had grade 1 erythema after 24 h, whereas after 48 h, 3/9 had $\pm$ erythema. Thus, diazinon causes a delayed skin sensitising (contact allergenic) potential in guinea-pigs.

Kuhn (1989e), US GLP statement provided: This study was conducted according to US EPA Guidelines 81-6. In a skin sensitisation study in male Hartley albino guinea pigs (approx. 7-10 weeks of age (285-360 g), supplied by Sasco Inc., The Woodlands, Texas; 10/group), no guinea pigs had any irritation response 48 h after challenge with 0.5 mL of 10% (v/v) technical diazinon (MG 8 FL-880045; batch code 790701, ML5755; purity 87.9%) in ethanol. Animals in the positive control group (0.6% (w/v) 1-chloro-2,4-dinitrobenzene in ethanol) gave a mean score of 3.3 after challenge relative to the naive control group score of 0.0. Although a guinea pig in the test group died on day 7 attributable to treatment, an irritation severity index of 0 was calculated for the test group at 48 h after challenge and therefore the diazinon formulation was classed a non-sensitiser.

Sachsse (1972i), Pre-GLP: In a study that lacks sufficient detail to be independently evaluated it was claimed that Pirbright White guinea pigs were devoid of any delayed skin sensitising activity after diazinon treatment.

3.1.3 Effect of Ageing on Acute Toxicity

Spindler (1969); Sterling (1972): During the late 1950s and early 1960s it became apparent that 'aged' technical diazinon could cause laboratory animal death at a lower concentration than had been observed using a freshly prepared product. Further investigation into the cause of this increased toxicity indicated that the presence of a small volume of water and oxygen promoted the formation of diethylphosphorothionate which in turn was further hydrolysed to diethylphosphate. However, both these intermediates are able, possibly under the catalytic influence of other by-products, to combine with itself or one another to form highly toxic tetraethyl-pyrophosphate (O,O-TEPP or TEPP), monothiono-tetraethyl pyrophosphate (O,S-TEPP) or dithiono-tetraethyl pyrophosphate (S,S-TEPP or sulfoTEPP); see Figure 2 below. These products were reported to an oral LD₅₀ of 1 mg/kg bw with the attendant increased ChE inhibition in rats. Many of the median lethal dose studies performed before 1963, when this degradation problem was recognised, were generally much lower than found in later studies using a stabilised formulation (see Table in section 3.1.1, ie. Bruce et al, 1955; Gaines 1960, 1969; Boyd & Carsky, 1969). Formation of O,O-TEPP, O,S-TEPP and S,S-TEPP was claimed to be reduced by the addition of a stabiliser, epoxidised soybean oil, immediately after manufacture.

Meir et al, (1979): SulfoTEPP (S,S-TEPP) in 3 commercial and 3 'military' diazinon formulations was detected by GC/MS. These were a dust, an EC and in an oil solution. Although the percentage of sulfoTEPP ranged between 0.2-0.71%, the lowest levels were detected in the dust formulations. On the basis that the oldest EC formulation tested had one the lower levels of sulfoTEPP it was reasoned that the formulation age was not a useful predictor for the estimating sulfoTEPP content in formulations. The authors concluded that sulfoTEPP was most likely to be formed during the synthesis of diazinon with diethyl thiophosphorylchloride.

Nichol et al, (1982): The acute oral LD₅₀ in rats (strain and source not specified) of HPLC-purified diazinon, 90% technical diazinon or 90% technical diazinon stored for 1 year was 470, 170 and 30 mg/kg bw respectively. The composition of 'new' and 'aged' 90% diazinon used in this study is shown in Appendix I and highlights increases in sulfoTEPP (4x), TEPP (7x) and isodiazinon (19x) concentration with ageing. The acute oral LD₅₀ of pure isodiazinon was 65 mg/kg bw and together with sulfoTEPP and TEPP may have been responsible for the increased toxicity of 'aged' diazinon.

Turle & Levac, (1987): Several different diazinon formulations (EC, LC and dust) at various strengths and available in Canada were tested by GC to determine their S,S-TEPP content. The maximum content found in these products was 0.53% and there was no apparent relationship between the date of manufacture and the S,S-TEPP content.

McDonald, (1993, 1994): Most of the data that were subsequently published in by Allender & Britt (1994, see below) were first presented as an interim report to the Australian Registration Liaison Committee (RLC; established to co-ordinate registration issues between the States and the Commonwealth of Australia). The following report details the background and presents the results of a national survey that monitored the quality of liquid diazinon products. This survey was co-ordinated by the Compliance Section of the NRA in Australia during 1993-4.

A media report in 1993 suggested that the cause of a companion animal death following exposure to a diazinon-containing dog wash was due to the presence of the impurity sulfoTEPP (S,S-TEPP). Whilst it was subsequently established that the particular dog wash causing the death had been an out-of-date product, there had been several other reports from State Agricultural Authorities (Victoria and South Australia) during 1991-2 which, based on state-wide sampling programs for diazinon impurity levels, suggested the stability of many in-date diazinon products was unsatisfactory. In response to these reports, the NRA requested that registrants provide information on the stability of their diazinon products and, additionally, instigated a small-scale nationwide product sampling program to ascertain the extent of the problem. Samples for testing were collected from all states throughout Australia, although almost two thirds of the total tested were sourced from only two states, namely Queensland and New South Wales. The unopened samples were selected at random and included the 23 most popular liquid diazinon products available for retail sale. A total of 48 different products containing either solutions (7 products), EC formulations containing less than 500 g/L (32 products) or EC formulations with greater than 500 g/L (9 products) were sampled. Samples from large volume containers (drums) were collected with polyethylene syringes and transferred to glass jars with teflon sealed lids for storage and transport to the testing facility in Sydney.

All of the registrants who responded to the request for information on the stability of their products indicated that they used TGAC sourced from either Makhteshim Agan or Ciba Geigy. The quantity of the stabiliser (epoxidised soyabean oil, or ESO) added by the two manufacturers to the TGACs following synthesis ranged between 2% and 6% depending on the proposed end use. Generally, product destined for agricultural application had the least ESO whereas that for a veterinary application had the most. Formulators indicated that they then routinely add some additional ESO (ranging between 2% and 8%) during formulation, depending on the end use. One registrant indicated that they routinely used an alternative stabiliser (or water scavenger) namely, 3,4-epoxycyclohexyl carboxylate and an antioxidant, butylated hydroxytoluene.

Of the 167 samples that had been collected from different batches of the 48 products (at the time of reporting), 157 were tested by GC for the diazinon concentration and the presence of two of

the three potential major impurities, namely monothiono-tetraethyl pyrophosphate (O,S-TEPP) or dithiono-tetraethyl pyrophosphate (S,S-TEPP or sulfoTEPP) [See Figure 2, section 3.1.3 for the proposed synthesis pathways]. The detection limit for O,S-TEPP and S,S-TEPP was 0.05 and 0.03 g/L respectively. The remaining 10 samples that had been collected were not tested because of leakage during transport (4), non receipt (5) or not being shipped (1). A third potential impurity in diazinon, namely tetraethylpyrophosphate (O,O-TEPP) was not able to be quantified due to the absence of an appropriate reference standard. For the benchmark impurity limits, the maximum permissible for S,S-TEPP was 2 g/kg a.i. (as listed in the former Appendix L of the SUSDP) and 0.2 g/kg a.i. for O,S-TEPP (from the FAO standard, CP/223). Only those batches (n=28) that were found to have either or both impurities are shown in the Table below. None of the other tested products had any detectable impurities.

Diazinon products with O,S-TEPP and/or S,S-TEPP

Product	Diazinon Label claim	Diazinon Measured	O,S-TEPP		S,S-TEPP	
	g/L	g/L	g/L	(g/kg ai)†	g/L	(g/kg ai)†
Sheepdip Blowfly Suppressant	96	21.0	5.49	261.4	0.48	22.86
	96	24.5	4.67	190.6	<0.03	-
	96	40.0	2.5	62.5	<0.03	-
Dog Wash	150	94.7	1.55	16.37	0.42	4.44
	200	195	1.24	6.36	0.63	3.23
Sheepdip Blowfly Suppressant	200	201.0	0.71	3.53	0.25	1.24
Dog Wash	100	97.2	0.14	1.44	0.27	2.78
	35	33.0	<0.05	-	0.34	10.3
Sheepdip Blowfly Suppressant	80	66.5	<0.05	-	0.32	4.85
	200	182.0	<0.05	-	0.75	4.12
	200	198.0	<0.05	-	0.74	3.74
Insecticide	300	238.0	<0.05	-	0.69	2.90
	300	239.0	<0.05	-	0.65	2.72
Dog Wash	200	197.0	<0.05	-	0.48	2.44
Sheepdip Blowfly Suppressant	80	72.0	<0.05	-	0.18	2.50
Insecticide	225	213.0	<0.05	-	0.34	1.60
Ant Spray	199	199.5	<0.05	-	0.25	1.25
	199	205.5	<0.05	-	0.24	1.17
Sheepdip Blowfly Suppressant	200	219.0	<0.05	-	0.17	0.78
	200	211.0	<0.05	-	0.17	0.81
Yard & Kennel Flea Control	200	198.0	<0.05	-	0.14	0.71
Insecticide	800	813.0	<0.05	-	0.45	0.55
Sheepdip Blowfly Suppressant	200	186.0	<0.05	-	0.10	0.54
Insecticide	800	759.5	<0.05	-	0.40	0.53
Yard & Kennel Flea Control	200	183.3	<0.05	-	0.09	0.49
Dog Wash	50	12.0	<0.05	-	0.02	0.4

Insecticide	800	754.0	<0.05	-	0.31	0.41
	800	808.0	<0.05	-	0.29	0.36

Figures shown in bold italics are those which exceeded the benchmark limits for impurities, ie. 0.2 g/kg ai and 2 g/kg ai for O,S-TEPP and S,S-TEPP respectively. † Calculated relative to the measured diazinon concentration.

Of the 157 batches tested, 26 (or 16.5%) contained S,S-TEPP, and 13 (or 8.3%) of these exceeded the benchmark limit. All 7 (or 4.4%) of the batches which were found to contain the highly toxic O,S-TEPP also exceeded the benchmark limit. Five of the 7 batches with O,S-TEPP also contained S,S-TEPP in excess of the benchmark. Hence, a total of 15 batches (or 9.5%) exceeded either one or both benchmark standards. The number of batches which exceeded the labelled diazinon concentration by $\pm 10\%$ was 35 (or 22%). Analyses performed by the registrants on retained product were reported to contain no S,S-TEPP in excess of the benchmark limit and none were found to contain any O,S-TEPP. However, there appeared to be a marked difference in the concentration of diazinon in the retained product relative to that in samples taken from the products in retail outlets.

Allender & Britt, (1994): In a small-scale nationwide diazinon product sampling survey sponsored by the NRA and reported by Allender and Britt (1994), random samples of several popular diazinon formulations were tested by GC for their diazinon concentration and the presence of O,S-TEPP and S,S-TEPP (see McDonald 1993, 1994 above for more details). Following the detection of both impurities in some samples, the possible relationship between the water content and the degree of degradation was investigated. The water concentration was measured by the Karl Fischer method only in those samples found to contain both S,S and O,S-TEPP. The results of this analysis are shown in the Table below.

Water content in diazinon formulations containing O,S-TEPP and S,S-TEPP

Product	Origin	Label claim Diazinon (g/L)	Water Content (mg/mL)	O,S-TEPP (g/L)	S,S-TEPP (g/L)	Diazinon (g/L)
Dog Wash	NSW	100	0.5	0.14	0.27	97.2
Dog Wash	NSW	150	0.7	1.55	0.42	94.7
Sheepdip Blowfly Suppressant	Qld	96	2.2	4.67	<0.03	24.5
Sheepdip Blowfly Suppressant	Qld	96	3.4	5.49	0.48	21.0
Stock Spray*	NSW	200	3.9	1.24	0.63	195.0
Insect Killer†	Qld	200	1.8	19.4	3.70	150.0
Insect Killer†	Qld	200	1.7	18.75	3.95	113.0
Insect Killer†	Qld	200	6.4	13.4	6.90	135.0

* Listed as dog wash in report to RLC; † Performed after the interim report to RLC

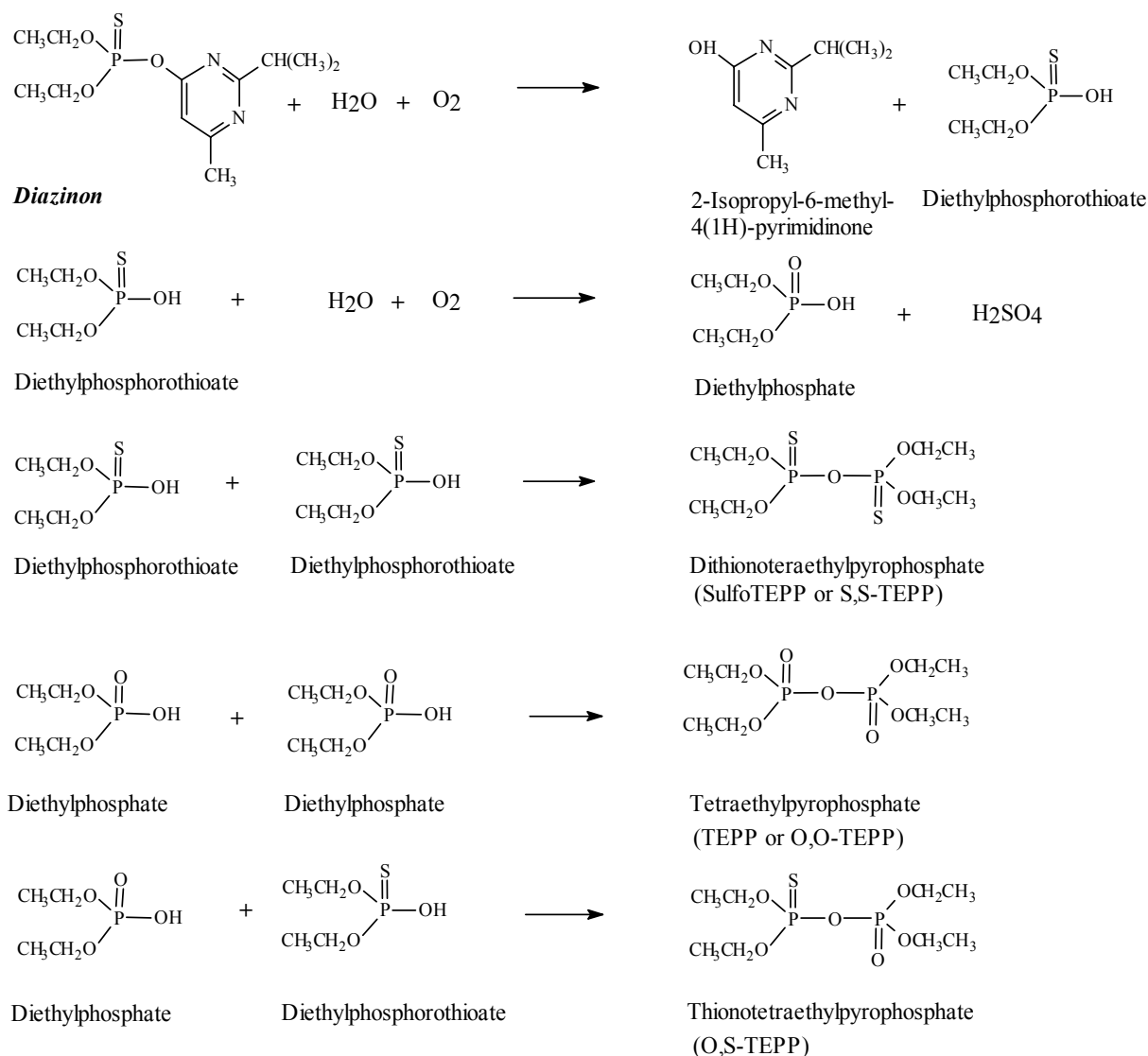
Of the 169 randomly selected samples which included some from the 23 most popular products, 26 (or 15.4%) **that also** failed to meet the $\pm 10\%$ label claim for diazinon were found to contain O,S-TEPP and/or S,S-TEPP. However, only eight (8) (or 4.7%) of these samples, three from New South Wales and five from Queensland had both degradation products. Eight of these 26 products, which included insect killer products (3), dog wash (2), sheep dip (2) and stock spray (1) also contained water (0.5-6.4 mg/mL or 0.05-0.64% w/v) together with high concentrations of O,S-TEPP and/or S,S-TEPP. There did not appear to be a clear relationship between water

content and percentage degradation although it was suggested that there was a link between the integrity of the container to prevent moisture access and the initiation of diazinon degradation.

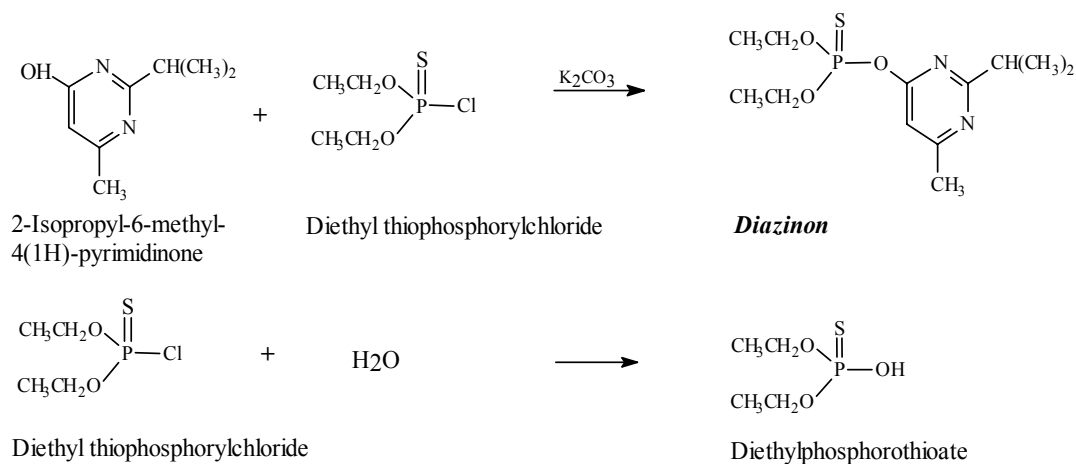
Figure 2

Proposed Formation of Diazinon Degradation Products

During Storage



As a By-Product of Manufacture



3.2 Isomers, Metabolites, and Degradation Compounds.

3.2.1 Oral Median Lethal Dose Studies.

A summary of submitted and published findings of acute median lethal dose studies for the impurities of technical diazinon is shown in the Table below.

Oral Median Lethal Dose Studies

Species [strain]	Sex	Group Size	Vehicle	Purity (%)	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
Degradation products Tetraethylpyrophosphate {O,O-TEPP}							
Rat [SD]	M/F	10/sex	None	NS	0.5, 1, 2, 3, or 4¶	0.95 (M), 0.66 (F)	Kuhn (1995a)‡
Monothionotetraethylpyrophosphate {O,S-TEPP}							
Rat [SD]	M/F	10/sex	None	NS	0.2, 0.5, 0.7, 0.8, 1, 2, 3, or 4 (M) 0.1, 0.3, 0.4, 0.5, 1, 2, 3, or 4 (F)¶	0.71 (M) 0.46 (F)	Kuhn (1995b)‡
Dithionotetraethylpyrophosphate {S,S-TEPP} [SulfoTEPP]							
Rat [NS]	NS	NS	NS	NS	NS	5 (NS)	Fest & Schmidt (1973)
Rat [NS]	NS	NS	NS	NS	NS	~10 (NS)	Tomlin (1994)
Rat [SD]	M/F	10/sex	None	NS	1, 2, 3, 4, 6, 6**, 10, or 10**¶	>10 (M), 3.48 (F)	Kuhn (1995c)‡
Metabolites 2-isopropyl-6-methyl-4-pyrimidinone (G27550)							
Rat [Wistar]	M	NS	NS	~100	NS	2700	Mücke et al (1970)
2-(α-hydroxyisopropyl) -6-methyl-4-pyrimidinone (GS31144)							
Rat [Wistar]	M	NS	NS	~100	NS	>5000	Mücke et al (1970)
Isomer (n-propyl) O,O-diethyl O-(6-methyl-2-propyl-4-pyrimidinyl) phosphorothioate (Pyrazinon)							
Rat [NS]	NS	NS	NS	NS	NS	261	Eto (1974)

NS=Not stated; ¶ US EPA guideline 81-1; ‡ Study performed in 1989 but not reported until 1995, ** Repeat dosing.

3.3 End Use Products.

3.3.1 Median Lethal Dose Studies.

3.3.2 Capstar CS 500 g/L.2.3

A summary of findings of acute dose studies with diazinon CS 500 (micro-encapsulated) formulation is shown in the Table below. In all studies, doses quoted refer to the Product.

Note: The formulation used in the following studies was 'Capstar' dog wash, a 470 g/L micro-encapsulated concentrate.

Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
PO	Rat [Tif:RAIf]	M/F	5/sex	500, 2000 & 5000*	>5000 (0/10 deaths)	Hartmann & Schneider (1987b)
Dermal	Rat [Tif:RAIf]	M/F	5/sex	4000 (24 h, with occlusion)**	>4000 (0/10deaths)	Hartmann & Schneider (1987c)
Inhalation (nose-only)	Rat [Tif:RAIf]	M/F	5/sex	Unable to achieve concentrations higher than 400 mg/m ³ and most droplets were >20 µm	Unable to be performed	Schneider & Gfeller (1988)

* OECD guideline no. 401; ** OECD guideline no. 402.

3.3.3 Eye and Dermal Irritancy & Sensitisation Studies

Irritation studies

Route	Species	Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	M	3	0.1 mL/conjunctival sac, unrinsed ¶	Slight	Schneider & Hartmann (1987a)
Dermal	Rabbit [NZW]	F	3	0.5 mL/non-abraded/4 h occlusive§	Slight	Schneider & Hartmann (1987b)

¶ OECD guideline no. 405; § OECD guideline no. 404

Sensitisation study

Skin	Guinea pig [PW]	M/F	10/sex	Induction applications†	Non-sensitiser	Schneider & Gfeller (1987)
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† OECD guideline no. 406; PW=Pirbright White

Schneider & Hartmann (1987a), OECD GLP statement provided: The eye irritancy potential of diazinon CS 500 g/L (batch no. P 703001) was examined in 3 male New Zealand White rabbits, of approximately 12-14 weeks of age (2.15 to 2.43 kg) and obtained from the Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland. The study was performed in accordance with OECD (no. 405) guidelines. Any ocular response following instillation of 0.1 mL of the formulation into the conjunctival sac of the right eye was examined at 1, 24, 48 and 72 h. Bodyweights recorded before and 3 days after treatment revealed that 1 rabbit had a slight and insignificant loss (2%) after treatment. No corneal changes occurred but grade 1 conjunctival redness was observed in 2/3 rabbits after 1 h although this had resolved by 24 h. Thus, this diazinon formulation is classified as a slight eye irritant in rabbits.

Schneider & Hartmann (1987b), OECD GLP statement provided: This study was performed according to OECD guidelines (no. 404). Three female New Zealand White rabbits (approximately 12-14 weeks of age and weighing between 2.17 to 2.32 kg) obtained from the Kleintierfarm Madoerin AG, Fuellinsdorf, Switzerland had 0.5 mL of micro-encapsulated diazinon CS 500 (batch no. P.703001) in a 20 cm² gauze patch applied to an area of clipped unabrased skin. The patch was covered with aluminium foil and held *in situ* with surgical adhesive tape for 4 h (details for clearing the residual diazinon were not reported). Rabbits were checked daily for clinical signs and mortality and the application sites were examined after 1 h and then 1, 2, 3, 7 and 10 days following patch removal. There were no deaths or signs of systemic toxicity and bodyweight measured before and 10 days after treatment showed no loss. Erythema (grade 1 - primary irritation index) was observed in 2 rabbits after 1 h. At 24 h this reaction had resolved in one but was evident in a third, previously unaffected rabbit (although absent again at 48 h). The severity of the erythema in the other rabbit that had a reaction at 1 h had increased to grade 2 at 24 h and was accompanied by grade 1 oedema. This reaction persisted at day 3 but declined to only grade 1 erythema by day 7 and no reaction on day 10. Therefore this diazinon formulation is a slight skin irritant in rabbits.

Hartmann & Gfeller (1987), OECD GLP statement provided: The skin sensitisation potential of micro-encapsulated diazinon (batch no. P.703001) was tested in albino Pirbright White guinea pigs (10/sex/group) of approximately 10 weeks of age (328-434 g), supplied by an in-house animal breeding facility, utilising OECD guideline no. 406. Microencapsulated diazinon did not sensitise the skin of guinea pigs, following induction with 10 intracutaneous injections, intracutaneous challenge 14 days later (0.1% solution in physiological saline), and epicutaneous challenge after 10 more days (1, 5, 10, 30% concentrations in Vaseline). There were no skin responses 24 or 48 h after intra- or epicutaneous challenge. Bodyweight gains of all treated animals were normal, and there were no other clinical signs of toxicity.

3.3.4 Diacap 300 CS FL-93048.

A summary of findings of acute dose studies with diazinon CS 300 (micro-encapsulated) formulation is shown in the Table below. In all studies, doses quoted refer to the Product.

Note: The formulation used in the following studies was 'Diacap', a 270 g/L micro-encapsulated concentrate.

Median lethal dose studies†

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw) or LC ₅₀ (mg/m ³)	Reference
PO	Rat [SD]	M/F	5/sex	5050*	>5050 (0/10 deaths)	Kuhn (1993a)
Dermal	Rabbit [NZW]	M/F	5/sex	2020 (24 h, with occlusion) [§]	>2020 (0/10 deaths)	Kuhn (1993b)
Inhalation (whole body)	Rat [SD]	M/F	5/sex	2228 mg/m ³ for 4 h [¶] (50% droplets <2.6 µm)	>2228 (0/10 deaths)	Holbert (1993)

† All studies conform with US GLP; * US EPA guideline 81-1; [§] US EPA guideline 81-2; [¶] US EPA guideline 81-3.

3.3.5 Eye and Dermal Irritancy & Sensitisation Studies

Irritation studies†

Route	Species	Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	M/F	3 M & 6 F	0.1 mL/conjunctival sac, unrinsed (3/sex), rinsed (3 F) [¶]	Slight	Kuhn (1993c)
Dermal	Rabbit [NZW]	M/F	3/sex	0.5 mL/non-abraded/4 h occlusive [§]	Slight	Kuhn (1993d)

† All studies conform with US GLP; [¶] US EPA guideline 81-4; [§] US EPA guideline 81-5

Sensitisation study‡

Skin	Guinea pig [H]	M/F	10/sex	Induction applications†	Non-sensitiser	Kuhn (1993e)
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‡ Study conforms with US GLP; † US EPA guideline 81-6; H=Hartley

Kuhn (1993c), US GLP statement provided: This study was conducted according to US EPA Guideline 81-4. Nine New Zealand White rabbits (approx. 12-24 weeks of age), supplied by Ray Nichols Rabbitry, Texas, had a diazinon microencapsulated formulation (Diacap 300 CS FL-93048; batch code P209001; purity not stated) instilled into one eye. A quantity of 0.1 mL of the test material was placed into the conjunctival sac of the right eye of all rabbits (6 F, 3 M) and then rinsed out of the eyes of 3 (F), 30 sec later. The other 6 rabbits (3 M, 3 F) had their eyes rinsed with water after the 24 h recording. Assessment of ocular irritation/damage was made at 1, 24, 48 and 72 h. For rabbits whose eyes were rinsed soon after instillation, reddened conjunctivae (grade 2) in all 3 rabbits and chemosis (grade 1) in 1 rabbit were observed at 1 h but not thereafter. Similarly, for those with unrinsed eyes, reddened conjunctivae (grade 1) was seen in all 6 rabbits at 1 h but not thereafter. Chemosis (grade 1) and discharge (grade 1) were also observed in 2/6 rabbits at 1 h but not thereafter. The maximum irritation score for rinsed eyes was

4.7 and 3.3 for those without rinsing. Based on these results, both treatments caused slight irritation.

Kuhn (1993d), US GLP statement provided: This study was conducted according to US EPA Guideline 81-5. New Zealand White rabbits (3/sex; approx. 12-24 weeks of age), supplied by Ray Nichols Rabbitry, Texas, had 0.5 mL of a micro-encapsulated diazinon formulation (Diacap 300 CS FL-93048; batch code P209001; purity not stated) in a 6.25 cm² gauze patch applied to an area of clipped unabraded skin. The patch was secured with a piece of surgical adhesive tape and the torso of each animal wrapped in an elastic corset. The patches were removed after 4 h and the residual test material removed by gentle swabbing with water. Application sites were examined for irritation at 3/4 h and then 1, 2 and 3 days following patch removal. Grade 1 erythema was observed in 5/6 and grade 2 erythema in the sixth rabbit after 3/4 h. Only the rabbit having grade 2 erythema after 3/4 h had grade 1 at 24 h. Oedema was observed in 4/6 rabbits at 3/4 h but not thereafter. The irritation score of 0.5 from a maximum of 8 at 1 h indicates that this diazinon formulation is classified as a slight dermal irritant in rabbits.

Kuhn (1993e), US GLP statement provided: This study was conducted according to US EPA Guideline 81-6. In a skin sensitisation study in Hartley albino guinea pigs (approx. 7-10 weeks of age (285-360 g), supplied by Sasco Inc., The Woodlands, Texas; 2/sex/group for range finding and 10/sex/group for the definitive study), no guinea pigs had any irritation response 48 h after challenge with 0.4 mL of the micro-encapsulated diazinon formulation (Diacap 300 CS FL-93048; batch code P209001; purity not stated). Animals in the positive control group (0.15% 1-chloro-2,4-dinitrobenzene in acetone) gave a mean score of 1.3 after challenge relative to the naive control group score of 0.1. An irritation severity index of 0 was calculated for the test group at 48 h after challenge and therefore the diazinon formulation was classed a non-sensitiser.

3.3.6 Knox-Out 2FM.

A summary of findings of acute dose studies with diazinon (micro-encapsulated) formulation is shown in the Table below. In all studies, doses quoted refer to the Product.

Note: The formulation used in the following studies was for a 240 mg/L micro-encapsulated concentrate.

Median lethal dose studies†

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
PO	Rat [SD]	M/F	5/sex	5000*	>5000 (0/10 deaths)	Mallory (1993a)
Dermal	Rabbit [NZW]	M/F	5/sex	2000 (24 h, with occlusion)§	>2000 (0/10 deaths)	Mallory (1993b)

† All studies conform with US GLP; * US EPA guideline 81-1; §US EPA guideline 81-2.

3.3.7 Eye and Dermal Irritancy & Sensitisation Studies

Irritation studies†

	Species	Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	M/F	3/sex	0.1 mL/conjunctival sac, unrinsed¶	Non-irritant	Mallory (1993c)
Dermal	Rabbit [NZW]	M/F	3/sex	0.5 mL/non-abraded/4 h occlusive§	Slight	Mallory (1993d)

† All studies conform with US GLP; ¶ US EPA guideline 81-4; § US EPA guideline 81-5

Sensitisation study‡

Skin	Guinea pig [H]	M/F	10/sex	Induction applications†	Non-sensitiser	Armondi (1993)
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‡ Study conforms with US GLP; † US EPA guideline 81-6; H=Hartley

Mallory (1993c), US GLP statement provided: This study was performed according to US EPA Guideline 81-4. New Zealand White rabbits (3/sex; approx. 12-24 weeks of age), supplied by Hazleton Research Products, Denver, had a 23% diazinon microencapsulated formulation (Knox-Out 2FM; batch code EBC-27H2-17; purity not stated) instilled into one eye. A quantity of 0.1 mL of the test material was placed into the conjunctival sac of the right eye of rabbits and an assessment of ocular irritation/damage was made at 1, 24, 48 and 72 h. No ocular reaction was observed in any rabbit up to 72 h after instillation. Based on these results, Knox-Out 2FM causes no ocular irritation.

Mallory (1993d), US GLP statement provided: This study was performed according to US EPA Guideline 81-5. New Zealand White rabbits (3/sex; approx. 12-24 weeks of age), supplied by Hazleton Research Products, Denver, had 0.5 mL of a 23% micro-encapsulated diazinon formulation (Knox-Out 2FM; batch code EBC-27H2-17; purity not stated) in a 6.25 cm² gauze patch applied to an area of clipped unabrased skin. The patch was secured with a dental dam and surgical adhesive tape. The patches were removed after 4 h and the residual test material removed by gentle swabbing with water. The application sites were then examined for irritation at 30-60 min and then 1, 2 and 3 days following patch removal. Grade 1 erythema was observed in 5/6 rabbits between 30 and 60 min but not thereafter. No oedema or more severe irritation was observed in any rabbit. The irritation score of 0.21 at 1 h indicates that this diazinon formulation is classified as a slight dermal irritant in rabbits.

Armondi (1993), US GLP statement provided: This study was performed according to US EPA Guideline 81-6. In the dose range-finding study, 2 males and 2 females were each exposed to different dilutions of the microencapsulated diazinon formulation 'Knox-Out 2FM' (batch code EBC-27H2-17; purity not stated) in distilled water, to determine the highest non-irritating dose. As a result of this study, the test article was applied undiluted in the main study. In the main study the test material was applied to shaved, occluded backskin in 10 male and 10 female Hartley guinea pigs (4-6 weeks old; Buckberg Lab Animals, Tomkins Cove, NY) for 6 h, once per week, for 3 weeks. Negative control animals (5/sex) received distilled water. Positive control animals (3 males and 2 females) were treated with 0.3% of 1-chloro-2,4-dinitrobenzene. The test animals were challenged at a naive skin site 14 days after the last induction, using the same

method of test material application as for induction. No positive responses were observed at 24 or 48 h in the test article treated group, or the negative control group. As expected, 5/5 in the positive control group responded. Therefore 'Knox-Out 2FM', containing 23% of microencapsulated diazinon, was not a skin sensitizer, as assessed by the Beuhler method, in male and female Hartley guinea pigs.

3.3.8 Duogard Collar Powder

Duogard Collar powder contains two active ingredients, diazinon (150 g/kg) and pyriproxyfen (2.5 g/kg). 2.6 Knox-Out 2FM.2.6 Knox-Out 2FM.2.6 Knox-Out 2FM.2.6 Knox-Out 2FM.2.6 Knox-Out 2FMA summary of findings of acute dose studies using Duogard Collar powder (Batch no. LC 5002-2) and performed with GLP compliance are shown below. In all studies, doses quoted refer to the Product.

Median lethal dose studies†

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
PO	Rat [SD]	M/F	5/sex	5000 in 1% aqueous CMC*	>5000 (0/10 deaths)	Mercier (1995a)
Dermal	Rabbit [NZW]	M/F	5/sex	2000 (24 h, with occlusion)§	>2000 (0/10 deaths)	Mercier (1995b)

† All studies conform with US & OECD GLP; * OECD guideline 401; § OECD guideline 402; CMC=carboxymethyl cellulose.

3.3.9 Eye and Dermal Irritancy & Sensitisation Studies

Irritation studies†

	Species	Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	M	6	0.1 mL/conjunctival sac, unrinsed¶	Slight	Mercier (1995c)
Dermal	Rabbit [NZW]	M	6	0.5 mL/non-abraded/4 h occlusive§	None	Mercier (1995d)

† All studies conform with US & OECD GLP; ¶ OECD guideline 405; § OECD guideline 404.

Sensitisation study†

Skin	Guinea pig [H]	M/F	10/sex	Induction applications	Non-sensitiser	Mercier (1995e)
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† Study conforms with OECD GLP and guideline 406; H=Hartley

Mercier (1995c), OECD & US GLP statements provided: This study was conducted according to OECD guideline 405. Six male NZW rabbits, obtained from E.S.D., Romans, France, of about 3 months of age (2-3 kg) had 80 mg (corresponding to a volume of about 0.1 ml) of Duogard powder introduced into the conjunctival sac of the right eye. The eyes were not rinsed and examined at 1, 24, 48 and 72 h and on days 7, 14 and 21 and reactions were scored according to

the OECD guideline. Slight redness (grade 1) of the conjunctiva was observed in 6/6 rabbits at 1 h, 1/6 at 24 h and 1/6 at 48 h. Slight conjunctival chemosis (grade 1) was seen in 5/6 rabbits at 1 h and in 2/6 at 24 h. Slight congestion (grade 1) of the iris was present in 3/6 rabbits at 1 h. Circumcorneal injection of the iris was reported in 4/6 rabbits at 1 h, 6/6 at 24 h, 5/6 at 48 h, 3/6 at 72 h, and 1/6 on day 7, but the reaction was not quantified. The mean score (of the 24, 48 and 72 h readings) was 0.11 for conjunctival redness and chemosis. Since the severity of circumcorneal injection, which persisted for up to 7 days, was not recorded, the eye irritation potential of ground Duogard cannot be determined.

Mercier (1995d), OECD & US GLP statements provided: This study was conducted according to OECD guideline 404. Six male NZW rabbits, obtained from E.S.D., Romans, France, of about 3 months of age (2-3 kg) were treated with 0.5 g Duogard powder prepared as a paste with 0.24 g water on the intact skin under semi-occlusive dressing for 4 h. No signs of irritation was observed at 1, 24, 48, or 72 h after dosing. Duogard was not a skin irritant in rabbits.

Mercier (1995e), OECD & US GLP statements provided: This study was conducted according to OECD guideline 406. The skin sensitisation potential of Duogard powder was studied in albino Hartley guinea pigs (obtained from Charles River France) of about 6 weeks of age using the Buehler test. As induction exposure, twenty guinea pigs (10/sex) were dermally treated with the test material as a 68% paste in water under occlusive dressing for 6 h. The dosing volume was 0.5 ml. The animals were treated at the same site 3 times at 7-day intervals. A naive control group of 10 animals (5/sex) were similarly treated with distilled water. Fourteen days after the final induction treatment, both the naive and treatment groups were challenged with 0.5 ml water and the test material as 68 % paste in water under occlusive dressing for 6 h. The skin reactions were examined at 24 and 48 h after the challenge exposure. No reactions were observed after challenge exposure. The test substance was not a skin sensitiser in guinea pigs. Although the study was claimed to be conducted in compliance with the OECD guideline, no positive control studies were reported.

3.3.10 Dotton Flea Control for Dogs

Dotton Flea Control (or Dimpylate 20% Spot On) for dogs is an EC formulation that contains diazinon (200 g/L) as the active ingredient. . A summary of findings of acute dose studies using Dotton Flea Control for dogs (Batch no. LC 9164; purity not reported) and performed at Hazleton, France with testing facility GLP and QA compliance are shown below. In all studies, doses quoted refer to the Product.

Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
PO	Rat [SD]	M/F	5/sex	1007, 1264, 1596, 1121, or 1416*	1207	Lheritier (1989a)
Dermal	Rabbit [NZW]	M/F	5/sex	1007, 1596, 2518, or 3981 (24 h, with occlusion)**	2559	Lheritier (1989b)

* OECD guideline no. 401; ** OECD guideline no. 402.

3.3.11 Eye and Dermal Irritancy & Sensitisation Studies**Irritation studies**

	Species	Sex	Group size	Method	Result	Reference
Ocular	Rabbit [NZW]	M	6	0.1 mL/conjunctival sac, unrinsed¶	Severe	Mercier (1989a)
Dermal	Rabbit [NZW]	M	6	0.5 mL/non-abraded/4 h occlusive§	Slight	Mercier (1989b)

¶ OECD guideline 405; § OECD guideline 404.

Mercier (1989a): This study was conducted according to OECD guideline 405. Six male New Zealand White rabbits (2.4-2.7 kg bw), supplied by Charles River France, Cléon, France, had 0.1 mL the EC formulation, Dotton Flea Control (or Dimpylate 20% Spot On), instilled into an eye. The test solution remained *in situ* for 24 h, after which it was rinsed with distilled water and examined for possible corneal erosion with the aid of a sodium fluorescein solution (2% w/v). Assessment of ocular irritation/damage were made at 1, 24, 48 and 72 h.

At 1 h, 5/6 and at all assessment times thereafter 6/6 rabbits had corneal ulceration that was accompanied by grade 2 (mean) opacity. The area of corneal opacity ranged between grade 1 (a quarter or less) and 4 (more than three quarters). Iridial changes, characterised by circumcorneal injections and congestion (not quantified), were evident in all (6/6) rabbits after 1 h and remained unchanged over the duration of the assessment period. Permanent myosis with preservation of a direct photomotor reflex was also observed in 2/6 rabbits at 1 h, 1/6 at 24 h, 2/6 at 48 h, and 1/6 at 72 h. Conjunctival reactions, ie. chemosis (oedema) and enanthema (redness), that were both grade 2 (ie. obvious swelling of the eyelids and diffuse crimson conjunctiva) in all (6/6) rabbits after 1 h showed a gradual reduction in mean intensity with time, ie. for chemosis it reduced from 1.82 at 24 h to 1.67 at 48 h, and 1.50 at 72 h whereas for enanthema the mean reduction was from 2 at 24 h to 1.83 at 48 h and 1.67 at 72 h. Thus, based on these results, the EC formulation Dotton Flea Control (or Dimpylate 20% Spot On) is a severe ocular irritant.

Mercier (1989b): This study was performed according to OECD guidelines (no. 404). Six male New Zealand White rabbits weighing between 2.3 and 2.5 kg bw and obtained from Charles River France, Cléon, France and E.S.D., Romans, France had 0.5 mL of the EC applied in a 6 cm² gauze patch to an area of clipped unabrased skin. The patch was covered with a semi-occlusive dressing (perforated adhesive tape) for 4 h (details for clearing any residual diazinon were not

reported). The rabbits were checked daily for clinical signs and mortality and the application sites were examined after 1 h and then at 24, 48 and 72 h following patch removal. Erythema (grade 1 - primary irritation index) was observed in 4/6 rabbits after 1 h. At all times thereafter no erythema was observed in any rabbit, however, slight desquamation was noted in 1/6 rabbits at 48 and 72 h. No oedema was evident at any time in any of the treated rabbits. Therefore based on these results this diazinon formulation is a slight skin irritant in rabbits.

Mercier (1990): Since only a summary of the skin sensitisation study was available, no independent evaluation was possible. However, a sensitization test that complied with OECD guideline 406 revealed that although there was a strong sensitisation reaction observed in Dunkin-Hartley guinea pigs with the positive control (0.5% 1-chloro-2,4-dinitrobenzene), none was evident with 0.5 mL of Dotton Flea Control (or Dimpylate 20% Spot On).

3.3.12 'Preventef' (Flea collar with 15% diazinon)

3.3.13 'Basudin 20ES' (20% EC)

3.3.14 'Basudin 20WP' (20% WP)

A summary of submitted and published findings of acute median lethal dose studies for some Products of diazinon, ie. 'Preventef' (Flea collar with 15% diazinon), 'Basudin 20ES' (20% EC) and 'Basudin 20WP' (20% WP) are shown in the Table below.

Median Lethal Dose Studies

Route	Species	Sex	Group Size	Doses Tested (mg/kg bw)	LD ₅₀ (mg/kg bw)	Reference
'Preventef' {Flea collar with 15% diazinon}						
PO	Mouse [NS]	M/F	NS	NS	635 (M) 517 (F)	Syntex (1985)
	Rat [NS]				973 (M) 843 (F)	
Dermal	Rabbit [NS]				>1250	
'Basudin 20ES' {20% EC}						
PO	Rat [Wistar]	M	6/group	NS	408	Edson & Noakes (1960)
Dermal				NS (20 h, with occlusion)	>1000 (0/6 deaths)	
'Basudin 20WP' {20% WP}						
PO	Rat [Wistar]	M	6/group	NS	293	Edson & Noakes (1960)
Dermal				NS (20 h, with occlusion)	>1000 (0/6 deaths)	

NS=Not stated

3.4 Antidote Studies.

Harris, LW, Fleisher JH, Innerebner TA, Cliff WI & Sim VM (1969) The effects of atropine-oxime therapy on cholinesterase activity and the survival of animals poisoned with

O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate. Medical Research Laboratory, Edgewood Arsenal, Maryland, USA. Toxicol Appl Pharmacol 15: 216-224

To test the effects of atropine and 2-PAM on ChE activity in rats and rabbits after acute diazinon poisoning, technical diazinon (91.9% purity, batch no. FL6199, Geigy Chem Corp, USA) in peanut oil was administered to groups of female albino rats or rabbits. Rats were poisoned with an oral dose equivalent to 0.8 LD₅₀ (ie. 235 mg/kg bw) followed 10 min later by an IM injection of atropine (16 mg/kg bw). Twenty-four hours later, 2 groups of 6 rats each were treated with 30 mg/kg bw of 2-PAM given orally and intravenously respectively. Cholinesterase activity in the diaphragm was measured in sacrificed rats 1 h after 2-PAM treatment.

For rabbits, diazinon was administered SC at 1600 mg/kg bw (LD₅₀=670 mg/kg bw) and after the first signs of poisoning, 16 mg (~6.7 mg/kg bw) of atropine was injected IM. Whole blood cholinesterase activity was measured at various time intervals with some coinciding with the administration of IV 2-PAM (30 mg/kg bw) or in combination of 30 mg/kg bw orally (~1, 3.5 & 19 h; from graph).

Rats treated with atropine and 2-PAM demonstrated significant reactivation of diaphragm ChE levels, ie. 45% and 35% after IV and oral administration respectively relative to 11% in controls.

In rabbits, 2-PAM administration resulted in a reactivation of inhibited blood ChE activity with a concurrent decrease in signs of toxicity. However, clinical signs and blood ChE inhibition reappeared within 2 h of 2-PAM administration.

Younger RL & Radeleff RD (1964) Use of pyridine-2-aldoxime methochloride in the treatment of organic phosphorus compound poisoning in livestock. Toxicological Investigations Laboratories, USDA, Kerrville, Texas, USA. Am J Vet Res 25: 981-987

2-PAM administration alone was effective in treating mild and moderate diazinon intoxication in cattle, sheep and goats. In severe intoxication, 2-PAM alone was ineffective for the immediate relief of poisoning effects, whereas combination therapy with atropine was effective.

3.5 Single Dose Metabolic Effects

3.5.1 Rat

Potrepka RF (1994) Acute cholinesterase inhibition time course study with D-Z-N® diazinon MG87%. Report no. F-00185. Lab: Ciba-Geigy Corp., Crop Protection Division, Environmental Health Center, Farmington, Connecticut, USA. Sponsor: Ciba-Geigy Corp., Crop Protection Division, Greensboro, North Carolina, USA. Study duration: 25 -29 Oct, 1993. Report date: 12 Jan, 1994. (US GLP statement provided)

To monitor the time to onset and the degree of ChE inhibition in plasma, erythrocytes, spinal cord and regional areas of the brain (cerebellum, cerebral cortex, striatum and hippocampus) following an exposure to an OP, technical diazinon (Ciba-Geigy Corp.; purity 88%; Lot no. FL-880045) in corn oil was administered by gavage to groups of 30 fasted Sprague-Dawley rats (Harlan Sprague-Dawley, Frederick, MD, USA; 15/sex) at 0, 2.5, 150, 300, or 600 mg/kg bw. Dose selection was based on a range-finding study (Study no. HWI 6117-221) where at 500 mg/kg bw in males and 250 mg/kg bw in females, whole brain ChE activity was inhibited by about 70%, 24

h after dosing. ChE activity estimations (by colorimetric assay) in 5 rats/group and clinical observations were made before treatment and then at 3, 9 and 24 h after dosing.

Although no deaths occurred, typical signs of OP poisoning (chromodacryorrhea, chromorhinorrhea, loose stools, general muscle fasciculations, diarrhoea, loose stools and/or salivation) were observed in males (~ 2-5/15 per sign) and females (1-5/15 per sign) at 300 and 600 mg/kg bw. These signs appeared first after 3 h at 600 mg/kg bw, though the majority of these effects were observed at 9 h at 300 and 600 mg/kg bw with some reduction in incidence by 24 h.

The mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition (mean percentage reduction)

ChE Location	Interval (h)	Male				Female			
		Dose (mg/kg bw)							
		2.5	150	300	600	2.5	150	300	600
Plasma	3	21**	66**	71**	72**	57**	74**	77**	79**
	9	30**	79**	80**	77**	60**	82**	85**	73**
	24	17**	76**	84**	88**	42**	89**	89**	91**
Erythrocyte	3	0	66**	82**	74**	[1]	42**	50**	73**
	9	[1]	76**	78**	81**	40**	68**	78**	74**
	24	11	68**	77**	76**	11	70**	68**	71**
Cerebellum	3	1	51**	76**	80**	7	54**	48**	66**
	9	6	59**	78**	84**	[2]	65**	79**	77**
	24	[3]	45**	60**	80**	0	68**	74**	81**
Cerebral Cortex	3	[16]	31	67**	75**	[4]	34*	35**	56**
	9	20*	62**	82**	85**	5	63**	75**	78**
	24	[23]	45**	60**	80**	1	73**	77**	85**
Striatum	3	0	28*	69**	75**	[13]	26*	31**	50**
	9	[10]	65**	77**	85**	[9]	66**	81**	83**
	24	[12]	43**	58**	85**	5	68**	84**	87**
Hippocampus	3	[5]	40**	70**	80**	10	46**	47**	56**
	9	5	57**	76**	84**	[5]	68**	81**	83**
	24	[25]	45**	62**	85**	1	65**	74**	81**
Thoracic spinal Cord	3	[10]	27	65**	77**	[8]	39**	33*	49**
	9	[8]	51**	76**	85**	[4]	63**	73**	78**
	24	[9]	42**	50**	81**	[3]	51**	46**	81**

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls;

* p≤0.05; ** p≤0.01 (Dunnett's t test).

Very little regional variation (cerebellum, cerebral cortex, striatum and hippocampus) in the degree of ChE inhibition was apparent in the brain. Significant ChE inhibition (>28%) occurred throughout the brain and spinal cord at doses in excess of 2.5 mg/kg bw. In contrast, significant ChE inhibition was observed in plasma at the lowest tested dose of 2.5 mg/kg bw but not in erythrocytes where significant inhibition became apparent at the next higher dose of 150 mg/kg bw. Irrespective of its location (brain, spinal cord, plasma or erythrocytes), ChE

activity became inactivated (significantly) after 3 h but the maximum inactivity was observed 9 h after dosing. Little or no recovery of ChE activity was evident 24 h after dosing.

Matin MA & Husain K (1987) Cerebral glucose and glycogen metabolism in diazinon-treated animals. Industrial Toxicology Research Center, Lucknow, India. J Biochem Toxicol 2: 265-270

Technical diazinon (batch and source not indicated) administered by intraperitoneal injection to 8 female Wistar rats (source not reported) at a dose of 40 mg/kg bw resulted in tremors and convulsions. Treated rats, sacrificed after these effects became maximal, ie. after 2 h, had significantly reduced ($p<0.05$) ChE (by 57%) activity but elevated fructose 1,6 diphosphatase activity (by 28%) in the brain relative to a corresponding number of untreated controls. Brain phosphoenolpyruvate carboxykinase or glucose-6-phosphate dehydrogenase activities measured at the same time were unchanged. Glycogen content in the brain was also significantly reduced (by 30%; $p<0.05$) after treatment together with a significant rise in lactate (by 40%; $p<0.05$) concentration. Pyruvate concentrations appeared unchanged whereas blood glucose was elevated (by 50%; $p<0.01$). It was speculated that these changes were a compensatory mechanism to provide extra energy to cerebral tissue as a result of the stimulatory effects of diazinon.

3.1.2 Dog

Dressel TD, Goodale RL, Arneson MA & Borner JW (1979) Pancreatitis as a complication of anticholinesterase insecticide intoxication. Department of General Surgery, University of Minnesota, Minnesota, USA. Ann Surg 189: 199-204

Mongrel dogs were used as a model to investigate the induction of pancreatitis by organophosphates; a complication associated with accidental or deliberate exposure in humans that sometimes results in death (see Section 11). A series of experiments were performed to study the effects of diazinon on intraductal pressure, secretory rate and histopathological changes in the canine pancreas.

Diazinon (25 mg/kg bw; Ciba-Geigy Corp.; purity and batch not stated) injected IV to 6 pentobarbital-anaesthetised dogs caused a significant increase in mean intraductal pressure (from 12 to 27.8 cm saline); an effect that was abolished by IV atropine (0.075 mg/kg bw). In a second group of 5 dogs having their minor pancreatic duct and pylorus clamped, secretin stimulated (1 U/kg/h) mean major-duct pancreatic secretion flow rate was increased from 0.13 to 0.56 mL/min after 3 consecutive doses of 25 mg/kg bw diazinon injected IV 5 min apart; this effect was also abolished by IV atropine (0.075 mg/kg bw).

In the third experimental series 2 of 8* dogs, infused with IV secretin (2 U/kg/h) for 1 h and given 3 bolus IV doses of diazinon (25 mg/kg bw) 5 minutes apart after 15 min, died 1 and 2 days after treatment respectively [* It was stated that 12 healthy dogs were divided into four treatment groups, ie. with or without diazinon and sacrificed after 2 or 72 h, yet the number of dogs in the groups totalled 13]. All 8 dogs developed acute hyperamylasemia (ie. mean serum amylase increased to 11,580 SU/dL at 2 h from a pre-infusion level of 1610 SU/dL; $p<0.02$) and hyperlipasemia (ie. mean serum lipase increased to 8.75 STU/mL at 2 h from a pre-infusion level of 0.41 STU/mL; $p<0.02$) during treatment. Serum lipase was still elevated 24 h later (2.13 STU/mL; $p<0.02$) and control dogs ($n=4$ or 5?) that were treated with secretin but not diazinon had no change in their serum amylase or lipase concentrations.

In the dog that died 1 day after treatment, postmortem autolysis prevented any meaningful histopathological investigation. For the other dog that died, the pancreas appeared grossly normal but microscopically had a mild lymphocytic infiltrate. Gross examination of the pancreata of the other diazinon-treated dogs revealed marked interstitial oedema after 2 h that were not evident in those sacrificed at 72 h. Under microscopic examination acinar cell vacuolization was the predominant finding in pancreata at 2 h, with most vacuoles occurring in the lateral or basilar portions of the cells displacing the nuclei; no inflammatory infiltrate was observed. Pancreata from diazinon-treated dogs after 72 h and all controls appeared normal.

[A case study detailing the accidental ingestion of fonofos resulting in hyperamylasemia, elevated temperature and persistent lethargy after the initial acute characteristic clinical signs of OP poisoning was also included in this report.]

Dressel TD, Goodale RL, Borner JW & Etani S (1980) A study of the cholinesterases of the canine pancreatic sphincters and the relationship between reduced butyrylcholinesterase activity and pancreatic ductal hypertension. Department of Surgery, University of Minnesota, Minnesota, USA. Ann Surg 192: 614-619

The major and minor pancreatic ampullae, excised from 4 pentobarbital-anaesthetised dogs 1 h following IV administration of diazinon (25 mg/kg bw; Ciba-Geigy Corp.; purity and batch not stated), were sectioned in a cryostat after freezing to enable tissue enzyme histochemistry to be performed. In 5 control dogs, butyrylcholinesterase activity was identified in the nerves of the duodenum, the pancreatic duct, and those nerves innervating the smooth muscle of the ampullary region. Acetylcholinesterase on the other hand, was found in the same locations except for the sphincteric or duodenal muscle tissue. In dogs treated with diazinon, no histochemically demonstrable butyrylcholinesterase activity was observed in either the smooth muscle or nerves whereas acetylcholinesterase, albeit with reduced activity, was still present. It was reasoned on the basis of these results that the pancreatic ductal hypertension that occurs following diazinon treatment is due to selective reduction in pancreatic smooth muscle butyrylcholinesterase activity.

Frick TW, Dalo S, O'Leary JF, Runge W, Borner JW, Baraniewski H, Dressel TD, Shearen JG & Goodale RL (1987) Effects of insecticide, diazinon, on pancreas of dog, cat and guinea pig. Department of Surgery, Laboratory Medicine and Pathology, University of Minnesota, Minnesota, USA. J Environ Path Toxicol Oncol 7: 1-12

Since OPs inactivate both acetylcholinesterase and butyrylcholinesterase it was reasoned that the comparative investigation of a species with an abundance of acinar cell (tissue) fixed butyrylcholinesterase (eg. guinea pig) with one having an absence (eg. cat) might help explain the propensity for dogs (with abundant tissue-fixed butyrylcholinesterase distribution) to develop pancreatitis after acute diazinon treatment.

In a second study in this series (see Dressel et al, 1979 this section), secretin (1 U/kg/h) was slowly administered IV by infusion together with 3 bolus IV doses (in different veins) of diazinon (25 mg/kg bw) 5 minutes apart to anaesthetised mongrel dogs (8), cats (3) and guinea pigs (3; strain and gender not stated) and all sacrificed after 3 h for dogs and 2 h for cats and guinea pigs. Another regimen, with diazinon alone, was given to 3 cats and 20 guinea pigs; cats were sacrificed at 3 or 6 h whereas for guinea pigs it was 3 (8 guinea pigs), 6 or 24 h (6 guinea pigs/group). Control dogs (8/group), cats (3/group) and guinea pigs (3/group) were treated with saline only.

Diazinon had previously been shown to increase the pancreatic duct pressure in dogs (see Dressel et al, 1979 this section). No change in mean intrapancreatic ductal perfusion pressure was observed in cats and a similar experiment in guinea pigs was not performed. Serum amylase activity increased from 607 U/L in controls to 4700 U/L in diazinon-treated dogs ($p < 0.001$) 3 h after administration but changes in the cat and guinea pig were not significant. In contrast, serum butyrylcholinesterase activity had significantly declined ($p < 0.001$) in the dog, cat and guinea pig by 81%, 82% and 70% respectively.

Histopathological investigation of the pancreas at 3 h confirmed the results of a previous study (see Dressel et al, 1979 this section) where dogs had disseminated vasculitis, focal perivascular exudation and basolateral vacuolisation of the acinar cells after diazinon treatment in the presence or absence of secretin though these changes were more pronounced with the combination treatment; atropine pretreatment (0.2 mg/kg bw) prevented formation of these lesions. In guinea pigs, vasculitis developed after 2 or 3 h in the presence or absence of secretin, and perivascular exudation and basolateral vacuolisation of the acinar cells became apparent at 3 and 6 h though some regeneration was apparent at 24 h; atropine prevented the development of these lesions. Degeneration of mitochondria was characterised by swelling and loss of cisternae; a lesion not observed in dogs, accompanied vacuolisation, oedema and vasculitis. Changes in the cat pancreas at 2, 3 and 6 h was limited to infrequent focal microcystic changes in the endoplasmic reticulum.

Goodale RL, Manivel JC, Borner JW, Liu S, Judge J, Li C & Tanaka T (1993) Organophosphate sensitizes the human pancreas to acinar cell injury: An ultrastructural study. Department of Surgery, Laboratory Medicine and Pathology, University of Minnesota, Minnesota, USA. Pancreas 8: 171-175

Pancreatic fragments taken from five human brain-dead donors and incubated *in vitro* in the presence of 100 μ M echothiophate (an OP) for 1 h and then with 10 μ M acetylcholine for 2 h had acinar cell damage characterised by vacuolisation. Although many of the changes were also observed in fragments incubated in either echothiophate or acetylcholine alone, the mean number of zymogen granules per acinar cell was significantly less ($p < 0.02$) for the combination treatment relative to untreated controls and substantially less than each individual treatment, ie. approx. a 2.5-fold reduction for each.

Woehrle F (1990) Dimpylate 20% Spot On. Safety study in the Beagle dog by the cutaneous route. Report no. 441059. Lab: Hazleton, France, L'Arbresle, France. Sponsor: Laboratoires Virbac, Carros, France. Study duration: 13 Jun – 12 Jul, 1989. Report date: 12 Jan, 1990. (Pre-GLP)

To assess the effects of a single dermal application of Dotton Flea Control (Dimpylate 20% Spot On, Virbac; purity 96.5%; Lot no. LC 9129) in Beagle dogs (Hazleton Research Products, Virginia, USA and Monte-Berico, Vicenza, Italy; 2/sex/group), 0, 20, 60, or 100 mg/kg bw of the formulation was applied to the unshaved neck (interscapular) region and monitoring was performed over 4 weeks. The test material was applied directly to the skin (by parting the fur) and remained unoccluded for the duration of the monitoring period.

Behaviour and general health were monitored daily and the dermal application site was also examined daily and any reactions scored. Food and water consumption were recorded daily and reported weekly whereas bodyweight was recorded and reported weekly; both measurements

commenced 2 weeks before treatment. Blood for clinical chemistry and haematology was collected once before treatment then on day 2, 15 and 29 thereafter. The following haematology parameters were determined; Hb, MCH, MCHC, erythrocyte count, WBC, Hct, reticulocytes, differential blood count, prothrombin time, activated partial prothromboplastin time, and platelets. Clinical chemistry measured sodium, potassium, inorganic phosphorus, calcium, chloride, total bilirubin, total protein, BUN, creatinine, serum glucose, AST, ALT, AP, alkaline phosphatase, albumin, globulin and A/G ratio together with erythrocyte and serum ChE activity. In addition to the 4 occasions on which blood was collected for clinical chemistry and haematology, blood was also collected for ChE activity determination (by a colorimetric method) on days 8 and 22 after treatment. Urine samples collected in a metabolism cage (for ~16 h) once pretest and then days 2, 15 and 29, was used to assess the volume, appearance, specific gravity, pH, glucose, blood, protein, urobilinogen, ketones, bilirubin and sediment.

Necropsy was performed on the dogs that either died or were sacrificed during the study as well as those at the terminal kill. Absolute and body-relative organ weights of the heart, liver, kidneys, testes/ovaries, adrenals, thymus, prostate, thyroids, uterus and brain were also recorded at the terminal kill. Histopathological investigations were performed on specimens of adrenal gland, aorta, bone (sternum), brain, oesophagus, eyes (with optic nerves), ovaries, uterus, uterine cervix, mammary gland, bone (sternum), gall bladder, heart, kidneys, large intestine (caecum, colon), liver, lungs, lymph nodes (mesenteric, mediastinal, cervical), prostate, testes, pancreas, peripheral (sciatic) nerve, pituitary, skeletal muscle, skin (treated and adjacent areas), small intestine (duodenum, jejunum, ileum), spinal cord, spleen, stomach, thymus, thyroid with parathyroid glands, epididymides, and urinary bladder.

There were no deaths or clinical signs associated with treatment. Similarly, there were no treatment-related changes in food or water consumption, bodyweight or in any of the measured haematology or clinical chemistry parameters except for reduced ChE activity in plasma. The mean percentage reduction in plasma ChE activity is shown in the Table below.

Plasma ChE Inhibition (mean percentage reduction)

Dose (mg/kg bw)	Gender	Day 2	Day 8	Day 15	Day 22	Day 29
20	M	81	62	48	39	28
	F	79	67	42	18	1
60	M	80	73	67	47	29
	F	86	77	74	53	25
100	M	82	73	67	32	7
	F	88	81	65	37	30

Similarly, there were no treatment-related changes in urinalysis, organ weights, gross pathology, or histopathology.

4. SHORT-TERM REPEAT-DOSE TOXICITY

4.1 Rat

4.1.1 Oral Administration

Davies DB & Holub BJ (1980a) Comparative subacute toxicity of dietary diazinon in the male and female rat. Department of Nutrition, University of Guelph, Guelph, Canada. Toxicol Appl Pharmacol 54: 359-367

Diazinon (Ciba-Geigy, Canada; 99.2% purity; batch not stated) was fed to Wistar rats (50/sex/group; Woodlynn Labs Ltd, Guelph, Canada) in a semi-purified diet at 0 or 2 ppm (equivalent to 0.2 mg/kg bw/day) for 7 days, or, 0 or 25 ppm (equivalent to 2.5 mg/kg bw/day) for 30 days. The diet was prepared prior to the commencement of the studies by mixing diazinon suspended in corn oil with a semi-synthetic diet. Diazinon concentration in the diet was measured and deemed to be acceptable after concentrations of 1.9 and 26.2 ppm respectively were recorded. Food consumption and rat bodyweight were recorded twice weekly and clinical signs were monitored daily.

Blood for plasma and erythrocyte ChE activity measurements were collected from random groups of 10 rats at various times (3-5 days apart) except for those in the 0 or 25 ppm study where at each bleed the same rats were sampled. At day 15 and 30 in the 30-day study, brain ChE activity was measured in 6 sacrificed rats per group. All ChE activities were measured using a radiometric method, with tritiated acetylcholine as the substrate.

There were no clinical signs observed at any dose level. Treated rats at 2 or 25 ppm had a similar food consumption and bodyweight gain except for female rats at 25 ppm where mean food consumption increased by 9% from day 15 onward. The maximum mean percentage reductions in ChE activity measured in the 2 studies are shown in the Table below.

Maximum ChE Inhibition (mean percentage reduction)

Dose (ppm)	Duration (days)	Plasma ChE		Erythrocyte ChE		Brain ChE	
		male	Female	male	female	male	female
2	7	5	29*	[12]	3	ND	ND
25	30	52*	76*	44*	85*	[3]	6

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; * p≤0.05, ND=Not determined.

Thus, relative to males, ChE activity in female rats appears to be more sensitive to inhibition after exposure to diazinon in the diet.

Chang JCF (1994) Cholinesterase inhibition in 28 day feeding study in rats. Report no. F-00186. Lab: Ciba-Geigy Corp., Crop Protection Division, Environmental Health Center, Farmington Connecticut, USA. Sponsor: Ciba-Geigy Corp., Crop Protection Division, Greensboro, North Carolina, USA. Study duration: 11 Jan - 10 Feb, 1994. Report date: 11 Jul, 1994. (US GLP statement provided)

To monitor ChE inhibition in the blood (ie. plasma and erythrocytes) and regional areas of the brain (cerebellum, cerebral cortex, striatum and hippocampus) of rats after diazinon ingestion, technical diazinon (Ciba-Geigy Corp.; purity 88%; Lot no. FL-880045) was administered in the feed to groups of 30 Sprague-Dawley rats (CrI:CD® BR; Charles River Laboratory, Raleigh, NC, USA; 15/sex) at 0, 0.3, 30, 300, or 3000 ppm for 28 days. Dose selection mimicked those given in a 90-day neurotoxicity study (Pettersen & Morrissey, 1994, Study no. F-00176; see section

10.1), hence at the highest non-lethal dose of 3000 ppm, neurotoxic effects were expected. Analysis of diazinon stability and homogeneity in the feed over 44 days indicated that these parameters were within 11% and 13% respectively of targets and therefore deemed acceptable.

Rats were observed twice daily (am & pm) for general health and behavioural changes and a general physical examination was performed weekly. Food consumption and bodyweight were recorded weekly during exposure. ChE activity in plasma, erythrocytes, spinal cord (thoracic region) and regional areas of the brain (cerebellum, cerebral cortex, striatum and hippocampus) were measured using a colorimetric assay on days 8, 15 and 29.

No deaths occurred and the predominant treatment-related clinical sign first observed on day 8 was muscle fasciculations in both sexes (3/15 males and 14/15 females) at 3000 ppm. Diarrhoea was also observed in 3/15 females from day 8. Bodyweight gain in males was significantly reduced ($p \leq 0.01$) by 54%, 29%, 26% and 26% respectively after each week of treatment at 3000 ppm. Females at the same dose also had a reduced weekly weight gain of 103%, 40%, 45% and 40% with all except the gain during week 3 being statistically significant ($p \leq 0.01$). Food consumption at 3000 ppm tended to be lower for females though this reduction was not significantly reduced ($p \leq 0.01$) except during week 1 for both males (21%) and females (27%). The average amount of diazinon consumed, calculated from food consumption and average bodyweight was 0.02, 2.3, 23, and 213 mg/kg bw/day for males at 0.3, 30, 300, 3000 ppm respectively. For females at the corresponding concentration levels, the actual diazinon doses were 0.02, 2.4, 23 and 210 mg/kg bw/day. The mean percentage reductions in ChE activities for each treatment group and averaged over the 3 measurements are shown in the Table below.

ChE Inhibition (mean percentage reduction)

ChE Location	Exposure (weeks)	Male				Female			
		Actual Dose (mg/kg bw/day)							
		0.02	2.3	23	213	0.02	2.4	23	210
Plasma	1	14*	59**	88**	96**	10	81**	95**	98**
	2	17	59**	84**	91**	2	81**	93**	97**
	4	5	51**	77**	87**	32	81**	94**	96**
Erythrocyte	1	1	39**	89**	94**	9	38**	86**	94**
	2	0	55**	83**	85**	8	59**	79**	89**
	4	4	58**	64**	74**	[5]	57**	88**	82**
Cerebellum	1	[4]	2	8*	67**	[12]	[14]	49**	88**
	2	6	1	22**	72**	[6]	6	60**	81**
	4	[2]	[6]	15*	70**	1	6	60**	94**
Cerebral Cortex	1	15	7	14	77**	[5]	1	47**	92**
	2	2	[16]	0	80**	[6]	[13]	62**	91**
	4	[4]	[3]	10	84**	[7]	6	72**	91**
Striatum	1	1	[4]	13	82**	6	0	58**	95**
	2	11	2	15	82**	4	[3]	73**	95**
	4	[3]	[5]	5	84**	4	9	78**	97**
Hippocampus	1	[5]	[6]	[2]	79**	[3]	[1]	51**	92**
	2	[5]	0	14	79**	5	2	72**	94**
	4	5	9	9	84**	[11]	[2]	71**	94**
Thoracic spinal cord	1	[15]	[10]	12	73**	10	8	44**	89**
	2	[1]	1	8	72**	[4]	1	76**	96**
	4	[3]	3	11	71**	[13]	[21]	62**	89**

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls;

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's t test).

Significant and dose-related inhibition of plasma and erythrocyte ChE was evident in males and females at concentrations equal to and greater than 30 ppm (2.4 mg/kg bw/day) from week 1 onwards. ChE inhibition in the brain showed little regional variation although females appeared to be more sensitive with significant dose-related inhibition ($p \leq 0.01$) being observed in all tested brain regions from week 1 at 300 ppm (23 mg/kg bw/day) whereas significance at the same dose was only apparent in the cerebellum of males.

Therefore, in both sexes, ChE inhibition in plasma and erythrocytes was at least an order of magnitude more sensitive to diazinon treatment than that observed in regional areas of the brain.

4.1.2 Inhalational Administration

Zak F, Luetkemeier B, Sachsse K & Hess R (1973) 21-Day inhalation study in the rat with technical diazinon. Ciba-Geigy Ltd, Toxicology Unit Sisseln. Project no. Siss 1679. Unpublished. (Pre-GLP)

Tif:RAIf rats (9/sex/group; Ciba-Geigy Breeding Unit) in a chamber were exposed (whole body) to an aerosol of technical diazinon (Ciba-Geigy; purity 97.1%, batch no. Mg 647) at concentrations of 0, 151, 245, or 559 mg/m³ for 6 h per day, 5 days per week for 21 days. The inhalational particle diameter distribution was assessed as being predominantly less than 7 microns (83.3-90.7%) with 34.6-43.5% being <1 µm. Eight rats (4/sex) from the control and 559 mg/m³ groups were kept for an additional recovery period of 25 days after the daily treatment phase, whereas all others were sacrificed on day 23.

Rats were observed daily for general health and behavioural changes. Food consumption and bodyweight were recorded daily and an ophthalmic examination was performed weekly. Haematology and clinical chemistry including ChE activity (colorimetric assay) were determined on day 18. Day 18 ChE activities in the brain and blood for the control and 559 mg/m³ groups were assessed using 5 and 9/group respectively with the surviving 4 rats/group being tested for brain and blood ChE activity after recovery on day 43. For the other treatment groups 9/group were used for all ChE activity estimations. Haematology measured Hct, Hb, erythrocyte count, WBC, a leucocyte differential count, thrombocyte count, Heinz body count, reticulocyte count, prothrombin time and erythrocyte sedimentation whereas clinical chemistry measured blood glucose, BUN, AP, A/G ratio, total protein, ALT and ChE activity.

Gross necropsies were performed on all rats killed at the end of treatment except for the 8 allowed to recover; these were sacrificed on day 25 of recovery. The following tissues were weighed then preserved for histopathological examination: brain, pituitary, eye, thyroid, oesophagus, trachea, lung, thymus, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, small intestine, large intestine, mesenteric lymph node, urinary bladder, prostate, testes with epididymis, or ovaries and uterus.

A control female died at some unspecified time after a blood collection on day 18; macroscopic examination revealed some haemorrhagic sites in the lungs and congestion of the organs. Exophthalmus, salivation, diarrhoea, ruffled fur and tonic-clonic muscle spasms were observed daily within 2 h after exposure at the highest concentration of 559 mg/m³; at 151 and 245 mg/m³, exophthalmus and diarrhoea were observed though surprisingly with a more rapid onset, ie. immediately after exposure. No clinical signs were evident during the 25-day recovery period. Food consumption for males and females at 559 mg/m³ was reduced during the first 3 days of treatment but thereafter appeared to be within the range of other groups. Probably reflecting reduced food-energy conversion, bodyweight gain of males and females at 559 mg/m³ (approx. 10% and 6% respectively) and males at 245 mg/m³ (approx. 10%) were reduced throughout treatment. During the recovery period, males and females gained bodyweight at a similar rate to controls except for females that appeared to have an accelerated rate from day 15 of treatment onward, so that by day 5 of recovery their mean bodyweight was the same as for controls. Ophthalmic examinations and haematology parameters were normal, while clinical chemistry changes were limited to reduced ChE activity in erythrocytes, plasma and brain. The mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition (mean percentage reduction)

Concentration (mg/m ³)	Duration (days)	Plasma ChE		Erythrocyte ChE		Brain ChE	
		male	female	male	female	male	female
151	18	9	3	[8]	[21]	9	29**
245	18	44**	42**	[3]	[9]	45**	43**
559	18	65**	62**	67**	67**	63**	64**
559	21 (+25 days recovery)	[9]	[21]	12	12	[9]	1

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; ** p≤0.01.

Although the investigators state in their summary that the ChE activity in the brain was significantly inhibited at all concentrations, irrespective of gender, inspection of the results (shown in the Table above) indicates that for males at the lowest dose tested of 151 mg/m³, no significant ChE inhibition occurred. Thus, given the clinical signs (exophthalmus and diarrhoea) at the lowest concentration (though the incidence for males and females were not separately reported) and the convincing dose-response relationship for the ChE inhibition in brain and plasma, it might be concluded that the ChE inhibition observed in the brain of females is correct. On the other hand, the good agreement between the degree of inhibition observed in plasma and brain at all concentrations except for females at 151 mg/m³ raises the issue of biological plausibility. How is it possible to have significant ChE inhibition in the brain (of female rats) in the absence of any significant corresponding ChE inhibition in blood (plasma and/or erythrocytes)? Inspection of the individual animal data for brain and plasma ChE activities does not reveal any widely divergent values that would help explain the result (ie. ChE activity (in Klett units) in females; plasma: control - 30, 41, 31, 42, 38, 38, 36, 40, 35, and at 151 mg/m³ - 35, 44, 37, 32, 33, 32, 35, 35, 40; brain: control - 244, 178, 194, 159, 194, and at 151 mg/m³ - 141, 156, 126, 120, 174, 128, 130, 144, 125).

No organ weight changes or macroscopic changes were observed at any concentration. Similarly, histopathological examination revealed no treatment-related changes.

Thus, rats exposed to an aerosol of diazinon at concentrations up to 559 mg/m³ were found to have clinical signs of poisoning (exophthalmus and diarrhoea) and a significant reduction (p≤0.01) in brain and plasma ChE activity at concentrations ≥ 245 mg/m³. Recovery of diazinon-inhibited ChE activity in blood and brain to control values after 25 days was also demonstrated. Although a significant reduction in brain ChE activity was also observed at the lowest concentration tested, namely 151 mg/m³, logically this cannot be considered to be biologically plausible due to the absence of a corresponding significant inhibition of blood ChE activity (ie. in plasma and/or erythrocytes). However, another inhalational (nose-only) study by Hartmann (1990; see below) indicates that brain and plasma ChE were both inhibited in males and females at concentrations below that used in this study, suggesting that the absence of ChE inhibition in plasma at 151 mg/m³ is, for some obscure reason, incorrect.

Hinkle DK, Suggs JE & Jackson MD (1980) Environmental and biological effects following application of diazinon impregnated strips within a laboratory animal room. Environmental Biology and Environmental Toxicology Division, US EPA, Research Triangle Park, North Carolina, USA. Lab Animal Sci 30: 981-983

Thirty-six polymeric plastic strips (25 sq. cm) impregnated with 10% diazinon and placed at 0.75 m intervals apart in a 25.92 m³ room housing 80 Sprague-Dawley rats (40/sex, Crl:COBS CD (SD)) resulted in a maximal diazinon concentration in air being detected 15-30 days later. Diazinon concentrations in air, sampled and measured (by GC) 0, 0.25, 1, 2, 3, 4, 7, 15 and 30 days after strip exposure, were found to be 0.01, 0.32, 0.56, 0.76, 0.60, 0.84, 0.90, 1.34 and 1.21 µg/m³ respectively. However, no corresponding ChE inhibition in plasma or erythrocytes was detected in rats (5/sex) using an automated pH stat method.

Hardy CJ, Clark GC, Street AE, Gibson WA, Lewis DJ & Gopinath C (1984) An investigation of the toxicity of diazinon administered by inhalation to rats over a 28-day period. Report no. VRB 3/831095. Lab: Huntingdon Research Centre Ltd, Cambridgeshire, England. Sponsor: Virbac Laboratories, Nice Cedex, France. Study duration: 27 Sep - 24 Oct, 1983. Report date: 4 Jan, 1984. (QA only)

Wistar rats (Crl:COBS WI BR strain; 10/sex/group; Charles River Ltd, UK) in chambers were exposed by a whole-body exposure method to an aerosol of technical diazinon (Virbac Ltd; batch no. 104639; purity not reported) for 6 h/day for 5 days/week for 28 days. Selection of appropriate target concentrations for the respirable aerosol, namely 0 (air-only), 15, 100, or 750 mg/m³, were based on a review of the results of an acute exposure study that determined a combined sex LC₅₀ of 4370 mg/m³ (see Hardy & Jackson, 1984; Section 3.1.1). The actual mean diazinon concentrations measured in the chamber by GLC analysis were found to be 15, 97 and 710 mg/m³ respectively for the treatment groups and the percentage of respirable aerosol particles (ie. <5.5 µm) among the groups was in the range of 93.5-94.7%.

Treated rats were observed during and after exposure, and twice daily on non-exposure days for health and behavioural changes. Food and water consumption, and bodyweight were recorded weekly starting 1 week before the first exposure. Haematology, urinalysis and clinical chemistry were performed during the last week of treatment. Haematology parameters measured were MCHC, MCV, Hct, Hb, erythrocyte and total and differential WBC counts, platelet count, and cell morphology (eg. Heinz bodies). Clinical chemistry measurements included blood glucose, BUN, AST, ALT, AP, albumin, total protein, globulins, A/G ratio, total bilirubin, total cholesterol and triglyceride, sodium, calcium, chloride, phosphorus, glutamyl transpeptidase, together with an assessment of plasma and erythrocyte ChE activity. Gross necropsies were performed on all rats at the terminal kill, 3 and 4 days after the final exposure for males and females respectively. Liver, kidneys, adrenals, testes, and lungs with trachea and larynx were removed, weighed and preserved for histopathological processing and examination. Other tissues collected and processed for histopathological examination were aorta, skin, pituitary, eye, thyroid, oesophagus, trachea, lung, thymus, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, small intestine, large intestine, brain, nasal passage, larynx, pharynx, urinary bladder, prostate, testes, epididymis, ovaries, uterus, mammary gland, axillary, cervical and tracheobronchial lymph nodes, sternum and marrow, femur, skeletal (thigh) muscle, aorta, submaxillary salivary gland, seminal vesicles, vagina, sciatic nerve, spinal cord, and tongue.

No deaths occurred during the study. Treatment-related clinical signs were most prevalent during exposure at 710 mg/m³ where abnormal respiratory movements, salivation and gasping, all indicative of respiratory tract irritation, together with whole-body tremors (especially frequent among females) were observed. The severity of these effects appeared to decrease with frequency of exposure. At other times, tremors and signs consistent with respiratory irritation were also observed at the highest concentration, though with markedly reduced severity. Faeces were

smaller and harder than controls for all rats at 710 mg/m³ and for females at 97 mg/m³. The significantly reduced (M 27%, F 21%; p<0.001 & p<0.01 respectively) mean bodyweight gain of the 710 mg/m³ rats throughout treatment was accompanied by a corresponding significant (p<0.05) reduction in food consumption during weeks 1, 2 and 3 in males (21%, 11% & 4%) and week 1 in females (29%). Water consumption for males during week 1 was also significantly (p<0.05) reduced (17%), although the overall consumption during treatment was similar to controls. In females at 710 mg/m³, significance for reduced water consumption was only achieved for the first day after the initial exposure. Urinalysis investigations on day 23 (M) and 24 (F) revealed that males at 710 mg/m³ had significantly reduced output (35%) with a corresponding increase in specific gravity and acidity (pH 6.5 relative to 7 in controls).

There were no consistent changes in haematology observed. Except for ChE activities in plasma and erythrocytes that were significantly (p<0.01) reduced in a clear dose-related manner, significant changes in other clinical chemistry parameters attributable to treatment were only observed at 710 mg/m³ where significant reductions (p<0.05) in total protein (M 6%, F 11%), globulin (M 15%, F 11%) and creatinine (M 17%) were associated with an elevated A/G (M 20%, F had reduced albumin - 11%) and AP (M 25%, F 43%) in plasma. The mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition (mean percentage reduction)

Concentration (mg/m ³)	Plasma ChE		Erythrocyte ChE	
	Male	female	male	female
15	44**	78**	63**	60**
97	58**	84**	70**	58**
710	87**	94**	76**	68**

** p≤0.01.

Macroscopic examination of tissues taken at the terminal kill revealed no lesions that could be attributed to treatment. Although the absolute mean weight of lungs in males and females and liver in females were significantly (p<0.05) heavier than controls, this was deemed (by the investigators) to be of no biological importance despite there being an increased incidence of focal squamous metaplasia of the epithelium in the ventro-lateral region of the larynx in males (4/10) and females (3/10) at 710 mg/m³. Epithelial hyperplasia over the arytenoid projection of the larynx was also detected in 3 other males at 710 mg/m³.

Thus, Wistar rats exposed to an aerosol of diazinon at concentrations up to 710 mg/m³ in a chamber had clinical signs of OP toxicity (tremors, abnormal respiratory movements, salivation and gasping) at 710 mg/m³ and significant dose-related reductions in erythrocyte and plasma ChE activities for at all tested concentrations (ie. 15, 97 and 710 mg/m³). Females tended to have more marked clinical signs together with a correspondingly increased degree of ChE inhibition. Other changes, possibly related to treatment at the highest concentration of 710 mg/m³, were significantly (p<0.05) reduced total protein (M 6%, F 11%), globulin (M 15%, F 11%), creatinine (M 17%), and elevated A/G (M 20%, F had reduced albumin - 11%) and AP (M 25%, F 43%) in plasma.

Hartmann HR (1990) 21-Day repeated exposure inhalation toxicity in the rat. Report no. 891205. Lab: Experimental Toxicology, Ciba-Geigy Ltd, Stein, Switzerland. Sponsor: Ciba-

Geigy Ltd, Agricultural Division, Basel, Switzerland. Study duration: 19 Sept - 7 Oct, 1989. Report date: 19 Jun, 1990. (Swiss GLP statement provided)

Tif:RAIf rats (10/sex/group; Ciba-Geigy Breeding Unit) were exposed in nose-only exposure chambers to a technical diazinon (Ciba-Geigy Corp.; batch no. FL880045; purity 88%)/ethanol aerosol (w/w ratio 0.01 except for 0.1 at 10 mg/m³) for 6 h/day for 5 days/week for 21 days in accordance with OECD guideline no. 412 and US EPA guideline 82-4. Selection of the target concentrations in an aerosol, namely 0.1, 0.3, 1.0, or 10 mg/m³, were based on a preliminary study (2/sex/group) that found a 62% inhibition of plasma ChE in both sexes at 3 mg/m³ and none at 0.3 mg/m³. Two control groups, namely air-only and ethanol at 24000 mg/m³ were also included in the definitive study. The actual mean diazinon concentration measured in the breathing zone by GC analysis were found to be 0.05, 0.46, 1.57 and 11.6 mg/m³ respectively for the treatment groups; the discrepancies were attributed to diazinon's affinity for the delivery pipe walls. The mass mean aerodynamic diameter of the aerosol particles was in the range of 0.7 to 1.4 µm.

The rats were observed for general health and behavioural changes during and after exposure, and once daily on non-exposure days. Food consumption and bodyweight were recorded weekly starting 22 and 20 days respectively before the first exposure. Ophthalmoscopy was performed before and after the final treatment. Haematology and clinical chemistry were performed at the terminal kill with a pretest sample of blood being collected to establish plasma and erythrocyte ChE activities. Haematology parameters were MCHC, MCV, Hct, Hb, erythrocyte and total and differential WBC counts, thrombocyte count, prothrombin time, and reticulocyte count. Clinical chemistry included blood glucose, BUN, AST, ALT, AP, albumin, total protein, globulins, A/G ratio, total bilirubin, total cholesterol and triglyceride, sodium, calcium, chloride, phosphorus, glutamyl transpeptidase, together with an assessment of plasma, brain and erythrocyte ChE activity. Gross necropsies were performed on all rats at the terminal kill (day 21). Heart, brain, liver, kidneys, adrenals, thymus, ovaries/testes, spleen and lungs were removed, weighed and preserved for histopathological processing and examination except for half the brain tissue that was used for a ChE activity determination. Other tissues collected and processed for histopathological examination were skin, pituitary, eye, thyroid, oesophagus, trachea, lung, thymus, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, small intestine, large intestine, mesenteric lymph node, urinary bladder, prostate, testes, epididymis, ovaries, uterus, mammary gland, axillary and popliteal lymph nodes, sternum and marrow, femur, skeletal muscle, aorta, submaxillary salivary gland, seminal vesicles, vagina, peripheral nerve, spinal cord, orbital gland, extraorbital lacrimal gland, Zymbals gland, muzzle and tongue.

Apart from a male rat at 0.46 mg/m³ that was found dead just prior to the terminal kill, no other deaths occurred during the study. There were no treatment-related clinical signs or ophthalmoscopic changes observed and although the mean bodyweight of 0.05 mg/m³ males was significantly reduced (6%; $p \leq 0.05$) relative to the ethanol controls but not air controls for week 2, this transient effect is unlikely to be treatment related because of the lack of any dose relationship. Similarly, reduced food consumption for males at 0.05 mg/m³ during weeks 1 and 2 but not for the 0.46, 1.57, or 11.6 mg/m³ groups suggests that this change is unrelated to treatment. Apart from minor (not exceeding 5% relative to either the air or ethanol controls) though in some cases significant changes ($p \leq 0.05$) in some erythrocyte parameters, ie. reduced RBC and Hb, and increased MCV in females at 11.6 mg/m³, no other consistent changes in haematology were observed. Changes in clinical chemistry were predominantly associated with ChE inhibition with other changes being attributable to group size because none appeared to be dose related except for reduced glucose in males at 1.57 and 11.6 mg/m³ (14 and 15%

respectively, $p \leq 0.05$). The mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition (mean percentage reduction)[¶]

Concentration (mg/m ³)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Male	female	male	female	male	female
0.05	[8], [9]	3, 13	[7], 1	1, 3	1, [6]	24**, 24**
0.46	5, 4	20*, 28**	5, 7*	[6], [4]	0, [7]	17*, 16
1.57	14*, 13**	27**, 35**	6, 8	10*, 12**	4, [3]	20*, 20*
11.6	19*, 18**	43**, 49**	36**, 38**	39**, 40**	0, [6]	31**, 36**

[¶] First figure in each column is the inhibition relative to the ethanol control whereas the second is relative to the air-only control; Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; * $p \leq 0.05$; ** $p \leq 0.01$.

Although sometimes statistical significance for ChE inhibition was achieved between 7 and 17% (eg. erythrocyte ChE activity in males at 0.46 mg/m³) and yet not for others with apparently a similar degree of inhibition (eg. 13% for plasma ChE at 0.05 mg/m³); can be explained by the degree of variability of individual results in the respective groups. Inhibition of this brain ChE activity in females at the lowest tested concentration of 0.05 mg/m³ seems to have no biological plausibility because it does not appear to be dose related and it occurred in the absence of significant inhibition in blood ChE (ie. in plasma and/or erythrocyte).

Macroscopic and microscopic examination of tissues taken at the terminal kill revealed no lesions that could be attributed to treatment as their incidence was comparable in control groups. Similarly, absolute organ weight or their ratio to brain or bodyweight were comparable with controls except for significantly heavier lungs relative to bodyweight in females exposed to 0.46 or 1.57 mg/m³ (6%, $p \leq 0.5$ and 9%, $p \leq 0.01$ respectively) but not at 11.6 mg/m³. The absence of a dose-response relationship and the comparable outcome with controls when compared with brain weight suggests these results are not biologically significant.

Thus, rats exposed by the nose-only route to an aerosol of diazinon at concentrations up to 11.6 mg/m³ had no clinical signs of poisoning but had statistically significant reductions in brain and plasma ChE activities at 0.46 mg/m³. Other findings at higher concentrations that may possibly be related to treatment were reduced serum glucose concentration in males at 1.57 and 11.6 mg/m³ and some erythrocyte parameters, ie. reduced RBC and Hb, and increased MCV in females at 11.6 mg/m³.

4.2 Rabbit

4.2.1 Dermal Application

Tai CN & Katz R (1984) Diazinon technical: 21-Day dermal toxicity study in rabbits. Report no. 842007. Lab: Ciba-Geigy Corp., Pharmaceuticals Research, Safety Evaluation Facility, Summit, NJ, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 23 Jan - 14 Feb, 1984. Report date: 11 Jun, 1984. (US GLP statement provided)

Technical diazinon (Ciba-Geigy Corp., Greensboro, NC; Batch no. FL-831737; purity 97.1%) diluted with 50% (w/v) polyethylene glycol 300 to give concentrations of 0 (vehicle only), 1, 5, or 100 mg/kg bw was applied for 5 days/week for 3 weeks to a shaved area not less than 10% of the total body surface area of New Zealand White rabbits (5/sex/group; H.A.R.E. Rabbits for Research, Hewitt, NJ, USA). The test material was applied directly to the skin then covered with gauze and held in place with non-irritating adhesive tape until the site was washed with water and dried, 6 h later.

Behaviour and general health were monitored twice daily, and the dermal application sites were examined and any reaction scored before each daily application. Bodyweight and food consumption were measured weekly with haematology and blood chemistry being assessed prior to and at the end of the study. The following haematology parameters were determined; Hb, erythrocyte count, WBC, Hct, reticulocytes, differential blood count, coagulation time, and platelets. Clinical chemistry measured sodium, potassium, inorganic phosphorus, calcium, chloride, total bilirubin, total protein, BUN, creatinine, serum glucose, AST, ALT, AP, albumin, globulin and A/G ratio together with erythrocyte, serum and brain ChE activity.

Necropsy was performed on the rabbits that either died or were sacrificed during the study as well as those at the terminal kill. Any gross lesions or tissue masses together with liver, kidneys, brain (with a half being processed for a ChE activity determination), and the application site skin specimens were processed for histopathological examination. Absolute and body-relative organ weights of the heart, liver, kidneys, testes/ovaries, pituitary, adrenals and brain (incl. stem) were also recorded at the terminal kill.

Since 4 of 5 males at 100 mg/kg bw/day died within the first 6 days (two on day 3 and one each on days 5 and 6), the highest dose was reduced for the survivors after the fifth treatment (day 7) to 50 mg/kg/day. Prior to the change in dosage, rabbits at 100 mg/kg bw/day displayed characteristic clinical signs of OP poisoning, namely anorexia, hypoactivity, ataxia, fasciculations, muscular hypotonia, tremors, salivation and diarrhoea. Thereafter survivors at 50 mg/kg/day and males at 5 mg/kg bw/day had slightly increased incidences of anorexia and diarrhoea throughout treatment. No gross or histopathologic changes were apparent in the male rabbits that died. Food consumption and bodyweight were similar among treatment groups.

Treatment-related grade 2 (well defined) erythema was observed in some diazinon-treated male and female rabbits at all doses. The incidence for males was 1, 3 and 1 at 1, 5 and 50 mg/kg bw/day respectively, though it was noted that 1 of the 3 rabbits at 5 mg/kg bw/day and the only surviving rabbit at 50 mg/kg bw/day had red foci. For females, the treatment group incidence was 2, 2 and 3 respectively, and 5/5 at 50 mg/kg bw/day had dry flaky skin from day 5 to 8-10. There was no apparent time to onset-dose relationship for either gender and lesions were observed for between 1 and 4 days. No oedema was evident in any treatment group and application site hyperkeratosis for the 50 mg/kg bw/day group was the only observed histopathological lesion attributable to treatment in all surviving rabbits (ie. 6/10).

Apart from a significant reduction ($p \leq 0.05$) in platelet count (23%) for females at the highest dose and in the eosinophil and basophil count for the sole male survivor at high dose, no other significant haematology changes were observed. Significant changes in clinical chemistry were associated with ChE inhibition in both sexes and an increased albumin concentration (8%) and A/G ratio (28%), and reduced sodium (2%) in females at the highest dose (100/50 mg/kg bw/day). The significant reduction in serum phosphorus observed for the sole male at 100/50

mg/kg bw/day is of doubtful biological significance. The mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition (mean percentage reduction)

Dose (mg/kg bw/day)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Male	female	male	female	male	female
1	4	32*	[6]	11	[14]	[4]
5	23	36**	1	14	[4]	18*
100/50 [†]	64**	60**	39	32**	28	43**

[†] Only 1 male in the group at 100/50 mg/kg bw/day; Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; * p≤0.05; ** p≤0.01.

Apart from diffuse dark red lesions (irritation) of the stomach in the male at 50/100 mg/kg bw/day, necropsy did not reveal any internal changes associated with the dermal application of diazinon. Reduced absolute kidney weight that appeared to be dose related in females (ie. 20%, 26% and 28% at 1, 5 and 100/50 mg/kg bw/day respectively) achieved significance (p≤0.05) relative to bodyweight at the highest dose tested; a similar trend was not observed in males. Therefore based on the significant inhibition of plasma ChE activity at the lowest tested dose of 1 mg/kg bw/day, no NOEL for this study can be established.

5. SUBCHRONIC TOXICITY

5.1 Rat

5.1.1 Gavage Studies

Rajendra W, Oloffs PC & Banister EW (1986) Effects of chronic intake of diazinon on blood and monoamines and amino acids. Departments of Biological Sciences and Kinesiology, Simon Fraser University, British Columbia, Canada. Drug Chem Toxicol 9: 117-131

In a study aimed to investigate changes in catecholamine and amino acid neurotransmitter concentrations in the rat cerebrum following long-term exposure to an OP, technical diazinon (source and batch not stated; purity 87%) in ethanol/water 1:1 (v/v) was administered by gavage to 12 adult male Wistar rats (Charles River, Ontario, Canada) for 28 weeks. Dosing was performed twice weekly with 1.75 mg/kg bw, so that the average daily dose was 0.5 mg/kg bw/day. Four rats each from a concurrent control group that were given vehicle only and the treatment group were sacrificed after weeks 7, 14 and 28 to enable blood and brain to be collected for a determination of monoamine (noradrenaline, adrenaline, dopamine and serotonin) and amino acid neurotransmitter (Asp, Glu, Tau, Gln and GABA) concentrations. ChE activities in plasma, erythrocytes and brain were also determined (by colorimetric assay). Bodyweight was recorded at 3-4 week intervals.

Although there were no clinical signs, rats lost bodyweight throughout treatment, with significance being achieved after week 14 (8% loss), 20 (10.7% loss) and 28 (10% loss). Although it is unclear from the report as to whether the time interval between dosing and blood and brain collection was consistent or varied between 3 and 4 days after dosing, the variable degree of ChE inhibition observed in blood and brain at weeks 7, 14 and 28 with significance

($p \leq 0.001$) only being observed for reduced plasma ChE activity (49%) after 28 weeks may also have been the result of a small group size ($n=4$). Similarly, for the same reasons, reductions of 28% for brain ChE activity at week 14, and 22% for plasma ChE activity at week 7 were not significant (at $p \leq 0.05$). Brain dopamine concentration, but not noradrenaline or dopamine, was found to be significantly elevated (by 3.7 fold; $p \leq 0.05$) at week 28. However, at week 28, all measured amino acids [Asp (by 26%), Glu (25%), Tau (18%), Gln (25%) and GABA (31%)] in the brain were significantly reduced ($p \leq 0.1$ except for GABA where $p \leq 0.05$) relative to the concurrent controls. In plasma, the serotonin concentration was elevated throughout treatment (ie. 48% and 60% at weeks 7 and 14 respectively) and this increase (2.27 fold) achieved significance ($p \leq 0.05$) at week 28.

On the basis of these results it was speculated that although ChE inhibition in plasma indicated exposure to an OP, it did not necessarily reflect the degree of neurological impairment (for humans) and this may be explained by the reduction in both brain excitatory neurotransmitters, ie. Asp and Glu, and inhibitory neurotransmitters, ie. Tau and GABA.

Anthony J, Banister E & Oloffs PC (1986) Effect of sublethal levels of diazinon: Histopathology of Liver. Departments of Biological Sciences and Kinesiology, Simon Fraser University, British Columbia, Canada. Bull Environ Contam Toxicol 37: 501-507

In a histopathological study, which appears to use tissue specimens from the same 12 treated male Wistar rats whose treatment was described in a previous study (Rajendra et al, 1986; see above), livers were taken after sacrifice and processed for light and electron microscopy to assess any changes arising from the 28-week diazinon treatment.

Light and electron microscopy revealed that intermittent (2/weekly) gavage dosing of diazinon resulted in hepatic vacuolation and lipid infiltration which was detected after 14 weeks and then progressed in severity during the following 14 weeks.

Wilkinson JG, Rajendra W, Oloffs PC & Banister EW (1986) Diazinon treatment effects on heart and skeletal muscle enzyme activities. Departments of Biological Sciences and Kinesiology, Simon Fraser University, British Columbia, Canada. J Environ Sci Health 21: 103-113

In a third study of this series, Wistar rats, treated with diazinon as described in a previous study (Rajendra et al, 1986; see above), were sacrificed and their left ventricle, soleus, medial gastrocnemius and plantaris muscles removed to enable phosphofructokinase, hexokinase, lactate dehydrogenase, and succinate dehydrogenase activities to be determined.

As described in Rajendra et al, 1986 (see above), there were no clinical signs and the diazinon-treated rats were 10% lighter than vehicle controls after 28 weeks of treatment. However, the weight of the heart, soleus, medial gastrocnemius and plantaris muscles were not significantly different from vehicle controls, suggesting that the bodyweight loss was not the result of changes in lean body mass. Phosphofructokinase, lactate dehydrogenase and succinate dehydrogenase activities in the muscles of treated rats were not significantly different from vehicle controls. Hexokinase activity in the plantaris muscle was significantly less than vehicle control, but not from a pre-treatment control. The biological significance of this observation is unclear.

5.1.2 Dietary Studies

Hussain MA, Oloffs PC, Blatherwick FJ, Gaunce AP & MacKenzie CJG (1981) Detection of incipient effects of anticholinesterase insecticides in rats and humans by electromyography and cholinesterase assay. Dept of Biological Sciences, Simon Fraser University, British Columbia, Canada. J Environ Sci Health 16: 1-19

Technical diazinon (purity 87%; source and batch not specified) on a slice of carrot was fed to male Wistar rats (source not specified) daily at 0.5 or 5 mg/kg bw/day for 26 weeks. Rats were weighed every second week. EMG measurements were made at either 2- or 4-week intervals, and the ChE activities in plasma and erythrocytes were determined every 2 weeks.

For the EMG determinations, rats were anaesthetised with ether and a stimulating electrode inserted near the hip to stimulate the sciatic nerve. The recording electrode was placed over the anterior tibialis muscle. Four supramaximal pulses of 0.05 msec duration was applied; there was little variation in the action potential produced by each of these pulses.

No clinical signs were observed during the feeding period. Bodyweight gain was significantly ($p < 0.001$) reduced by feeding diazinon. The decrease was in the order of 20% (estimated from a graph); the actual bodyweights and individual animal results were not reported. Plasma ChE activity was reduced by approximately 70% relative to controls throughout the study at both 0.5 or 5 mg/kg bw/day. Erythrocyte ChE activity was reduced by more than 20% relative to controls from week 2 at 5 mg/kg bw/day and from week 20 at 0.5 mg/kg bw/day. The erythrocyte ChE activity was reduced to approximately 20% of control values by both doses by the end of the study. There was no change in the amplitude of the action potential in EMG examinations throughout the study. This indicates that, in rats, EMG measurement may not be a useful indicator of exposure to diazinon. In this 26-week study, inhibition of plasma and erythrocyte ChE activities were seen at 0.5 mg/kg bw/day, hence no NOEL could be established.

Davies DB & Holub BJ (1980b) Toxicological evaluation of dietary diazinon in the rat. Department of Nutrition, University of Guelph, Guelph, Canada. Arch Environ Contam Toxicol 9: 637-650

Diazinon (Ciba-Geigy, Canada; 99.2% purity; Lot #140/101) was fed to female Wistar rats (50/group; Woodlynn Labs Ltd, Guelph, Canada) in a semi-purified diet at 0, 5, 10, or 15 ppm for 92 days. Female rats were selected for study because a previous short-term study had shown them to be more sensitive than males to ChE inhibition (see section 4.1, Davies & Holub, 1980a). In order to increase the accuracy for determining a NOEL based on ChE inhibition in plasma, erythrocytes and brain, a second and third study, each with a reduced duration and diazinon concentration, were performed. The second study involved groups of 16 rats being fed diazinon at 0, 1, 2, 3, or 4 ppm for 42 days while groups of 10 rats in the third study were fed 0, 0.1, 0.5, 1, or 2 ppm for 35 days. The diet was prepared once before commencement of each study series by mixing diazinon suspended in corn oil with a semi-synthetic diet. Diazinon stability in the diet was checked and found to be suitable for at least 4 months if stored at 3-4 °C after preparation. The measured diazinon concentration in the diet indicated that actual levels were within $\pm 5\%$ of the targets. Food consumption and bodyweight were recorded twice weekly and clinical signs were monitored daily.

In the 3 studies, blood for plasma and erythrocyte ChE activity determinations were collected from groups of 8-10 rats at various time (approximately 2-5 days apart). Brain ChE activity determinations were confined to the 2 longer duration studies where satellite groups of 6 rats were sacrificed at various times (5-14 day intervals). ChE activities were measured using a radiometric method with tritiated acetylcholine as the substrate.

There were no clinical signs observed at any dose in the 3 studies. Treated rats in all studies had similar food consumption and bodyweight gain except for rats at 0.5 ppm (3rd study) where mean bodyweight gain and food consumption were 12% and 10% respectively less than controls. The maximum mean percentage reductions in ChE activity measured in the 3 studies are shown in the Table below.

Maximum ChE Inhibition (mean percentage reduction)

Dose (ppm)	Duration (days)	Plasma ChE	Erythrocyte ChE	Brain ChE
0.1	35	4	0	-
0.5	35	16*	0	-
1	35	28*	16	-
2	35	42*	12	-
1	42	33*	8	NS
2	42	51*	16*	NS
3	42	65*	20*	NS
4	42	61*	22*	NS
5	92	75*	18*	2
10	92	80*	38*	6
15	92	85*	55*	2

* $p \leq 0.05$; NS=Approximately equivalent to controls.

Thus, there is a marked difference in the sensitivity of female rats to the inhibition of ChE activity in plasma relative to that in erythrocytes or brain. Therefore a NOEL for this study can be established at 0.1 ppm (equivalent to 0.01 mg/kg bw/day) based on a statistically significant inhibition of plasma ChE activity at the next higher dose of 0.5 ppm.

Weir RJ (1957a) Diazinon 25W: Subacute administration - rats. Report no. (not stated). Lab: Hazleton Laboratories, Falls Church, VI, USA. Sponsor: Geigy Agricultural Chemicals, McIntosh, Alabama, USA. Study duration: Mar - Jun 1956. Report date: Original Jul 16, 1956; Revised Feb 4, 1957. (Pre-GLP)

Diazinon 25W (a 25% wettable powder; Geigy Agricultural Chemicals, McIntosh, AL, USA; Batch no. MFL-1102; purity 22.0%) mixed with the diet was fed to Sprague-Dawley rats (15/sex/group; 125 g bw; Charles River, Kingston, NY, USA) at concentrations of 0, 0.5, 1, 2, or 4 ppm (a.i.) for 13 weeks. However, diazinon homogeneity, stability and concentration in the food admixture were not assessed. Food consumption and bodyweight were measured weekly and clinical monitoring was performed daily. After 28, 54 and 89 days respectively, the number of rats in each treatment group was reduced by 5 to enable ChE activity in the brain and blood

(plasma and erythrocytes) to be assessed; ChE activities were determined using an electrometric technique (Δ pH/h method).

Although 2 rats at 0.5 ppm died, one from pneumonia and the other because of physical entrapment in a wire food speller, none were attributable to diazinon treatment. There was a high incidence of respiratory disease in the colony affecting both control and treatment rats at similar rates and the symptoms associated with this condition (and confounding clinical signs possibly attributable to treatment) were rapid and laboured respiration, wheezing, rough fur, bloody discharge from the eyes and nose. The only sign apparently attributable to treatment (because individual results were not reported) occurred at 4 ppm where 2 males had slight tremors during the third week of treatment. Food consumption and bodyweight gain were unaffected by treatment. The mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition (mean percentage reduction)[¶]

Concentration (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Male	female	male	female	male	female
0.5	15	16	5	1	[2]	[1]
1	25	19	1	12	0	3
2	18	37	2	6	4	5
4	38	61	7	11	0	4

[¶] Overall mean of determinations made on days 28, 54 and 89; Values in square brackets indicate the extent (%) to which the measured activity was greater than controls.

The absence of a dose-response relationship for the inhibition of plasma ChE activity in males suggests that the NOEL for this study is 1 ppm (approx. equivalent to 0.1 mg/kg bw/day) based on substantial inhibition of plasma ChE activity (37%) at 2 ppm in females.

Singh AR, McCormick GC & Arthur AT (1988) Diazinon (MG8): 13-Week feeding study in rats. Report no. 882011. Lab: Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 8 Jan - 12 Apr, 1988. Report date: 4 Aug, 1988. (US GLP statement provided)

Diazinon was fed to Sprague-Dawley Crl:CD®(SD)BR rats (15/sex/group; Charles River, Kingston, NY, USA) in the diet at concentrations of 0, 0.5, 5, 250, or 2500 ppm for 13 weeks. Technical diazinon (Ciba-Geigy Corp.; purity 87.7%; Lot no. FL-872049) in acetone was mixed with rodent chow and air dried overnight to remove the acetone. This admixture was found to be suitable with respect to homogeneity, stability and concentration for 20 weeks at room temperature. Food consumption and bodyweight were measured weekly for 2 weeks prior to and during treatment, and clinical monitoring was performed daily. Water consumption and urine volume were measured during the week before treatment and then again during week 12. Similarly, ophthalmoscopy and an auditory/physical examination were also performed before treatment and then again during weeks 12 and 14 respectively. Haematology (Hct, Hb, erythrocyte count, WBC, a leucocyte differential count, thrombocyte count, (Heinz body and reticulocyte counts were performed in the control and 2500 ppm groups only), platelet count and prothrombin time) and clinical chemistry including ChE activity (by colorimetric assay) were determined during week 13. Clinical chemistry measured blood glucose, BUN, total serum protein, bilirubin, AP, ALT, AST, sodium, potassium, chloride, calcium, phosphorus, cholesterol,

creatinine, albumin, gamma globulins and the A/G ratio. Urinalysis included an assessment of the specific gravity, pH, glucose, ketones, protein, bilirubin and an examination of the sediment.

There were no treatment-related deaths. Clinical signs that appeared to be treatment-related occurred only at 2500 ppm with soft faeces and a degree of hypersensitivity to touch and sound being intermittently observed throughout treatment in both sexes (10/15 males and 15/15 females; 12/15 males and 15/15 females, affected respectively); aggressive behaviour was also noted in 3/15 males. Bodyweight loss resulting from treatment was observed at 2500 ppm where a reduced weight gain was most evident (significant; $p < 0.01$) from day 14 to 42 in males and day 7 to 49 in females, so that at the end of treatment males and females were 6% and 13% respectively lighter than concurrent controls. Although no significant changes in water consumption among groups occurred, food consumption was reduced for rats at 2500 ppm, but only during the first week of treatment in males (17%; $p < 0.01$) and for the first two weeks in females (31% and 13% respectively; $p < 0.01$). Calculations involving the diazinon concentration in food, the weight of food consumed and the average group midperiod bodyweight, enabled the dosage level in each treatment group to be calculated. For males this was 0.03, 0.3, 15 and 168 mg/kg bw/day respectively whereas for females it was slightly higher at 0.04, 0.4, 19 and 212 mg/kg bw/day.

Ophthalmoscopic examination did not reveal any changes attributable to treatment. The haematological assessment in females revealed dose-dependent changes in erythrocytic parameters (ie. erythrocyte count, 1.3, 1.8, 2.8 and 9.5% respectively; Hb, 1.5, 2, 3.6 and 4.1% respectively; Hct, 1.8, 1.8, 4.4 and 7.7% respectively), although only Hct at 250 ($p < 0.05$) and 2500 ppm ($p < 0.01$) achieved significance. A corresponding significant ($p < 0.01$) increase in reticulocytes (3.3-fold) was also observed in females at 2500 ppm (changes at lower concentrations were not examined). Increased WBC ($p < 0.05$) in females at 2500 ppm and eosinophil count ($p < 0.05$) in males at 0.5 ppm are probably incidental findings as there did not appear to be any dose-response relationship. Clinical chemistry changes were also characterised by a lack of any dose-response relationship so that in males the reduced cholesterol (by 18%; $p < 0.05$), elevated ALT (by 16%; $p < 0.05$) at 2500 ppm, and reduced sodium (by 1%; $p < 0.05$) observed at 5 ppm may not be attributable to treatment. Similarly, in females, reduced ALT (by 46%; $p < 0.01$), sodium (1.2%; $p < 0.05$), chloride (3.4%; $p < 0.05$) and elevated phosphorus (24%; $p < 0.01$) at 2500 ppm, and reduced ALT at 250 (by 36%; $p < 0.05$) and 0.5 ppm (by 39%; $p < 0.05$) may likewise be unrelated to treatment. However, there were reduced ChE activities in erythrocytes, plasma and brain that were attributable to treatment. These mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition (mean percentage reduction)

Concentration (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE	
	Male	Female	male	female	male	female
0.5	[10]	12	[4]	4	[7]	[2]
5	26**	78**	4	17**	[5]	0
250	89**	97**	27**	41**	4	41**
2500	97**	98**	26**	42**	49**	57**

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; ** $p \leq 0.01$.

There was significant inhibition of ChE activity in the erythrocytes of females and the plasma of males and females at 5 ppm whereas ChE activity in the brain was significantly inhibited once the

diazinon concentration in the diet reached 250 ppm in females and 2500 ppm in males. Apart from a significantly increased specific gravity of the urine (M 2.2%, F 1.6%; $p < 0.01$ for both) at 2500 ppm that was associated with non-significant reductions in urine volume (M 20%; F 17%) and water consumption (M 17%; F 12%), urinalysis was similar among treatment groups.

Macroscopic inspection of organs at necropsy revealed no gross abnormalities, although the absolute (15%; $p < 0.05$) and bodyweight-relative (20%; $p < 0.01$) weight of the liver was significantly increased in females at 2500 ppm. Males in the same group also had increased absolute (4%) and bodyweight-relative (7%) liver weights though both these changes were not significant. Although neither males nor females at 2500 ppm had significantly increased liver weights relative to brain weight, an increase of 6.5% and 12% respectively suggests a physiological adaptation, an assertion consistent with centrilobular hepatocellular hypertrophy observed in 13/15 females at 2500 ppm (and 3/15 at 250 ppm). The only other significant ($p < 0.05$) organ weight change was observed for the body-weight relative increase in weight of the kidneys (12%) in females at 2500 ppm.

In conclusion, rats fed diazinon in the diet at 2500 ppm lost bodyweight, were hypersensitive to touch and sound, and excreted soft faeces. In females at 2500 ppm, increased liver weight resulting from hepatocellular hypertrophy was also observed. However, the NOEL for the study was established at 0.5 ppm (equal to 0.03 mg/kg bw/day in males and 0.04 mg/kg bw/day in females) based on significant plasma ChE inhibition in both sexes and erythrocyte ChE inhibition in females at 5 ppm.

Singh AR, McCormick GC & Arthur AT (1988) Diazinon (MG8): 13-Week feeding study in rats. Report no. 882011. Lab: Ciba-Geigy Corp., Research Department, Pharmaceutical Division, Summit, New Jersey, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 8 Jan - 12 Apr, 1988. Report date: 4 Aug, 1988. (US GLP statement provided)

Sprague-Dawley Crl:CD®(SD)BR rats (15/sex/group; Charles River, Kingston, NY, USA) were fed diazinon in their diet at concentrations of 0, 0.5, 5, 250 or 2500 ppm for 13 weeks. Technical diazinon (Ciba-Geigy Corp.; purity 87.7%; Lot no. FL-872049) in acetone was mixed with rodent chow and air dried overnight to remove the acetone. This admixture was found to be suitable with respect to homogeneity, stability and concentration for 20 weeks at room temperature. Food consumption and body weight were measured weekly for 2 weeks prior to and during treatment, and clinical monitoring was performed daily. Water consumption and urine volume were measured during the week before treatment and then again during week 12. Similarly, ophthalmoscopy and an auditory/physical examination were also performed before treatment and then during weeks 12 and 14 respectively. Haematology (Hct, Hb, erythrocyte count, WBC, a leucocyte differential count, thrombocyte count, (Heinz body and reticulocyte counts were performed in the control and 2500 ppm groups only), platelet count and prothrombin time) and clinical chemistry including ChE activity (colorimetric assay) were determined during week 13. Clinical chemistry measured blood glucose, BUN, total serum protein, bilirubin, AP, ALT, AST, sodium, potassium, chloride, calcium, phosphorus, cholesterol, creatinine, albumin, gamma globulins and the A/G ratio. Urinalysis included an assessment of the specific gravity, pH, glucose, ketones, protein, bilirubin and an examination of the sediment. There were no treatment-related deaths. Clinical signs that appeared to be treatment-related occurred only at 2500 ppm where soft faeces and a degree of hypersensitivity to touch and sound were observed intermittently throughout treatment in both sexes (10/15 males and 15/15 females; 12/15 males and 15/15 females, affected respectively); aggressive behaviour was also noted in 3/15 males.

Bodyweight loss resulting from treatment was observed at 2500 ppm where reduced weight gain was most evident (significant; $p < 0.01$) from day 14 to 42 in males and day 7 to 49 in females, so that at the end of treatment males and females were 6% and 13% respectively lighter than concurrent controls. Although no significant changes in water consumption among groups occurred, food consumption was reduced for rats at 2500 ppm, but only during the first week of treatment in males (17%; $p < 0.01$) and for the first two weeks in females (31% and 13% respectively; $p < 0.01$). Calculations involving the diazinon concentration in food, the weight of food consumed and the average group midperiod bodyweight, enabled the dosage level in each treatment group to be calculated. For males this was 0.03, 0.3, 15 and 168 mg/kg bw/day respectively whereas for females it was slightly higher at 0.04, 0.4, 19, 212 mg/kg bw/day. Ophthalmoscopic examination did not reveal any changes attributable to treatment. Haematological assessment in females showed dose-dependent changes in erythrocytic parameters (ie. RBC, 1.3, 1.8, 2.8 and 9.5% respectively; Hb, 1.5%, 2%, 3.6% and 4.1%; Hct, 1.8%, 1.8, 4.4% and 7.7%), although only Hct at 250 ($p < 0.05$) and 2500 ppm ($p < 0.01$) achieved significance. A corresponding significant ($p < 0.01$) increase in reticulocytes (3.3-fold) was also observed in females at 2500 ppm (changes at lower concentrations were not examined). Increased WBC ($p < 0.05$) in females at 2500 ppm and eosinophil count ($p < 0.05$) in males at 0.5 ppm are probably incidental findings as there did not appear to be any dose-response relationship. Clinical chemistry changes were also characterised by a lack of any dose-response relationship so that in males the reduced cholesterol (by 18%; $p < 0.05$) and elevated ALT (by 16%; $p < 0.05$) at 2500 ppm, and reduced sodium (by 1%; $p < 0.05$) observed at 5 ppm may not be attributable to treatment. Similarly, in females, reduced ALT (by 46%; $p < 0.01$), sodium (1.2%; $p < 0.05$), chloride (3.4%; $p < 0.05$) and elevated phosphorus (24%; $p < 0.01$) at 2500 ppm, and reduced ALT at 250 (by 36%; $p < 0.05$) and 0.5 ppm (by 39%; $p < 0.05$) may likewise be unrelated to treatment. However, there were reduced ChE activities in erythrocytes, plasma and brain that were attributable to treatment. These mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition (mean percentage reduction)	Concentration (ppm)	Plasma ChE	female	Erythrocyte ChE	female	Brain ChE	female
0.5	[10]	12	[4]	4	[7]	2	526**
5	[10]	12	[4]	4	[7]	2	526**
25	[10]	12	[4]	4	[7]	2	526**
250	[10]	12	[4]	4	[7]	2	526**
2500	[10]	12	[4]	4	[7]	2	526**

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; ** $p \leq 0.01$.

There was significant inhibition of ChE activity in the erythrocytes of females and the plasma of males and females at 5 ppm whereas ChE activity in the brain was significantly inhibited once the diazinon concentration in the diet reached 250 ppm in females and 2500 ppm in males. Apart from a significantly increased specific gravity of the urine from rats (M 2.2%, F 1.6%; $p < 0.01$ for both) at 2500 ppm that was associated with non-significant reductions in urine volume (M 20%; F 17%) and water consumption (M 17%; F 12%), urinalysis was similar among treatment groups. Macroscopic inspection of organs at necropsy revealed no gross abnormalities, although the absolute (15%; $p < 0.05$) and bodyweight-relative (20%; $p < 0.01$) weight of the liver was significantly increased in females at 2500 ppm. Males in the same group also had increased absolute (4%) and bodyweight-relative (7%) liver weights though both these changes were not significant. Although neither males nor females at 2500 ppm had significantly increased liver weights relative to brain weight, an increase of 6.5% and 12% respectively suggests a physiological adaptation; an assertion consistent with centrilobular hepatocellular hypertrophy observed in 13/15 females at 2500 ppm (and 3/15 at 250 ppm). The only other significant ($p < 0.05$) organ weight change was observed for the body-weight relative weight of the kidneys (12%) in females at 2500 ppm. In conclusion, rats fed diazinon in the diet at 2500 ppm lost

bodyweight and were hypersensitive to touch and sound, and excreted soft faeces. In females at 2500 ppm, increased liver weight resulting from hepatocellular hypertrophy was also observed. However, the NOEL for the study was 0.5 ppm (equal to 0.03 mg/kg bw/day in males and 0.04 mg/kg bw/day in females) based on significant plasma ChE inhibition in both sexes and erythrocyte ChE inhibition in females at 5 ppm.

Edson EF & Noakes DN (1960) The comparative toxicity of six organophosphorus insecticides in the rat. Medical Department, Chesterford Park Research Station, Essex, England. Toxicol Appl Pharmacol 2: 523-539

Diazinon EC (Basudin 20ES, JR Geigy, Basle, batch no. not given) mixed with the diet was fed to groups of 10 male Wistar rats (source not given) at concentrations of 0, 1, 5, 25, or 125 ppm (equal to 0.102, 0.44, 2.26, or 11.7 mg/kg bw/day; calculated as a.i.) for 16 weeks. Dilutions of the formulation were prepared at 7 day intervals whereas the feed was prepared daily. Confidence in the stability of the formulation was based on two median lethal dose studies that yielded similar results irrespective of using either a 1-week old or a freshly prepared formulation. ChE activity in pooled erythrocytes and plasma of 4 rats per treatment group was measured after 1, 2, 4, 8 and 13 weeks of treatment by a manometric technique. At the end of the study relative organ weights were recorded and the ChE activity in plasma, erythrocytes and brain measured in all treated rats.

No treatment-related deaths or clinical signs were observed. At the conclusion of the study erythrocyte ChE activity was depressed to 90% and 81% of controls at 1 and 5 ppm respectively. At 25 ppm and 125 ppm, erythrocyte ChE activity decreased to 54% and 20% respectively of the controls after 4 weeks and remained low throughout the remaining study period. Plasma ChE activity was reduced to 83% and 48% at 15 and 125 ppm respectively but remained unchanged at 1 and 5 ppm. At all concentrations, the plasma ChE activity was increasing toward the end of the study period. Brain ChE activity was only marginally reduced (6%) at the highest tested concentration of 125 ppm. Thus, the NOEL can be set at 5 ppm (approximately 0.44 mg/kg bw/day), based on significant inhibition of erythrocyte ChE activity in male rats at 25 ppm.

5.2 Dog

Weir RJ (1957b) Diazinon 25W: Subacute administration - dogs. Report no. (not stated). Lab: Hazleton Laboratories, Falls Church, VI, USA. Sponsor: Geigy Agricultural Chemicals, McIntosh, Alabama, USA. Study duration: Mar - Jun 1956. Report date: Original Oct 9, 1956; Revised Feb 1, 1957. (Pre-GLP)

Diazinon 25W, a 25% wettable powder (Geigy Agricultural Chemicals, McIntosh, AL, USA; Batch no. MFL-1102; purity 22.0%) was mixed with Fuller's earth (1% or 10% w/w) and administered orally to groups (2/sex) of mongrel dogs in gelatin capsules at 0.02, 0.04 or 0.08 mg/kg bw/day for 6 days/week for 90 days. Dosing of the 0.02 mg/kg bw/day group commenced 21 days after the other 2 treatment groups and there did not appear to be a concurrent control group included in the study. Bodyweights were measured weekly and clinical signs were monitored daily. Blood for plasma and erythrocyte ChE activity measurements were collected 2 h after dosing on day 0, 1, 3, 8, 16, or 17, 31 or 33, 64, or 66 and 90 of the study. ChE activities in blood were measured using a manometric technique and compared with the pretest activities.

There were no deaths or changes in bodyweight, appetite or behaviour following treatment, though none of these parameters were reported with any detail. A pregnant female at 0.04 mg/kg bw/day uneventfully delivered 6 pups on day 26 of the study (although it is unclear as to whether this pregnancy was known prior to the commencement of the study). Another dog at 0.04 mg/kg bw/day had a sore throat while a third was treated with 400000 U of penicillin/streptomycin for a cold. Three of four dogs at 0.08 mg/kg bw/day had respiratory infections; apparently attributable to the rather high incidence of kennel cough in the colony. The maximal mean ChE inhibition in plasma ranged from 28% after 16 days at 0.02 mg/kg bw/day to 38% after 8 days at 0.04 mg/kg bw/day and 62% at day 33 at 0.08 mg/kg bw/day. In erythrocytes, ChE activity was in excess of the pretest value at all doses and all sampling times except for 25% inhibition at 0.08 mg/kg bw/day on day 8.

This study is not considered adequate for regulatory purposes for the following reasons: i) low number of dogs per group (2/sex); ii) no concurrent control group; iii) a number of the dogs had respiratory infections; iv) one dog was pregnant.

Williams MW, Fuyat HN & Fitzhugh OG (1959) The subacute toxicity of four organic phosphates to dogs. Department of Pharmacology, Bureau of Biological and Physical Sciences, Food and Drug Administration, Department of Health, Education and Welfare, Washington DC, USA. Toxicol 1: 1-7

This study also described treatment with Dipterex, chlorthion and parathion-methyl, however, only results associated with diazinon treatment are reported in this summary.

In a study designed to monitor plasma and erythrocyte ChE inhibition, diazinon 25W (24.1% purity; source and batch not specified) at 0, 0.25, 0.75, or 75 ppm (as a.i.) was fed to mixed-breed dogs [1/sex/group and 5 in the control group (gender not stated)] in their regular ground dog chow for 12 weeks. ChE inhibition was measured at the end of the first week and every second week thereafter, which included a 6-week recovery period.

Treatment-related effects other than ChE inhibition were not measured or reported. Although the results for plasma and erythrocyte ChE inhibition were presented only as graphs, it appears that for treatment at 0.75 and 75 ppm, activity was significantly inhibited in plasma by up to 35% and 95% respectively. In erythrocytes, the activity was only significantly inhibited (by 55%) at 75 ppm. Recovery in plasma was complete after about 4 weeks whereas the activity in erythrocytes was still less (by about 10%) than control values.

Barnes TB, Hazelette JR & Arthur AT (1988) Diazinon (MG8): 13-Week oral toxicity study in dogs. Report no. 882012. Lab: Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 26 Jan - 29 Apr, 1988. Report date: 4 Aug, 1988. (US GLP statement provided)

In a study designed to establish the maximum tolerated dose and a NOEL, diazinon was fed to Beagle dogs (4/sex/group; Laboratory Research Enterprises Inc, Kalamazoo, Michigan, USA) in the diet at concentrations of 0, 0.1, 0.5, 150, or 300 ppm (equal to 0.0034, 0.020, 5.9, or 10.9 mg/kg bw/day) for 13 weeks. The technical diazinon (Ciba-Geigy Corp.; purity 87.7%; Lot no. FL-872049) in acetone was mixed with canine diet and air dried overnight to remove the acetone. This admixture was found to be suitable with respect to homogeneity, stability and concentration for 27 days at room temperature. Food consumption and bodyweight were

measured weekly for 2 weeks prior to and during treatment, and clinical monitoring was performed daily. Urine was collected either by catheterisation or in a plastic liner during the week before treatment and then during weeks 5, 9 and 14. Similarly, ophthalmoscopy and an auditory/physical examination were also performed before treatment and then again during weeks 14 and 13 respectively. Haematology (Hct, Hb, erythrocyte count, WBC, a leucocyte differential count, thrombocyte count, (Heinz body and reticulocyte counts were performed in the control and 300 ppm groups only), platelet count and prothrombin time) and clinical chemistry were determined during treatment weeks 5 and 9, and then prior to termination (week 13) except for ChE activity (by colorimetric assay) in the brain that was measured after the terminal kill (week 14). Clinical chemistry measured blood ChE activity, glucose, BUN, total serum protein, bilirubin, AP, ALT, AST, sodium, potassium, chloride, calcium, phosphorus, cholesterol, creatinine, albumin, gamma globulins and the A/G ratio. Urinalysis included an assessment of the colour, clarity, specific gravity, pH, glucose, ketones, protein, urobilinogen, bilirubin and an examination of the sediment.

There were no treatment-related deaths and although emesis (both after food and at other times) and bloody faeces were observed in 2/4 males at 300 ppm it was not considered treatment related because a female (1/4) at 150 ppm had similar signs. Reduced bodyweight gain resulting from treatment were observed in females (by 34%) at 150 ppm and in both sexes at 300 ppm (M, 33%; F, 45%), however, by the end of treatment their bodyweights were not significantly different from concurrent controls. Food consumption among treatment groups did not appear to be significantly different from controls.

Ophthalmoscopic examination did not reveal any changes attributable to treatment. The haematological assessments revealed no dose-related changes and all significant differences observed were transient. In males, reduced clotting time at 150 and 300 ppm (by 25% and 24% respectively; $p < 0.05$) together with reduced prothrombin time (by 8%; $p < 0.05$) at 300 ppm were observed on day 56. A slightly increased neutrophil count (17%; $p < 0.05$) was associated with a reduced number of lymphocytes (by 41%; $p < 0.01$) in 0.5 ppm males on day 86. On day 29, females at 0.1, 0.5, 150 and 300 ppm had reduced WBC (by 28%, 46%, 34% and 39% respectively; $p < 0.01$); a reduced reticulocyte count at 300 ppm; a slightly increased percentage (0.5%; $p < 0.05$) of nonsegmented cells at 150 ppm; and a reduced neutrophil count (by 20%; $p < 0.05$) with a concomitant increase in the lymphocyte count (by 41%; $p < 0.05$).

Clinical chemistry changes were characterised by an absence of a dose-response relationship and most were transient. However, one change that was probably related to treatment involved a reduced serum protein concentration at 300 ppm. Males had reduced albumin concentrations on days 29, 56 and 86 (12%, 16%, 11%) and although significance ($p < 0.01$) was achieved on days 29 and 56, the increased variability at day 86 (ie. SD=0.2 relative to 0.03 for control) suggests that with a greater number of dogs per group significance would have been achieved. This assertion is further supported by the observation that the total protein concentration on days 29, 59 and 86 was significantly ($p < 0.01$) reduced (by 8.4%, 15% and 10% respectively) at 300 ppm and by 1.4% ($p < 0.05$) at 150 ppm on days 29 and 56. Transient changes in males, probably unrelated to treatment, were an elevated albumin concentration (7%; $p < 0.05$) at 0.1 ppm; increased calcium concentration on days 29 (5%; $p < 0.05$) and 56 (6.3%; $p < 0.01$) at 300 ppm; and an increased bilirubin (18%; $p < 0.05$) at 300 ppm on day 29. In females the albumin concentration was significantly reduced (by 10%; $p < 0.05$) at 150 ppm and 300 ppm on day 29 and at 300 ppm (8%; $p < 0.05$) on day 56. Electrolyte changes in females, also probably unrelated to treatment, were observed on day 29 for sodium (increased by 2%; $p < 0.01$) at 0.1 ppm and for calcium (5%; $p < 0.05$) at 150 ppm on days 29 and 56. However, clear treatment-related changes were observed

for ChE activities in erythrocytes, plasma and brain. These mean percentage reductions in ChE activities are shown in the Table below.

ChE Inhibition after 5, 9 and 13 weeks of treatment (mean percentage reduction)

Concentration (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE†	
	male	Female	male	female	Male	Female
0.1	20, 20, 18	[6], [4], [5]	2, 6, [3]	[3], [2], 0	4	[4]
Mean	19	[5]	2	[2]	4	[4]
0.5	29*, 27, 30*	17, 16, 15	4, 8, 18	7, [2], 4	[4]	[3]
Mean	29*	16	10	3	[4]	[3]
150	81**, 78**, 80**	79**, 77**, 81**	26**, 25**, 25**	31**, 31**, 31**	31**	30**
Mean	80**	79**	25**	31**	31**	30**
300	80**, 75**, 85**	80**, 79**, 83**	33**, 28**, 31**	33**, 37**, 31**	42**	45**
Mean	80**	79**	31**	34**	42**	45**

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; † Week 13; * $p \leq 0.05$; ** $p \leq 0.01$.

The 3 entries for ChE activity in plasma and erythrocytes shown in the Table above are those recorded after 29, 56 and 86 days of treatment respectively. The mean values for the 3 respective entries are also shown. Urinalysis revealed a slightly increased specific gravity for both males and females at 300 ppm throughout treatment, however, these increases only achieved significance at day 92 for females (2.2%: $p < 0.05$) treated at 150 and 300 ppm.

Macroscopic examination of the tissues removed at the terminal kill revealed no changes attributable to treatment. Similarly, although a number of isolated incidences of statistically significant changes in organ weight were observed (epididymus, a 26% increase relative to bodyweight at 0.1 ppm; prostate, a 63% increase relative to bodyweight and 64% relative to brain weight at 0.5 ppm; salivary glands in females, a 26% reduction relative to bodyweight and 29% relative to brain), none were dose-related and so are most likely to be unrelated to treatment. Histopathology identified atrophy of the pancreatic acini in one male at 300 ppm, a result consistent with similar lesions observed after a single dose (25 mg/kg bw; Frick et al, 1987, see section 3.1.2) in a published study.

In conclusion, dogs of both sexes fed diazinon in the diet at 300 ppm (equal to 10.9 mg/kg bw/day) had reduced bodyweight gain whereas males also had a reduction in serum

albumin concentration and an incidence of atrophy of the pancreatic acini. Thus, 300 ppm in the diet is likely to be the maximum tolerated dose. However, the NOEL for the study was established at 0.1 ppm (equal to 0.0034 mg/kg bw/day) based on significant plasma ChE inhibition in males at 0.5 ppm.

Earl FL, Melveger E, Reinwall JE, Bierbower GW & Curtis JM (1971) Diazinon toxicity-Comparative studies in dogs and miniature swine. Division of Pharmacology and Toxicology, Food and Drug Administration, Department of Health, Education and Welfare, Washington DC, USA. Toxicol Appl Pharmacol 18: 285-295

Technical diazinon (Ciba-Geigy Corp., Ardsley, New York, USA; purity not reported; Lot no. M.S. 402702) containing 5% epoxidised soybean oil (as stabiliser) was diluted 1:100 in corn oil and administered in capsules to Beagle dogs (3/sex/group; FDA breeding colony, Washington DC, USA) at a concentration of 0 (corn oil only), 2.5, 5, 10, or 20 mg/kg bw/day for 8 months. Dose selection was based on a dose-ranging study in which survival, necroscopy and histopathology at doses up to 500 mg/kg bw/day were assessed. At a dose of 25 mg/kg bw/day or greater, 1 or both dogs at each of the tested doses died. Necropsy, performed on dogs surviving more than a day (ie. at ≤ 100 mg/kg bw/day), revealed haemorrhage on the dura mater and congestion or haemorrhage of the intestine that ranged from petechial and paint brush haemorrhage of the mucosal and serosal surface to frank haemorrhage on the mucosal surface. Histopathology revealed several minor findings among the 8 examined dogs, however, the major findings were haemorrhage in the colon, and a yellow-brown pigment in the cytoplasm of hepatic parenchymal cells and cortical renal epithelium.

Dogs in the main study were fed normal dog chow at the rate of 35 g/kg bw/day and any uneaten food was weighed and recorded. In addition to daily observations for behavioural changes, dogs were physically examined each day prior to dosing. For those killed at the conclusion of the study or found moribund (and then sacrificed) or dead during the study, a necropsy was performed. The number of tissues on which a histopathological examination was performed was reduced with dose so that for the 20 mg/kg bw/day group, brain, spinal cord, pituitary, thymus, thyroid, lungs, heart, liver, gallbladder, pancreas, spleen, kidney, adrenal, urinary bladder, stomach, small intestine, colon, testes or ovary and uterus, sciatic nerve, and skeletal muscle were examined, whereas at 10 mg/kg bw/day, thyroid, heart, liver, kidney, adrenal, gall bladder, pancreas, spleen, stomach, small intestine, colon, and testes or ovary were examined. At 2.5 and 5 mg/kg bw/day, only testes were examined.

Clinical chemistry measured AP, blood nitrogen, glutamic oxaloacetic transferase, lactate dehydrogenase, ornithine carbamoyl transferase, creatinine phosphokinase, lipase, amylase and total lipids in blood drawn twice before treatment and then at monthly intervals thereafter. Haematology included Hb, Hct, erythrocyte and reticulocyte counts, WBC, differential and platelet counts, and partial thromboplastin time. The myeloid:erythroid ratio was determined from 1000 rib marrow cells.

At the highest dose of 20 mg/kg bw/day, all 3 males and a female died or were sacrificed *in extremis* during treatment. The males died on days 14, 24 and 166 and the female on day 19. Reduced food intake, emesis with occasional diarrhoea and body fasciculations was observed among the dogs prior to death or sacrifice. Similar cholinergic signs were noted in a dog (1/6) at 10 mg/kg bw/day during the first 45 days but it survived. Although bodyweight losses occurred in some dogs, the number, dose group and extent were not reported. In view of these deaths and a lack of appetite at 20 mg/kg bw/day, an additional 3 dogs were recruited to determine the effects

of emaciation on haematological, biochemical, and pathological parameters. Treatment duration (with diazinon) for these additional dogs was the same as for the original group.

There were no significant changes in any of the measured haematology parameters although there was a marked increase in myeloid elements (100-150 fold) in the dogs that died or were sacrificed. Clinical chemistry changes apart from serum amylase were limited to a male dog that died after 166 days at 20 mg/kg bw/day and a second male that died 5 days before the completion of the study (232 days) following exposure at 10 mg/kg bw/day. Although the extent of the increases were not reported, alkaline phosphatase, lactate dehydrogenase and ornithine carbamoyl transferase concentrations were claimed to be elevated in these 2 male dogs. Markedly increased serum amylase were also observed in these 2 dogs that died as well as in the 3 surviving dogs at 10 mg/kg bw/day and another at 5 mg/kg bw/day (extent and dog gender not reported).

Gross pathological and histopathological changes were observed mainly among dogs treated at 20 mg/kg bw/day where lesions affecting the GI tract and associated organs were evident. In 5 of 6 dogs, marked oedematous thickening of the jejunum or duodenum occurred with one having a ruptured duodenum resulting in peritonitis while yet another had rupture of the pyloric portion of the stomach. Moderate cirrhosis of the liver was observed in 3/6 dogs. The only other probable treatment-related changes occurred in an emaciated dog at 10 mg/kg bw/day in which the thyroids were soft, the pancreas, spleen and testicles had atrophied and the liver had a yellow fatty appearance. Histopathological examination confirmed atrophic changes in the acinar cells and interstitial fibrosis of the pancreas, and in the pulp of the spleen; spermatogenesis had been completely arrested. The liver had parenchymal atrophy and hepatic cell dissociation.

Thus, in the absence of any determination of ChE inhibition, the NOEL can be set at 2.5 mg/kg bw/day based on an elevated amylase activity in the serum at the next higher tested dose of 5 mg/kg bw/day.

5.3 Pig

Earl FL, Melveger E, Reinwall JE, Bierbower GW & Curtis JM (1971) Diazinon toxicity-Comparative studies in dogs and miniature swine. Division of Pharmacology and Toxicology, Food and Drug Administration, Department of Health, Education and Welfare, Washington DC, USA. Toxicol Appl Pharmacol 18: 285-295

[This summary describes the second part of a comparative study in dogs and miniature pigs, ie. the miniature pig component.]

Technical diazinon (Ciba-Geigy Corp., Ardsley, New York, USA; purity not reported; Lot no. M.S. 402702) containing 5% epoxidised soybean oil (as stabiliser) was diluted 1:100 in corn oil and administered in capsules to Hormel-Hanford pigs (3/sex/group; source not reported) at concentrations of 0 (corn oil only), 1.25, 2.5, 5, or 10 mg/kg bw/day for 8 months. Dose selection was based on a dose-ranging study in which survival, necroscopy and histopathology of 4 pigs (2/sex) at doses up to 25 mg/kg bw/day were assessed. Groups were reduced to one male at 50 and 100 mg/kg bw/day, and two for each at 300 (1/sex) and 500 (2 males) mg/kg bw/day. At a dose of 25 mg/kg bw/day or greater, pigs at each of the tested doses either died or were sacrificed *in extremis*, except for one in the 500 mg/kg bw/day group that survived treatment. Necropsy on pigs treated at 5, 10, 25, or 50 mg/kg bw/day, revealed similar lesions as for dogs (see section 5.2), ie. haemorrhage on the dura mater and congestion or haemorrhage of the intestine that ranged from petechial and paint brush haemorrhage of the mucosal and serosal surface to frank

haemorrhage on the mucosal surface. Additionally haemorrhage of the heart, large fatty areas in the pancreas and congestion of the stomach were observed. Histopathology revealed several minor findings probably unrelated to treatment, however, a major finding that was related to treatment was the marked depletion of rib marrow cells in 6 of 8 pigs in which sections were prepared and examined.

In addition to daily observations for behavioural changes, pigs were physically examined each day prior to dosing. For those killed at the conclusion of the main study or found moribund (and then sacrificed) or dead during the study, a necropsy was performed. The number of tissues on which a histopathological examination was performed was reduced with dose so that for the 20 mg/kg bw/day group, brain, spinal cord, pituitary, thymus, thyroid, lungs, heart, liver, gallbladder, pancreas, spleen, kidney, adrenal, urinary bladder, stomach, small intestine, colon, testes or ovary and uterus, sciatic nerve, and skeletal muscle were examined, whereas at 10 mg/kg bw/day, thyroid, heart, liver, kidney, adrenal, gall bladder, pancreas, spleen, stomach, small intestine, colon, and testes or ovary were examined. At 5 mg/kg bw/day, liver, kidney and adrenals were examined whereas at 1.25 mg/kg bw/day only livers were examined.

Clinical chemistry measured alkaline phosphatase, blood nitrogen, glutamic oxaloacetic transferase, lactate dehydrogenase, ornithine carbamoyl transferase, creatinine phosphokinase, lipase, amylase and total lipids in blood drawn twice before treatment and then at monthly intervals thereafter. Haematology included Hb, Hct, erythrocyte and reticulocyte counts, WBC, differential and platelet counts, and partial thromboplastin time. The myeloid:erythroid ratio was determined from 1000 rib marrow cells.

At the highest dose of 10 mg/kg bw/day, only 1 female survived treatment. The other 5 pigs in the group that died on days 12, 16, 20, 25, and 38 had characteristic cholinergic signs prior to death, though there was no apparent relationship between time to onset of these clinical signs and time to death. The surviving pig, although having similar signs for 15 days, was remarkable by virtue of the delayed onset, ie. after 4 months of treatment. At 5 mg/kg bw/day there were no deaths and only 1 pig had signs that persisted for the first 6 months of treatment, whereas at 2.5 mg/kg bw/day the only pig that developed clinical signs after 19 weeks was sacrificed *in extremis* during week 20. In 4 of the 5 pigs that died at 10 mg/kg bw/day, oedematous thickening of the jejunum wall was observed, and 1 pig had localised mucosal erosion that penetrated into the muscular layers resulting in a marked mucosal seepage throughout the intestines. Three of the 5 pigs had also formed ulcers in the duodenum and all had somewhat firm livers that were apparently difficult to cut. This liver hardening was probably in part the result of interlobular connective tissue thickening in 3/6 pigs at 20 mg/kg bw/day as revealed by histopathological examination. For the sacrificed pig at 2.5 mg/kg bw/day, ascites harvested from its abdominal cavity clotted after exposure to air; it also had a mottled liver. It is unclear though as to whether the thickening of the interlobular connective tissue and lobular congestion observed during histopathological examination in 1 pig were associated with this sacrificed pig (no individual animal data).

Haematological parameters were normal except for a slight elevation in the myeloid:erythroid ratio (2-fold) and a reduced reticulocyte count (4-fold) among the pigs that died at 10 mg/kg bw/day. Apparently (though not reported in detail) erratic elevated levels of ornithine carbamoyl transferase, creatinine phosphokinase and amylase were observed in some pigs from all treatment groups.

Noteworthy gross pathological changes in pigs treated at 5 mg/kg bw/day were limited to oedema in the wall of the jejunum of a pig and a friable liver with focal subscapular haemorrhage in another. It is unclear (because individual animal data is not present) whether the friability was associated with the lobular congestion observed under microscopic examination in a pig from this treatment group.

The absence of the individual amylase activities (and other clinical chemistry parameters) indicates that a NOEL cannot be established for pigs in this study.

6. CHRONIC TOXICITY

6.1 Mouse

Wheeler RJ, Bernal E, Ball RA, Storrs EE & Fitzhugh OG (1979) Bioassay of diazinon for possible carcinogenicity. Publication no. (NIH) 79-1392. Lab: Gulf South Research Institute, New Iberia, Louisiana, USA. Sponsor: NCI Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA. Study duration: not stated. Report date: not stated. (Pre-GLP)

Technical diazinon (Ciba-Geigy Corp., Greensboro, North Carolina, USA; purity 98%; Lot no. FL-741306) was fed to B6C3F1 mice (50/sex/group except for controls that had 25/sex; NCI Frederick Cancer Research Center, Frederick, Maryland, USA) in the diet at concentrations of 0, 100, or 200 ppm for 103 weeks. Diazinon in acetone was mixed with rodent chow and then air dried to remove the acetone. This admixture was found to be suitable with respect to homogeneity, stability and concentration when stored at room temperature for the 7 days between fresh batches. Using vapour phase chromatography, the purity of the diazinon used in this study was assessed and found to be unchanged after storage for 4 years. Dose selection was based on a dose-ranging study in which survival and bodyweight at concentrations up to 3200 ppm were assessed after 13-weeks treatment. At 200 ppm, there was little change in survival or bodyweight whereas at 400, 800, 1600, or 3200 ppm, survival and/or bodyweight were reduced. Food consumption and body weight were measured weekly for 2 weeks prior to and during treatment, and clinical monitoring was performed twice daily. For mice, killed at the conclusion of the study (103 weeks treatment + 1 week recovery) and for any found moribund (and then killed) or dead during the study, a necropsy was performed. Histopathological examination involved skin, lungs (incl. bronchi), trachea, bone (incl. marrow), spleen, lymph nodes, heart, salivary gland, gall bladder, liver, intestine, pancreas, stomach, small and large intestine, kidney, bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate (or uterus), testis (or ovary), and brain.

Although survival and bodyweight were unaffected by treatment, hyperactivity was evident among all treated mice. The only treatment-related non-neoplastic lesion observed was an increase in cystic hyperplasia of the uterus/endometrium. The incidences were 22/46 at 200 ppm and 1/44 at 100 ppm relative to 0/22 in controls. Apart from an increased incidence of hepatocellular carcinoma at 100 ppm, (ie. 4/21, 20/46 ($p=0.046$) and 10/48 for control, 100 and 200 ppm groups respectively) that was not associated with a corresponding increase in hepatocellular adenoma (2/23, 0/47, 3/49 for control, 100 and 200 ppm groups respectively) liver adenomas, there was no treatment-related increase in the total number of tumours in treated mice relative to controls. Therefore, under conditions of this assay, the NOEL for carcinogenicity in mice was at the maximal tested of 200 ppm (30 mg/kg bw/day). However, a NOEL for clinical signs could not be established.

Kung AHC, Campbell WR, Barnett JW & Ellis JF (1980) Carcinogenicity evaluation with diazinon technical in albino mice. Report no. 8580-09381. Lab: Industrial Bio-Test Laboratories, Neillsville, Wisconsin, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 17 Nov, 1976 - 30 Jun, 1978. Report date: 7 July, 1980. Histopathology was performed in May, 1980 by JF Hardisty at Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina, USA. (Validated and Pre-GLP.)

Technical diazinon (Ciba-Geigy Corp., Greensboro, North Carolina, USA; purity 87.6%; Lot no. FL-761264) mixed with corn oil was added to dry rodent chow and fed to Swiss CD-1 white mice (60/sex/group; Charles River, Portage, Michigan, USA) at concentrations of 0 (because controls were shared with another unrelated study the vehicle was acetone and not corn oil as for the treatment groups), 4, 20, or 100 ppm (equivalent to 0.6, 3, or 15 mg/kg bw/day). A rationale for the dose selection was not given. Furthermore, the dietary admixtures were found (by the sponsors) to have been prepared at irregular intervals ('as needed' basis) prior to July 6, 1977. Batches had been prepared at intervals ranging between 7 and 157 days, however, a stability analysis had shown that by day 7, 2.7% had degraded and by day 157 it was 39%. After this problem was recognized, batches were prepared weekly and stored frozen, although the impact of the preceding 8 months on the study outcome could not be determined. Similarly, because it was not specified by the sponsors in the original protocol, food consumption had not been recorded over the same time period. It was mutually decided that measuring food consumption for the remaining duration of the study would not enable the overall dosages to be calculated and so was not pursued. However, sampling of the prepared feed batches at monthly intervals after month 9 revealed that apart from a batch prepared during month 19, the actual concentrations were within 31% of the targets but the overall mean concentrations (excluding month 19) were 3.7, 19.3, 99.1 ppm respectively. The month 19 batches had undetectable levels in the 4 ppm feed and 0.68 and 11 ppm respectively for the 20 and 100 ppm feeds. The impact of this reduced intake on the study outcome was not possible to determine.

During the first 11 months of treatment, daily monitoring (excluding weekends and holidays) was limited to reporting mortality. Thereafter (at the behest of the sponsor), more detailed observation and reporting of clinical signs, that included palpation to detect tumors, was initiated. After month 14, the frequency of palpation and clinical signs monitoring was reduced to monthly intervals. Bodyweight and bodyweight gain were recorded before treatment and then at weekly intervals throughout treatment. Necropsy and tissue specimen processing of decedents and those sacrificed at the scheduled kill were performed at the IBT labs, however, the histopathological examination of the prepared sections was not performed until 2 years later at Experimental Pathology Laboratories. Aside from any tissue masses detected by palpation other tissues examined were adrenal gland, aorta, brain (cerebral and cerebellar cortex, medulla/pons), oesophagus, eye (with optic nerve), gonads, uterus, mammary gland, marrow, heart, kidney, large intestine (caecum and colon), liver, lung, lymph nodes (cervical and mesenteric), prostate, pancreas, peripheral nerve, pituitary, salivary (submaxillary) gland, skeletal muscle, skin, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, stomach (cardia, fundus and pylorus), thyroid gland, parathyroid gland, trachea, and urinary bladder.

Apart from a marked increase in the number of deaths following a 3-week episode of elevated humidity in the room housing the mice, there was no treatment-attributable increase in mortality rate. Since this episode caused a higher death rate among mice in all groups (during weeks 80-81), the median survival were comparable in all groups, ie. at 0, 4, 20 and 100 ppm it was 459, 500, 501 and 510 days respectively in males, and 546, 539, 507 and 538 days respectively in

females. Since clinical signs were monitored during the latter stages of the study, those noted were generally associated with aging mice, ie. alopecia, skin lesions, and skin irritation and piloerection.

Significant ($p < 0.05 - 0.01$) loss of bodyweight was observed among all treated males during the first 2 months and then (transiently) at months 7, 9 and 14 for the 20 and 100 ppm groups. In contrast, a significantly ($p < 0.05 - 0.01$) reduced bodyweight was observed among all treated females (ie. 13%, 11% and 12% at 4, 20 and 100 ppm respectively at month 18) throughout the study except for months 6, 13 and 19. Macroscopic examination of tissues taken from decedents and those at the scheduled kill revealed mainly isolated incidences of kidneys with a discoloration or a granular surface, and enlarged spleen among males and females; urine stained perineum and subcutis oedema among males and missing eyes, ovarian cysts and enlarged uterine horns or uteri among females. However, none of these findings were related to dose and many occurred with equal frequency as for controls. There also appeared to be a good correlation of gross lesions with histopathological findings. In summary, there was no evidence of any inflammatory, degenerative, proliferative or neoplastic lesions among treatment male and females.

In view of the uncertain doses actually administered, this study can only be considered as supplementary data for to assess carcinogenicity potential. However, rats fed diazinon in their diet at a concentration that caused significant bodyweight loss, no increase in tumour incidence was observed.

Goldsmith LA & Craig DK (1983) Lifetime carcinogenicity study in mice. Diazinon. Report no. 21099. Lab: Litton Bionetics Inc, Kensington, Maryland, USA. Sponsor: Nippon Kayaku Co. Ltd, Tokyo, Japan. Study duration: 25 Jul, 1980 - 5 Aug, 1982. Report date: 4 Aug, 1983. (Although company QA was performed, no GLP statement was provided)

Technical diazinon (Nippon Kayaku Co. Ltd, Tokyo, Japan; purity not stated; Lot no. P-604) mixed with corn oil was added to dry rodent chow and fed to B6C3F1 mice (60/sex/group except for males at 200 and 300 ppm that had 59/sex; Charles River, Portage, Michigan, USA) at concentrations of 0 (with vehicle), 100, 200, or 300 ppm in males (equivalent to 15, 30, or 45 mg/kg bw/day) and 0 (with vehicle), 100, 200, or 400 ppm in females (equivalent to 15, 30, or 60 mg/kg bw/day) for 104 weeks. A rationale for the dose selection was not given. The dietary admixture was found to be suitable with respect to homogeneity, stability and concentration when stored frozen for the 7 days between fresh batches. Mice were monitored for death and clinical signs twice daily for the first 2 and last 9 weeks of treatment; at other times monitoring occurred once daily. In order to detect any tissue masses, mice were palpated at weekly intervals throughout treatment. Bodyweight and food consumption were recorded at weekly intervals

Haematology (Hct, Hb, erythrocyte and reticulocyte counts, total and differential leucocyte counts and platelet count) was assessed at 12 and 24 months on 10 rats/sex that were randomly selected from each group. At the terminal kill, some organs were removed, ie. adrenal glands, brain with medulla, heart, kidneys, liver, lungs, ovaries/testes and spleen and weighed before fixation in preparation for a histopathological investigation.

Mice bled at 12 months (for haematology) and all survivors after 2 years of treatment were sacrificed and a gross necropsy performed. A histopathological investigation was performed on any gross lesions (incl. tissue masses) and on specimens of adrenal gland, brain (at least 3 levels from the forebrain, midbrain and hindbrain), oesophagus, eyes (and the contiguous Harderian

gland), ovaries, uterus (corpus and cervix), mammary gland, bone (incl. marrow from sternum, vertebrae or tibiofemoral joint), heart, kidneys, large intestine, liver (at least 2 lobes), lungs (incl. all lobes and bronchi), lymph nodes, prostate, testes, pancreas, sciatic nerve, pituitary gland, major salivary glands, skeletal muscle, skin (mammary area), gall bladder, small intestine, spleen, stomach (squamous and fundic), thymus, thyroid with parathyroid gland, trachea, and urinary bladder.

Although for all groups 34 mice were found dead, 4 were accidentally killed and another 57 were sacrificed *in extremis*, none of these deaths could be attributed to treatment because the incidences were similar between sexes and among groups. Clinical signs, though also probably unrelated to treatment because of comparable incidences in all groups, were limited to alopecia and some tail injuries. Similarly, although small palpable masses were more numerous among males (61 from a total of 77), their distribution among groups (ie. 21, 15, 12 and 13 for the 0, 100, 200 and 300 ppm groups respectively) suggests that this observation was unrelated to treatment. Food consumption was significantly ($p < 0.05$) reduced on 33 of 58 occasions throughout treatment in males at 300 ppm and on 11 occasions in females at 400 ppm. The report, however, indicates that no precautions had been taken to minimise food spillage so that estimates made before week 17 were unlikely to be accurate, hence for males, significance was still achieved on 25 of 43 occasions in males and 7 in females. However, although the overall food consumption in males was approx. 13% less than controls, the concomitant bodyweight loss was only 4%, whereas for females whose overall consumption was reduced by 2% the loss was 7%. Actual dosages achieved appear to have exceeded targets because no adjustment to the diazinon concentration in the diet was made during treatment and because food spillage was not controlled, the actual mean daily dose cannot be accurately determined.

There were few haematological changes that appeared to be related to treatment except for a reduced number of segmented neutrophils in females after 24 months. This reduction, ie. 41%, 43% and 51% at 100, 200 and 400 ppm respectively achieved significance ($p < 0.05$) only at the highest dose. Absolute heart weight was increased for all treatment mice after 12 and 24 months, however, significance ($p < 0.05$) was not achieved except for females at 400 ppm (16%) after 12 months and in males at 100 (7%) and 400 ppm (9%) after 24 months. Although significance was abolished when compared with changes in bodyweight except for females at 400 ppm after 24 months, the consistent trend for increased absolute heart weight suggests a treatment-related effect. Other significant organ weight reductions, ie. liver (by 12%) and kidney (by 11%) in females at 400 ppm after 24 months are also possibly treatment related.

No consistent treatment-related increase in incidence of palpable masses among mice, the number per mouse or percent with masses over the duration of the study. The numbers of mice with benign and malignant neoplasms after 104 weeks of treatment were as follows:

Summary of Neoplasia Incidence

No. of rats with observ'n	Treatment Group (ppm)					
	Sex	0	100	200	300	400
Number examined	M	59	60	59	56	ND
	F	57	59	60	ND	60
Benign neoplasms	M	13	14	7	13	ND
	F	7	17	8	ND	12
Malignant neoplasms	M	11	17	5	10	ND
	F	17	22	18 [†]	ND	18
Total of neoplasms	M	24	31	12	23	ND
	F	34	39	26	ND	30

† Includes 1 with an uncertain benign or malignant tumor.; ND=Not determined.

A statistical analysis of the tumor incidence data did not reveal any increase in incidence for any treatment group relative to controls. Hence, under the conditions of this study, there was no evidence that diazinon at dietary concentrations up to 300 ppm in males and 400 ppm in females causes an increase in the number of tumors in B6C3F1 mice.

6.2 Rat

Wheeler RJ, Bernal E, Ball RA, Storrs EE & Fitzhugh OG (1979) Bioassay of diazinon for possible carcinogenicity. Publication no. (NIH) 79-1392. Lab: Gulf South Research Institute, New Iberia, Louisiana, USA. Sponsor: NCI Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA. Study duration: not stated. Report date: not stated. (Pre-GLP)

The protocol for this study was the same as described for a study in B6C3F1 mice (see section 6.1), except that diazinon was administered in the feed at concentrations of 0, 400, or 800 ppm to (Fischer) F344 rats for 103 weeks (103 weeks treatment + 2 weeks recovery for males or 1 week for females). The dose-ranging study found that at 800 ppm, there was no change in survival and bodyweight whereas at 1600 ppm and 3200 ppm, both were reduced.

Results

Although rat survival and bodyweight were unaffected by treatment, hyperactivity was evident among all treated males and among females at 800 ppm. It was stated that there was an increased incidence of tachypnea among rats at 800 ppm though there were no data supplied to indicate the actual number of affected rats. Similarly, bloating, vaginal discharge and/or bleeding were observed with greater incidence among treated females.

Palpable tissue masses were said to be more prevalent among females at 400 ppm and males at 800 ppm relative to controls, though the histopathology examination revealed an approximately equal incidence of neoplastic and non-neoplastic lesions among all groups. There was no specific neoplasia type that appeared to be attributable to treatment, although the lymphoma incidence increased in males at 400 ppm (25/50; $p=0.011$) relative to controls (5/25) and males at 800 ppm (12/50). The biological significance of this observation is uncertain. Endometrial stromal polyp formation in females is possibly related to treatment in that for the 400 and 800 ppm groups the incidence was 8/43 (19%) and 11/49 (22%) respectively, whereas in concurrent controls only

2/23 (9%) were affected. These increased tumour incidences, although not statistically significant, were also accompanied by a shorter time to onset (of a palpable mass), ie. 62 and 72 weeks for the 400 and 800 ppm groups respectively whereas for controls it was 104 weeks. The investigators dismissed these observations on the basis that this lesion is common among F344 rats and fall within the historical control incidence. However, this assertion could not be verified because historical control data were not supplied. Similarly, it was not possible to investigate a possible link between the vaginal bleeding and/or discharge, and the presence of endometrial polyps because the individual animal data for clinical signs were not available.

Thus, under the conditions of this assay, diazinon has an uncertain carcinogenic potential. The NOEL for the study was established at 400 ppm because of hyperactivity observed at the next higher concentration of 800 ppm.

Anon (1955) Chronic oral administration of diazinon 25% wettable powder to rats for 104 weeks. Report no. not stated. Lab: Hazleton Laboratories, Falls Church, VI, USA. Sponsor: not stated. Study duration: not stated. Report date: 22 Dec, 1955, Histology supplement - 30 Dec, 1955, Storage stability in tissues and urinary excretion supplement - 10 Jan, 1956. (Pre-GLP)

N.B. Only a summary of this study was available to the reviewer.

Diazinon (source, purity and batch were not stated) fed to albino rats (strain, source and group size were not stated) in the diet at concentrations of 10, 100, or 1000 ppm for 104 weeks did not cause any treatment-related deaths, clinical signs or changes in food consumption relative to controls. Apparently the only haematological change at the terminal kill was a reduced haematocrit in males at 1000 ppm. Reductions in ChE activity in brain, erythrocytes and plasma were apparently dose related, with the greatest reduction being observed in plasma followed in order by that in erythrocytes and the brain.

The reporting of the gross and histopathological observations were so brief that no independent evaluation of the study is possible. Apparently though, there was no evidence of any treatment-related tumour formation. Therefore, a NOEL for this study cannot be established in the absence of any data for independent evaluation.

Ashby R & Danks A (1987) Diazinon: Combined toxicity and oncogenicity study in rats. Amended report amalgamating studies 83/NKL002/322 & 82/NKL002/268. Report no. 87/NKL002/378. Lab: Life Science Research Ltd, Eye, Suffolk, England. Sponsor: Nippon Kayaku Co. Ltd, Tokyo, Japan. Study durations: 8 Aug, 1978 - 16 Dec, 1980 & 9 Oct, 1979 - 3 Nov, 1981. Report date: 14 Jul, 1987. (Pre-GLP)

Two studies (83/NKL002/322 & 82/NKL002/268) that investigated the effects of diazinon in the feed of rats over differing concentration ranges were initially reported separately, however, in order to conform with EPA registration requirements the individual reports have been combined and submitted as an amended report. The following summary of the 2 studies was derived from the amended report.

Technical diazinon (Nippon Kayaku Co. Ltd, Japan; purity 97.02%; Lot no. not reported) in the diet was fed to (Fischer) F344 rats (75/sex/group in study 83/NKL002/322 and 65 females/group in study 82/NKL002/268; Charles River Breeding Laboratories, Wilmington, Mass, USA) at concentrations of 0, 0.1, 1.5, or 22.5 mg/kg bw/day in one study (study 83/NKL002/322) and 0 or

0.025 mg/kg bw/day in a second (study 82/NKL002/268) for 104 weeks. Homogeneity of diazinon in the feed was checked before treatments and its stability was monitored at intervals not exceeding 15 weeks throughout the 2 studies. The feed admixtures were found to be suitable with respect to homogeneity (6% variability), stability (5%) and concentration (coeff. var. 21%) when stored at room temperature. However, no justification for the doses selected in either study was reported. Rats were monitored daily for death and clinical signs, and palpated weekly for swellings. Bodyweight and food consumption were recorded weekly for the first 26 weeks (or 24 weeks in the second study) and fortnightly thereafter. Ophthalmoscopy performed prior to treatment revealed non-resolving lesions in 7 rats. These rats were replaced and only the eyes of the control and highest dose (22.5 mg/kg bw/day) rats were examined during weeks 26, 50, 78 and 102 of the first study. Owing to the absence of any intergroup difference, ophthalmoscopy was not extended to other groups.

Haematology (Hct, Hb, erythrocyte counts, MCV, MCH, MCHC, WBC, differential leucocyte and platelet counts), clinical chemistry (glucose, BUN, total protein, A/G, bilirubin, AP, ALT, AST, CPK, LDH, cholesterol, sodium, potassium, calcium, chloride, phosphorus, uric acid and creatinine) and urinalysis (specific gravity, occult blood, pH, glucose, ketones, protein, urobilinogen, cells and cellular debris) parameters were assessed on 10/sex/group (or 10 females/group in study 2) after week 12, 25, 51, 102 and 120 (or 121 for clinical chemistry) in the first study only. ChE activities (ie. using acetyl and butyrylcholine substrates) were measured (by colorimetric analysis) at the same time as for other clinical chemistry parameters except that additional blood was collected after 17 and 38 weeks, and not at week 120 for the assay measuring acetyl ChE activity and at week 17, 25, 51 and 102 for butyryl ChE activity. In the second study, blood for the ChE activity measurements were collected from 8 females/group after 13, 26, 39, 52, 65, 78, 91 and 104 weeks. Brain ChE activity (acetyl and butyrylcholine) was measured in 10 rats/sex at the interim kills in the first study (weeks 18, 27, 52 and 104) and from 8 females at the terminal kill in the second study. At the terminal kill in both studies, some organs were removed, ie. adrenal glands, brain, heart, kidneys, liver, lungs, ovaries/testes, pituitary gland, spleen, thyroid and parathyroid, and weighed before fixation (except for thyroid and parathyroid which was weighed after fixation) in preparation for a histopathological investigation. The aortic arch, seminal vesicles and epididymides were also collected, fixed and stored but not sectioned and examined for lesions.

Histopathological investigations were performed on any gross lesions (incl. tissue masses) and on specimens of adrenal gland, turbinate bone, brain, oesophagus, eyes (with optic nerves), ovaries, uterus, uterine cervix, mammary gland, bone, Harderian gland, heart, kidneys, large intestine (caecum, colon), liver, lungs, lymph nodes (mesenteric, cervical), prostate, testes, pancreas, peripheral (sciatic) nerve, pituitary, salivary gland, skeletal muscle, skin, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, stomach, thymus, thyroid with parathyroid glands, tongue, trachea, oral cavity, nasal cavity, nasopharynx, middle ear, and urinary bladder.

Although it was claimed that survival among groups in the first study was not significantly affected by treatment, the cumulative number of deaths, namely 67, 59 and 76 relative to 61 in concurrent controls (combined sex) suggests that the increased mortality (ca. 20%) at the highest dose of 22.5 mg/kg bw/day is a treatment-related effect. There was no apparent gender difference in mortality at any dose and for the females in the second study there was no apparent difference in cumulative deaths between the treatment (21) and concurrent control groups (20). Apart from an increased number of lesions in the stomach, ie. ulceration (M, 12; F, 9 relative to 4 and 3 respectively in controls), acanthosis (15, 12 relative to 6 and 9), hyperkeratosis (15, 14 relative to 6 and 9), granulated submucosal tissue (16, 11 relative to 6 and 6) and hyperplasia of epithelium

(7, 6 relative to 0 and 1), among rats of either sex at 22.5 mg/kg bw/day that died between weeks 105 and 120, macroscopic and microscopic investigations did not reveal an increased incidence of neoplastic or non-neoplastic lesions attributable to treatment among the rats that had died or had been killed *in extremis* before the terminal or interim kills. Whilst all lesion incidences were elevated relative to controls, only mucosal granulation and the epithelial hyperplasia among males achieved significance ($p < 0.05$).

There were numerous significant ($p < 0.05$ or 0.01) weekly increases in the incidence of periorbital staining (or ocular discharge), urogenital and perianal staining among females in the 22.5 mg/kg bw/day group. Time to onset for periorbital and urogenital staining was 17 and 16 weeks respectively whereas significance for the incidence of perianal staining was delayed until week 43. For males, the incidence of perianal staining also achieved significance, however unlike females, all treatment groups were affected. Somewhat surprisingly, the incidence among males in the control group was initially significantly higher than those in the treatment groups, though after 10 weeks this reversed and remained significantly less than observed at 0.1 and 1.5 mg/kg bw/day for the next 14 weeks and for 23 weeks at 22.5 mg/kg bw/day. The time to onset (for significance to be achieved) in all treatment groups was 48 weeks, ie. slightly delayed relative to females at 22.5 mg/kg bw/day. No signs were reported for the second study.

Food consumption for males and females at 22.5 mg/kg bw/day increased progressively throughout treatment and this increase achieved significance during weeks 53-104 in males and 105-120 in females. The overall food consumption, ie. for weeks 1-120 was increased by 5% ($p < 0.01$) in males and 4% ($p < 0.05$) in females. This progressive increase in food consumption also resulted in a slightly increased bodyweight over the duration of treatment (3% in males, 4% in females; not significant) that only achieved significance ($p < 0.05$) during weeks 26-52 in females and weeks 26-78 in males. Despite the increased food consumption, the achieved mean dosage (because of adjustments) was only marginally increased for the 22.5 mg/kg bw/day group, ie. 0.025, 0.01, 1.5 and 22.6 mg/kg bw/day respectively. Water consumption recorded for weeks 1, 12, 25, 51, 77 and 101 revealed a reduced consumption for the 22.5 mg/kg bw/day group rats on most occasions with significance being achieved for week 12 in females ($p < 0.001$) and week 1 in males ($p < 0.05$). The increased water consumption for males ($p < 0.05$) relative to controls during weeks 51 (12%) and 101 (22%) but not relative to other treatment groups suggests a non-treatment related effect.

Probable treatment-related changes in haematology were observed for the erythrocytic parameters and lymphocytes in males after 12 weeks of treatment. The RBC, Hb and Hct were reduced in an approximate relationship with dose, ie. at 0.1, 1.5 and 22.5 mg/kg bw/day respectively, RBC was 3%, 6%** and 7%**, whereas Hb was 5%*, 6%[¶] and 5%[¶], and Hct was 2%*, 6%** and 6%** (* $p < 0.05$; [¶] $p < 0.01$; ** $p < 0.001$). The lymphocyte counts were similarly reduced for the 3 respective treatment groups, ie. 10%, 20%* and 20%*. All erythrocytic and lymphocytic counts had recovered to be within the normal range by week 25 of treatment. There were no clear treatment-related effects in urinalysis, however, at 22.5 mg/kg bw/day, alkaline phosphatase activity was significantly reduced (M 11%, $p < 0.05$; F 22%, $p < 0.01$) after 12 weeks and maintained until week 51. Other changes in clinical chemistry that were possibly related to treatment was a significant increase ($p < 0.001$) in the concentration of uric acid at 25 weeks, ie. 2-fold in males and 1.7-fold in females. However, there were clear treatment-related changes in ChE activity at all dose levels. These mean percentage reductions in ChE activities expressed relative to concurrent untreated controls, are shown in the Table below.

ChE Inhibition after 13, 26, 39, 52, 65, 78, 91 and 104 weeks of treatment (mean percentage

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reduction)

Dose (mg/kg bw/day)	ChE	Plasma		Erythrocyte		Brain†	
		male	female	male	female	male	female
0.025	Butyryl	-	21**, 15**, [2], 15**, 28**, 22 [¶] , 10 [¶] , 11	-	0, 0, 4, 0, 6, [2], 8, 0	-	3
	Mean	-	15	-	2	-	3
	Acetyl	-	16**, 12**, 6*, 19**, 16**, 21 [¶] , 8*, 8*	-	2, 1, [6], 14, 1, [4], 7, 2	-	[5]
	Mean	-	13	-	2	-	[5]
0.1	Butyryl	21**, 19*, 10, 30**, 24**, 52**	45**, 58**, 71**, 68**, 61**, 48**	81, 14, 0, 3	0, 17, 18, 19	7, [56], [29], 2	16, 9, [1], 11
	Mean	26	59	24.5	14	[19]	9
	Acetyl	13**, 13**, 13**, 24**, 30**, 39**	39**, 57**, 66**, 62**, 64**, 41**	[5], 19**, [11], [8], [20], 2	[4], 0, [1], 7, 9, 9	2, [14], [3], 4	0, 9, 12, [4]
	Mean	22	55	[4]	3	3	4
1.5	Butyryl	38**, 37**, 48**, 65**, 87**, 80**	85**, 86**, 95**, 91**, 87**, 88**	81, [13], 4, [34]	[20], [25], 23, 23	[7], [56], 25 [¶] , 49**	11, [7], 23*, 22*
	Mean	59	89	10	0	3	12
	Acetyl	39**, 48**, 50**, 58**, 70**, 71**	77**, 88**, 86**, 87**, 91**, 85**	24**, 59**, 28**, 53**, 58**, 49**	28*, 40**, 15, 51**, 46**, 38**	1, [7], 5, 3	7, [5], 26 [¶] , [14]
	Mean	56	86	45	36	0	4
22.5	Butyryl	38**, 44**, 64**, 67**, 90**, 83**	87**, 91**, 97**, 91**, 87**, 92**	81, 29, 0, [7]	[64], 17, 9, 37*	27, 23, 54**, 45**	30 [¶] , 0, 40**, 57**
	Mean	64	91	26	0	37	32
	Acetyl	75**, 81**, 80**, 83**, 95**, 99**	91**, 96**, 95**, 94**, 97**, 94**	58**, 75**, 68**, 75**, 84**, 69**	61**, 68**, 68**, 80**, 73**, 76**	15, 28, 25, 72**	47**, 42, 65**, 63**
	Mean	86	95	72	71	35	54

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; † At weeks 18, 27, 52 and 104-108; * p≤0.05; [¶] p≤0.01; ** p≤0.001.

From the Table, it is apparent that a significant reduction in plasma ChE activity was observed at all doses tested, with females being appreciably more sensitive to the inhibition. In contrast,

acetyl-ChE inhibition in erythrocytes achieved significance at 1.5 mg/kg bw/day in both sexes. In the brain, ChE activity was significantly inhibited in both sexes at the highest dose tested, ie. 22.5 mg/kg bw/day.

No consistent treatment-related increase in incidence of palpable masses, macroscopic changes and organ weight were observed at 0.5, 1.5, or 22.5 mg/kg bw/day at the terminal kill. Similarly, there was no increase in the incidence of neoplasms attributable to treatment. However, at 22.5 mg/kg bw/day there was an increased incidence of submucosal granulation tissue (8 relative to 0 in controls), acanthosis (9 relative to 0 in controls) and hyperkeratosis (8 relative to 0 in controls) of the keratinised cells in the stomach that achieved significance ($p < 0.05$) among females.

The numbers of rats at 0.5, 1.5, or 22.5 mg/kg bw/day with benign and malignant neoplasms after 120 weeks of treatment were as follows:

Summary of Neoplasia Incidence

	Sex	Treatment Group (mg/kg bw/day)				
		0	0.025†	0.1	1.5	22.5
Number examined	M	22	-	17	19	13
	F	22	-	21	28	23
Benign Neoplasms	M	60	-	50	49	38
	F	36	-	41	45	36
Malignant Neoplasms	M	8	-	6	9	6
	F	10	-	7	17	8
Total Neoplasms	M	68	-	56	58	44
	F	46	-	48	62	44

† Not done.

In conclusion, there was no evidence of carcinogenicity in rats treated with diazinon at doses that caused increased mortality, cholinergic clinical signs, increased bodyweight and food consumption, and ulceration of the keratinised region of the stomach. However, a NOEL for this study could not be established because significant ChE inhibition was observed in the plasma of female rats at the lowest tested dose of 0.025 mg/kg bw/day.

Kirchner FR, McCormick GC & Arthur AT (1991) One/two-year oral toxicity study in rats. Study no. 882018. Lab: Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 28 Jun, 1988 - 18 May, 1990. Report date: 14 Jun, 1991. (US GLP statement provided)

and

Mann PC (1993) Histopathological assessment of potential ocular toxicity of four organophosphate insecticides. Lab: Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina, USA. Sponsor: Ciba-Geigy Corp., Greensboro, North Carolina, USA. Report date: 3 Aug, 1993.

Technical diazinon (Ciba-Geigy Corp., Greensboro, North Carolina, USA; purity 87.7%; Lot no. FL-872049) in the diet was fed to Sprague-Dawley (CrI:VAF/Plus CD [SD] Br) rats (30/sex/group except for controls and the 250 ppm group that had 40/sex/group; Charles River

Laboratories, Kingston, New York, USA) at concentrations of 0, 0 (ie. with 26.5 ppm of epoxidized soybean oil (ESO; the stabiliser present in the technical formulation), 0.1, 1.5, 125, or 250 ppm a.i. (equal to 0.004, 0.06, 5 and 10 mg/kg bw/day for males and 0.005, 0.07, 6 and 12 mg/kg bw/day for females) for 98/99 weeks. Diazinon in acetone was mixed with rodent chow and air dried to remove the acetone. This admixture was found to be suitable with respect to homogeneity, stability and concentration (ie. within 15% of the target except for 2 batches of the 0.1 ppm feed that exceeded the target by 19% and 26% respectively) when stored at room temperature between batches. Dose selection was based on the results of (a) subchronic study/ies (although not explicitly stated, this reference probably relates to the 13-week feeding study by Singh et al, 1988; see section 5.1.2) so that for chronic dosing the no-observable effect level (NOEL) was anticipated to be 0.1 ppm whereas the second lowest concentration of 1.5 ppm was anticipated to be the no-observable adverse effect level (NOAEL) with only serum ChE activity being affected. ChE activities in brain, erythrocytes and plasma were all anticipated to be affected at the highest concentration of 250 ppm (ie. the MTD according to EPA guidelines). Rats were monitored daily for mortality and clinical signs. Bodyweight and food consumption were recorded before treatment and then weekly for the first 13 weeks and monthly thereafter. Physical and auditory examinations were performed 2 weeks before testing, and then during weeks 12, 26, 39, 52, 57 (for recovery rats only), 65, 79, 92, 97 (0.1 ppm males only) and 98. After 52 weeks of treatment, satellite groups of 10 rats/sex/group were sacrificed for an interim necropsy and another 10 rats/sex in the control, vehicle control (ie. with ESO) and 250 ppm groups were fed the untreated diet for a 4-week recovery period. Interim recovery rats were then sacrificed for necropsy during week 57. All other rats were sacrificed and necropsied during weeks 98-99 of treatment.

Urine volume and water consumption were recorded 2 weeks before exposure and then on weeks 15, 24, 49-50, 55 (recovery rats only) 77-78, and 97. Haematology and biochemistry (including plasma and erythrocyte ChE activities) parameters were assessed in weeks 13-14, 26-27, 51-52, 79-80, 97 (0.1 ppm males only) and 98. Urinalysis was performed during weeks 12-13, 28, 51, 79-80, 97 (0.1 ppm males only) and 98. Ten control rats/sex were used to establish baseline values for haematology, biochemistry and urinalysis 1 week before treatment; these animals were then killed. At sacrifice in the main study, bodyweight was measured and the main organs (ie. adrenal glands, brain (including brainstem), epididymides, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes, thymus, and uterus) removed and weighed before fixation.

Histopathological investigation was performed on any gross lesions (incl. tissue masses) and on specimens of adrenal gland, aorta, brain (cerebral and cerebellar cortex, medulla/pons), oesophagus, eyes (with optic nerves), ovaries, uterus, vagina, mammary gland, femur with marrow and joint, Harderian gland, heart, kidneys, lacrimal (exorbital) gland, large intestine (caecum, colon, rectum), liver, lungs, lymph nodes (mesenteric, submaxillary), epididymidis, prostate, seminal vesicle, testes, pancreas, peripheral (sciatic) nerve, pituitary, salivary (mandibular) gland, skeletal muscle (thigh), skin (abdominal region), small intestine (duodenum, jejunum, ileum), spinal cord (cervical, lumbar, thoracic), spleen, sternum with marrow, stomach (glandular, nonglandular), thymic region tissue, thyroid with parathyroid glands, tongue, trachea, and urinary bladder.

Owing to a reduced survival among males at 0.1 and 125 ppm (30% and 35% respectively), that was unrelated to treatment, the study was terminated (with US EPA concurrence) earlier than anticipated, ie. during weeks 98-99. Most deaths in all groups, irrespective of gender, were the result of pituitary adenoma and/or senile nephropathy. Both these conditions are apparently associated with senescence in this strain of rats.

Auditory and ophthalmologic examinations did not reveal any treatment-related findings. However, there was a progressive increase in the incidence of foot sores among 250 ppm males after 56 weeks so that by week 98, 6/11 (54%) survivors were affected, whereas at 0, 0 (ESO), 0.1, 1.5 and 125 ppm, 2/12 (17%), 1/9 (11%), 1/6 (17%), 3/10 (30%) and 1/7 (14%) respectively were affected. These foot sores among males at 250 ppm were considered to be secondary to a treatment-related increase in bodyweight despite there being a greater bodyweight gain at lower concentrations (ie. 34, 32, 17, and 23% at 0.1, 1.5, 125, and 250 ppm respectively). It seems that most of the increase in bodyweight at the end of treatment was attributable to the presence (or palatability) of ESO in the formulation because the gain relative to the ESO-control group (ESO concentration is equal to that present for the 250 ppm group) was reduced to 17, 15, 0, and 6% at 0.1, 1.5, 125, and 250 ppm respectively. A corresponding increase in food consumption among males approximately matched the observed increase in bodyweight. There was no change in water consumption among treatment groups and females did not have any treatment-related foot sores or corresponding increased food consumption with accompanying weight gain.

There were no treatment-related changes (ie. time patterns or dose-response relationships) evident in haematology (Hct, Hb, erythrocyte count, WBC, differential leucocyte count, Heinz body, platelet count, reticulocyte count, prothrombin time, mean corpuscular volume and mean corpuscular haemoglobin concentration) or urinalysis (colour/clarity, pH, specific gravity, protein, glucose, ketones, urobilinogen, bilirubin, occult blood and a microscopic examination) parameters. Similarly there were no treatment-related changes observed in the measured clinical chemistry parameters (ie. LDH, AST, ALT, AP, γ -glutamyl transferase, glucose, BUN, total bilirubin, cholesterol, albumin, globulin, A/G ratio, triglycerides, total protein, creatinine, CPK, sodium, potassium, chloride, calcium and phosphorus) except for ChE activity. These mean percentage reductions in ChE activities, expressed relative to concurrent untreated controls, are shown in the Table below.

ChE Inhibition after 13, 26, 51, 79 and 98 weeks of treatment (mean percentage reduction)

Concentration (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE†	
	male	female	male	female	male	female
0.1	7, 29, 1, 36, 43	26, 25, 17, 17, 16	0, 1, [6], 2, [7]	2, 1, [5], 5, 3	4, 3	5, [1]
<i>Mean</i>	15	20	[2]	1	4	2
1.5	29*, 12, 19, 14, 51*	68**, 54**, 52**, 45*, 30	0, [2], [7], [1], 5	4, 5, 0, 6*, 3	[1], 2	6, [6]
<i>Mean</i>	25	50	[1]	4	0	0
125	79**, 66**, 30, 86**, 89**	96**, 94**, 96**, 94**, 94**	16**, 16**, 16**, 28**, 21**	24**, 24**, 22**, 25**, 25**	2, 24**	26**, 29**
<i>Mean</i>	70	94	19	24	13	28
250	87**, 81**, 88**, 92**, 93**	98**, 97**, 97**, 96**, 95**	20**, 14**, 11**, 28**, 21**	26**, 22**, 20**, 29**, 25**	10, 42**	40**, 48**
<i>Mean</i>	88	97	19	24	26	44

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; † At weeks 52 & 98-99; * p≤0.05; ** p≤0.01.

The multiple entries for ChE activity in plasma and erythrocytes shown in the Table above are those recorded during treatment weeks 13, 26, 51, 79, and 98 respectively. Rats at 250 ppm that were allowed to recover for 4 weeks after 52 weeks of treatment had reduced inhibition of plasma (to [6%] in males and 10% in females), erythrocyte (1% in males and 7% in females) and brain ([5%] in males and 9% in females) ChE. Despite the increased activity, significance (p≤0.01) was still achieved for impaired brain and erythrocyte ChE activities in females. There was no inhibition of ChE activity in controls fed the diet supplemented with ESO.

The increased incidence of skin ulceration (pressure sores) observed on the feet of the 250 ppm males (10/20) relative to untreated (2/20) and vehicle controls (3/20) was dismissed as being related to treatment on the basis of an absence of a dose-incidence relationship. No other treatment-related changes were observed. All groups had a high incidence of pituitary tissue masses, including both control groups.

No treatment-related change in incidence of neoplasms was observed. The numbers of rats (20/group) with benign and malignant neoplasms after the second year of treatment were as follows:

Summary of Neoplasia Incidence

No. of rats with observ'n	Treatment Group						
	Sex	0 ppm	ESO (0)	0.1 ppm	1.5 ppm	125 ppm	250 ppm
Benign neoplasms	M	16	17	13	14	14	18
	F	18	16	15	17	17	17
Malignant neoplasms	M	5	10	5	6	5	5
	F	9	8	8	9	5	5
Total with neoplasms	M	16	20	17	16	17	19
	F	19	18	17	19	18	17

ESO=epoxidized soybean oil (the stabiliser present in the technical formulation)

No treatment-related effects were observed for tissue weight or on histopathological examination of tissues from treated and control rats. However, the following statistically significant findings were reported, although they are considered random (ie. not dose related) and unrelated to treatment. In males 250 ppm, focal islet cell hyperplasia in the pancreas (7/20 relative to 1/20 in vehicle controls), and stomach ulcers (2/20 relative to 0/20 in controls). In females, the ESO control group at the 1 year interim sacrifice had an increased incidence of squamous metaplasia in the uterus (5/9 relative to 1/10 in the untreated control), however there was no increase in squamous cell carcinoma incidence. At the terminal sacrifice, the 125 ppm group females had increased skin lipoma (3/30 relative to 0/40 in untreated controls), and in the 250 ppm group had an increased incidence of stomach oedema (2/20 relative to 0/20 in controls).

Following literature reports of possible retinal degeneration following administration of organophosphates, eyes and optic nerves (where available) from this study were re-assessed 2 years later by different pathologist from an independent laboratory (Mann PC, Experimental Pathology Laboratories, Inc). The conclusion drawn after examining sections from control, vehicle control and the 250 ppm group was that there were no significant ocular lesions in either the interim or interim recovery necropsy, and despite the presence of cataracts (unilateral and bilateral), inflammatory changes (iridocyclitis, hypopyon, phthisis bulbi and keratitis) and diffuse, bilateral retinal degeneration (a male and female in the control group and a female in the vehicle control) at the terminal kill, none were increased relative to the control incidence. Although no optic nerve lesions were found, these results are of doubtful value because almost half of these specimens were unavailable for examination for both the control and 250 ppm groups.

In conclusion, there was no evidence of carcinogenicity in rats at concentrations that caused a significant reduction of ChE activities in brain, erythrocytes and plasma of both sexes. The NOEL was 0.1 ppm (equal to 0.004 mg/kg bw/day for males and 0.005 mg/kg bw/day for females) based on significant plasma ChE inhibition at the next higher dose of 1.5 ppm.

6.3 Dog

Rudzki MW, McCormick GC & Arthur AT (1991) 52-week oral toxicity study in dogs. Study no. 882014. Lab: Ciba-Geigy Corp., Research Department, Pharmaceuticals Division, Summit, New Jersey, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 29 Aug, 1988 - 30 Aug, 1989. Report date: 14 Jun, 1991. (US GLP statement provided)

and

Mann PC (1993) Histopathological assessment of potential ocular toxicity of four organophosphate insecticides. Lab: Experimental Pathology Laboratories, Inc., Research Triangle Park, North Carolina, USA. Sponsor: Ciba-Geigy Corp., Greensboro, North Carolina, USA. Report date: 3 Aug, 1993.

Technical diazinon (Ciba-Geigy Corp., Greensboro, North Carolina, USA; purity 87.7%; Lot no. FL-872049) in the diet was fed to Beagle dogs (4/sex/group; Marshall Farms, North Rose, New York, USA) at concentrations of 0, 0.1, 0.5, 150, or 300 ppm a.i. (equal to 0.0032, 0.015, 4.7, or 7.7 mg/kg bw/day for males and 0.0037, 0.02, 4.5, or 9.1 mg/kg bw/day for females) for 52 weeks. Owing to a lack of bodyweight gain for dogs at 300 ppm, the dose was reduced to 225 ppm after 14 weeks. Dog food (chow) with diazinon was prepared by the addition of an acetone/diazinon solution and then allowing the admixture to air dry to remove the acetone. This admixture was found to be suitable with respect to homogeneity, stability and concentration (ie. within 15% of the target except for 1 batch of the 0.1 ppm feed at week 2 that exceeded the target by 21%) when stored at room temperature between batches. Dose selection was based on the results of a 13-week feeding study by Barnes et al, (1988; see section 5.2) so that for chronic dosing the no-observable effect level was anticipated to be 0.1 ppm whereas the second lowest concentration of 0.5 ppm was anticipated to be the no-observable adverse effect level with only serum ChE activity being affected. ChE activities in brain, erythrocytes and plasma were all anticipated to be affected at the highest concentration of 300 ppm (ie. the MTD according to EPA guidelines). Dogs were monitored daily for mortality and clinical signs. Bodyweight and food consumption were recorded before treatment and then weekly for the first 16 weeks and monthly thereafter. Physical and auditory examinations were performed 4 weeks before testing, and then during weeks 12, 26, 39 and 51. All dogs were sacrificed and necropsied during week 52 of treatment.

Clinical laboratory determinations (haematology, biochemistry (including plasma and erythrocyte ChE activities) and urinalysis were performed 4 weeks before treatment and then at weeks 13, 26, (27 & 38, urinalysis only), 39 and 52. At the terminal kill, bodyweight was measured and the main organs (ie. adrenal glands, brain (including brainstem), epididymides, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes, thymus, and uterus) removed and weighed before fixation.

Histopathological investigation was performed on specimens of adrenal gland, aorta, brain (cerebral and cerebellar cortex, medulla/pons), oesophagus, eyes (with optic nerves), ovaries, uterus, vagina, mammary gland, femur with marrow and joint, gall bladder, heart, kidneys, lacrimal (exorbital) gland, large intestine (caecum, colon, rectum), liver, lungs, lymph nodes (mesenteric, submaxillary), epididymidis, prostate, seminal vesicle, testes, pancreas, peripheral (sciatic) nerve, pituitary, salivary (mandibular) gland, skeletal muscle (thigh), skin (abdominal region), small intestine (duodenum, jejunum, ileum), spinal cord (cervical, lumbar, thoracic), spleen, sternum with marrow, stomach (glandular, nonglandular), thymic region tissue, thyroid with parathyroid glands, tongue, trachea, and urinary bladder.

One female at 0.5 ppm died on day 12 whereas a male *in extremis* after 1 day at 300 ppm was sacrificed on day 2. Clinical signs preceeding death were recumbency, hypoactivity, pallor, hypoactivity, diarrhoea, blood in faeces, vocalization, hypo- and hyper-thermia, labored and irregular breathing and salivation in the male, and diarrhoea with blood in the female. Necropsy revealed serosanguinous fluid in the pleural cavity, a firm lung lobe and a diffusely red colon in the male and reddening of the duodenal and jejunal mucosa in the female; both findings being consistent with the diagnosis as a generalised infection of the GI tract and therefore unrelated to treatment. The 2 dead dogs were replaced on days 15 and 4 respectively but their treatment was not extended so that data collected and reported for these groups refers to study days and not actual days of dosing.

Apart from a marked reduction in bodyweight gain among dogs at 300 ppm, (ie. 10% and 24% that for controls in males and females respectively) that indicated a dose in excess of the MTD and therefore necessitated a reduction to 225 ppm after 14 weeks (day 99) of treatment, significant reduction ($p < 0.05$ or 0.01) in bodyweight gain was also observed at among males at 150 ppm from day 14 to day 70. This reduced weight gain for males at 150 ppm resulted in a mean bodyweight difference of 16% by the end of treatment. Although food consumption was significantly reduced ($p < 0.05$ or 0.01) at day 21, 28 and 35 for 150 ppm males, the total mean consumption was reduced by 23% over the duration of the study. Females at 150 ppm also had reduced food consumption that achieved significance on days 35, 42, 70, 84, 98, 140, 168, 308 and 336, however, the overall mean reduction in consumption (22%) relative to controls was similar to males.

Auditory and ophthalmologic examinations (at week 23 and 53) did not reveal any treatment-related findings. There were no treatment-related changes (ie. time patterns or dose-response relationships) evident in haematology (Hct, Hb, erythrocyte count, WBC, differential leucocyte count, Heinz body, platelet count, reticulocyte count, clotting time, prothrombin time and erythrocyte morphology) or urinalysis (volume, colour/clarity, pH, specific gravity, protein, glucose, ketones, urobilinogen, bilirubin, occult blood and a microscopic examination) parameters. Similarly there were no treatment-related changes observed in the measured clinical chemistry parameters (ie. LDH, AST, ALT, AP, γ -glutamyl transferase, glucose, BUN, total bilirubin, cholesterol, albumin, globulin, A/G ratio, triglycerides, total protein, creatinine, CPK, sodium, potassium, chloride, calcium and phosphorus) except for amylase and ChE activities. The mean percentage reductions in ChE activities, expressed relative to concurrent untreated controls, are shown in the Table below.

ChE Inhibition after 13, 26, 39 and 52 weeks of treatment (mean percentage reduction)

Concentration (ppm)	Plasma ChE		Erythrocyte ChE		Brain ChE†	
	male	female	male	female	male	female
0.1	[7], [5], [15], [8]	18*, 9, 29*, 9	[10], [10], [5], [6]	2, [1], 2, 3	[9]	[4]
<i>Mean</i>	[9]	16	[8]	2	[9]	[4]
0.5	22, 24*, 5, 23	32**, 37**, 32**, 19**	[2], 4, 12, 12	[5], 2, 6, [1]	6	3
<i>Mean</i>	19	30	7	0	6	3
150	80**, 78**, 74**, 78**	81**, 75**, 86**, 78**	26**, 25**, 33**, 28**	26**, 32**, 32**, 33**	15	25*
<i>Mean</i>	78	80	28	31	15	25
300/225	78**, 70**, 65**, 74**	85**, 79**, 88**, 78**	21**, 27**, 24**, 28**	32**, 29**, 35**, 34**	25	35**
<i>Mean</i>	72	83	25	33	25	35

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; † At week 52; * $p \leq 0.05$; ** $p \leq 0.01$.

Apart from significant plasma ChE inhibition among females at 0.5 ppm, all dogs had significant ChE inhibition in plasma and erythrocytes at the next higher tested concentration of 150 ppm. In keeping with the enhanced ChE sensitivity observed among females, brain ChE activity was also significantly inhibited at a lower concentration (150 ppm) than for males, where appreciable inhibition was only observed at the highest tested concentration of 300/225 ppm. Increased serum amylase activity appeared to mimic plasma ChE inhibition in females, ie. a dose-response relationship with appreciably elevated activities being observed at the equivalent diazinon concentration; this effect can be seen in the Table below. Amylase activity among males was elevated at all concentrations though not in exact dose relationship, however, significance ($p \leq 0.05$) was only occasionally achieved among both males and females. A possible explanation to account for this lack of significance despite the 2-3 fold activity increase was the rather large intragroup variability (as shown by a large standard error among treatment groups); typically a phenomenon observed when group size is small (here $n=4$). There were no corresponding pancreatic lesions associated with these increased amylase activities.

Group Serum Amylase Activity (after 13, 26, 39 and 52 weeks of treatment)

Concentration (ppm)	Amylase activity increase (%)			
	male	mean	female	mean
0.1	8, 12, 7, 14	10	[4], 0, [4], [7]	[4]
0.5	9, 9, 5, 8	8	13, 13, 9, 4	10
150	37, 28, 22, 22*	27	27, 26, 19, 23	24
300/225	64, 13, 13, 12	26	35*, 29*, 63, 20	37

Values in square brackets indicate the extent (%) to which the measured activity was less than controls; * $p \leq 0.05$

Apart from significantly reduced ($p < 0.05$ or 0.01) absolute and brain weight-relative lung and mandibular salivary gland weight among females at 150 and 300/225 ppm (absolute lung weights by 19% and 28% respectively, relative weight by 19% and 29% respectively; absolute salivary gland, 27% and 22% respectively, relative weight by 28% and 23% respectively) that appeared normal by gross and histopathological examination, all other tissues were little changed with respect to weight, appearance and lesions.

Following literature reports of possible retinal degeneration following administration of organophosphates, eyes and optic nerves (where available) from the dogs in this study were re-assessed by a different pathologist from an unrelated laboratory (Mann PC, Experimental Pathology Laboratories, Inc) 2 years after the study had been completed. The conclusions drawn after re-examining sections from control and 300/225 ppm groups confirmed the earlier finding, namely that there were no significant ocular or optic nerve lesions at the terminal kill.

In conclusion, diazinon administered to dogs in their food caused significant bodyweight loss in males and inappetence in both sexes at 150 ppm (equal to 4.7 mg/kg bw/day in males). The NOEL for the study was established at 0.1 ppm (equal to 0.0037 mg/kg bw/day in females) based on a dose-related elevation in serum amylase activity and significant plasma ChE inhibition in females at the next higher concentration of 0.5 ppm.

6.4 Monkey

Cockrell KO, Woodard MW & Woodard G (1966) Diazinon 50W. Safety evaluation by repeated oral administration to monkeys for 106 weeks. Final report. Report no. not stated. Lab: Woodard Research Corp., USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, USA. Study duration: not stated. Report date: 1 Jun, 1966. (Pre GLP)

Diazinon 50W (Ciba-Geigy Corp.; purity 48.6%; Lot no. 72237) was administered PO by stomach tube to Rhesus monkeys (3/sex/group; source not stated) at doses of 0, 0.05, 0.5, or 5 mg/kg bw/day (as a.i.) for 6 days/week. The protocol indicates that the originally selected doses were twice those indicated (here) and were administered for 34 days before reducing them for the remainder of the study, ie. to week 106. The reason for this change to the original protocol was not disclosed but is likely to be based on clinical signs because ChE activities were not measured (by the Δ pH/h method) until week 66 of the study. The rationale for the dose selection were not detailed in the final report, though there is a suggestion that it may have been in the 26- and/or 52-week interim reports that were not submitted for review. Bodyweight was measured at week 26, 52, 78 and 106 and clinical monitoring was performed (daily?). Ophthalmoscopy and a neurological assessment (involving motor and sensory activity, and reflexes) were performed at week 106. Haematology (Hct, Hb, WBC, a leucocyte differential count, and sedimentation rate) and clinical chemistry (blood glucose, BUN, AP, ALT, AST, and ChE activities in erythrocytes and plasma) parameters were determined at week 66, 78, 91 and 104. ChE activity in the brain was measured at the terminal kill. Qualitative urinalysis (parameters were not detailed) was performed at week 65, 78, 93 and 104.

Although 4 monkeys died (a male and female at 5 mg/kg bw/day, a female at 0.5 mg/kg bw/day and a male at 0.05 mg/kg bw/day) during the study, none were attributed to treatment despite the absence of any deaths in the control group. Necropsy did not establish a cause of death in 3 of 4 though it was concluded, on the basis of significant bodyweight loss (emaciation) prior to death together with a more rapid sedimentation rate and an elevated WBC, that intercurrent infections were the most likely cause.

Clinical signs that appeared to be treatment-related were predominantly observed at 0.5 and 5 mg/kg bw/day where tremors, soft faeces and a degree of hypersensitivity to touch and sound were transiently observed throughout treatment. The number and gender of monkeys having these signs was not reported except for hyperesthesia that was observed in 1 monkey at 5 mg/kg bw/day and 0.05 mg/kg bw/day respectively. Reduced bodyweight gain was observed in all

groups throughout treatment but the reduced mean bodyweight after 106 weeks only achieved significance (14%) for the 5 mg/kg bw/day group. The ophthalmoscopic examination or neurological assessment did not reveal any changes attributable to treatment. Similarly, although no data were shown, the summary indicated that no changes in haematology, urinalysis or clinical chemistry were observed except for ChE activity in plasma and erythrocytes. These ChE reductions measured at week 66, 78, 91 and 104 are shown as a median percentage in the Table below. No data for brain ChE activity was supplied but the summary indicated that a significant reduction was observed in 1 monkey at the highest dose and in 2 monkeys that died during the study following exposure at 0.05 and 0.5 mg/kg bw/day respectively.

**ChE Inhibition after 66, 78, 91 and 104 weeks of treatment
(median percentage reduction for each group)**

Dose mg/kg bw/day)	Plasma ChE	Erythrocyte ChE
0.05	8, 9, 10, 1	0, 0, 0, 0
0.5	84, 46, 82, 8	33, 7, 0, 1
5	82, 84, 86, 20	88, 70, 67, 62

As shown in the Table, plasma ChE activity was substantially depressed at 0.5 mg/kg bw/day, whereas ChE activity in erythrocytes became substantially inhibited at the highest dose of 5 mg/kg bw/day. Gross necropsy, absolute and body-relative organ weights (ie. liver, heart, thyroids, brain, kidneys, lung pituitary and adrenals), and histopathology (ie. adrenals, aorta, brain, oesophagus, eye, ovary, uterus, mammary gland, bone marrow, heart, kidney, large intestine, liver, lung, lymph node, prostate, seminal vesicles, testes, pancreas, peripheral nerve, pituitary, salivary gland, skeletal muscle, skin, small intestine, spinal cord, stomach, thymus, thyroid, trachea, gall and urinary bladder for the control and 5 mg/kg bw/day groups) revealed no treatment-related changes.

The reporting of many important parameters in this study were so limited that no independent assessment of treatment-related changes other than for ChE inhibition was possible. However, since plasma ChE inhibition is normally the most sensitive marker of OP exposure, a NOEL based on ChE inhibition can be established at 0.05 mg/kg bw/day due to the reduction of ChE activity in plasma at 0.5 mg/kg bw/day.

7. REPRODUCTIVE TOXICITY

7.1 Mouse

Spyker JM & Avery DL (1977) Neurobehavioral effects of prenatal exposure to the organophosphate diazinon in mice. Department of Pharmacology, University of Arkansas for Medical Sciences, Little Rock, Arkansas. J Toxicol Environ Health 3: 989-1002

Groups of six adult female F₂ hybrid mice (National Center for Toxicological Research, Jefferson, Arkansas; age not stated) were mated 1:1 with F₂ males. Mated females were given diazinon (research lot MG8-FL741305; Ciba-Geigy) at doses of 0 (control), 0.18 or 9.0 mg/kg bw/day until parturition. The test material was mixed with peanut butter to give a daily administration volume of 1 mL. No information was provided on the stability of diazinon in the feed mixture. Animals were weighed daily during gestation. At birth, pups were examined for viability and gross physical defects. Within 6 h of birth, pups were randomised within treatment groups to give each

treated or control dam four male and four female pups from a like-treated dam. Offspring were weighed daily and examined for morbidity and mortality. Reflex ontogeny was evaluated by daily scoring for the presence or absence of physical or behavioural markers of development, namely simple righting, acceleration righting, contact placing, physical maturity (opening of the eyes and ears and first appearance of hair on the dorsal surface) and sexual maturity (date of vaginal opening or testes descent).

Offspring were weaned on postnatal day (PN) 28. On PN 38, visual, auditory and olfactory system functions were examined. Neuromuscular coordination was assessed on PN 50 by scoring swimming ability. Swimming activity was expressed as the amount of time spent swimming over a 10-minute period, and a qualitative score was also given for swimming style. Locomotor strength and endurance were tested on PN 60 by using a rod cling apparatus. On PN 65 a second endurance test (rotarod) was conducted. Other tests were conducted at PN 70 (inclined plane apparatus), PN 75 (exploratory activity/open-field apparatus), and PN 76 (open-field test repeated). Beginning on PN 87, animals were deprived of feed and reduced to 80% of their free-standing weight, then trained to run in a 130-cm long alley for food reinforcement. All animals were killed at 101 days of age. Brain sections were examined microscopically.

Results

Maternal and offspring data: As shown in the summary (Table) below the group mean bodyweight gain during gestation was about 14% lower in groups given 0.18 and 9.0 mg/kg bw/day diazinon, but there was no dose-response relationship with this effect, and the toxicological significance of this finding is not clear. No data on the group mean bodyweights were provided, and it is not possible to tell if the mean bodyweights were similar in all groups prior to treatment. A significant ($p < 0.05$) decrease in the mean litter size was observed at 0.18 mg/kg day only, and in the absence of any dose-response, this finding was not considered to be treatment-related. Offspring bodyweights at birth were not affected by treatment, but there was a slight but statistically significant decrease in mean pup bodyweights between weeks 1 and 3. Due to the graphical presentation of the data, the magnitude of this effect is not clear, but it appears as though the bodyweights were reduced by about 10-20% compared with controls. By the end of the study, group mean bodyweights were similar in control and high-dose groups. However, as the group mean bodyweights in animals given 0.18 mg/kg bw/day diazinon were generally higher than controls throughout the study, the toxicological significance of these bodyweight changes is equivocal.

Summary of maternal and offspring data (mean $\pm$ SD)

	Dose (mg/kg bw/day)		
	0	0.18	9.0
Maternal weight gain during pregnancy (g)	17.9 $\pm$ 1.0	15.4 $\pm$ 0.9*	15.4 $\pm$ 0.6*
Litter number	8.3 $\pm$ 0.3	6.6 $\pm$ 0.7*	8.3 $\pm$ 0.2
Gestation duration (days)	19.3 $\pm$ 0.2	19.7 $\pm$ 0.1	19.6 $\pm$ 0.1
Number of dams	22	21	19
Number of offspring	159	133	132

* $p < 0.05$ (t-test)

Developmental landmarks: As shown in the Table below significant ($p < 0.05$) retardation of development was confined to slight increases in the time of appearance of contact placing and the

onset of sexual maturity in animals at 0.18 mg/kg bw/day only. There was no dose-response relationship demonstrated, and no effects at the high-dose level. As such, these findings were not considered to be treatment-related.

Summary of developmental landmarks (postnatal day of appearance; mean $\pm$ SD)

	Dose (mg/kg bw/day)		
	0	0.18	9.0
Simple righting	3.2 $\pm$ 0.4	3.0 $\pm$ 0.1	2.7 $\pm$ 0.4
Hair coat	4.9 $\pm$ 0.2	4.9 $\pm$ 0.1	4.2 $\pm$ 0.1
Contact placing	11.0 $\pm$ 0.4	13.3 $\pm$ 0.4*	9.1 $\pm$ 0.3
Acceleration righting	10.9 $\pm$ 0.3	11.2 $\pm$ 0.9	11.1 $\pm$ 0.4
Eye opening	13.6 $\pm$ 0.4	13.3 $\pm$ 0.1	14.3 $\pm$ 0.3
Ear opening	13.9 $\pm$ 0.3	13.3 $\pm$ 0.2	14.7 $\pm$ 0.3
Sexual maturity	28.4 $\pm$ 0.4	30.0 $\pm$ 0.4*	26.8 $\pm$ 0.6
Number of offspring	67	48	44

*p<0.05 Mann-Whitney U-test

Endurance and coordination: Swimming activity was decreased at 0.18 mg/kg bw/day but increased at 9.0 mg/kg bw/day (see Table below). No toxicological significance was given to these findings. Slight increases (p<0.05) in rod cling endurance and slight decreases (p<0.05) in inclined plane measurements were not dose-dependent, and were not considered to be treatment-related. Large, dose-related decreases in rotarod endurance were not statistically significant, indicating that the data variability was probably large, and this finding was not considered to be treatment-related.

Male and female offspring from dams exposed to 9.0 mg/kg bw/day displayed impaired running performance in a Lashley III maze, but only in the last 10 of 45 trials (data presented graphically only). In the first 35 trials, the running speed of offspring from treated dams was similar to or greater than the speeds of control offspring. The toxicological relevance of the findings from the last 10 trials is not known. Open-field data were unaffected by treatment.

Summary of endurance and coordination (mean $\pm$ SD)

Dose (mg/kg bw/day)	0	0.18	9.0
Swimming activity (sec)	200 $\pm$ 23	190 $\pm$ 19	243 $\pm$ 34*
Rod cling endurance (sec)	65 $\pm$ 4	78 $\pm$ 6*	78 $\pm$ 8*
Rotarod endurance (sec)	1006 $\pm$ 621	407 $\pm$ 161	103 $\pm$ 56
Inclined plane (degrees)	120 $\pm$ 7	104 $\pm$ 8**	109 $\pm$ 8**
Number of offspring tested	40	20	20

*p<0.05, **p<0.01 (by one-way analysis of variance and post-hoc analysis)

Pathology: It was reported that morphological abnormalities were observed in the brain tissue of offspring from dams treated with diazinon at 9.0 mg/kg bw/day, with focal defects in the forebrain in the area extending from the anterior commissure to the anterior olfactory nucleus. In 5/8 brains examined, dense aggregations of chromatin-containing cells were reported. These findings were not observed in offspring born to control dams or dams exposed to 0.18 mg/kg bw/day. The low level of reporting, and the lack of pathology data, makes the relevance of these findings unclear. The report was not adequate for regulatory purposes.

Summary

Female mice were exposed to diazinon throughout gestation at doses of 0, 0.18 or 9.0 mg/kg bw/day. Delayed bodyweight gains were seen in high-dose offspring, and this delay in development might have been treatment-related, but the level of reporting was not adequate for regulatory purposes. No other clear dose-related effects on pup development, endurance or coordination were seen at any dose. A reduction in running speed in a number of trials, and the presence of some chromatin-containing cells in the brain tissues of some high-dose pups were noted, but the toxicological relevance of these findings was not clear (Spyker & Avery, 1977).

Spyker Cranmer J, Avery DL, Grady RR & Kitay JI (1978) Postnatal endocrine dysfunction resulting from prenatal exposure to carbofuran, diazinon or chlordane. Department of Pharmacology, University of Arkansas for Medical Sciences, Little Rock, Arkansas, and Departments of Physiology and Internal Medicine, University of Virginia, Charlottesville, Virginia, USA. J Environ Path Toxicol 2: 357-369.

Groups of six adult female F₂ hybrid mice (National Center for Toxicological Research, Jefferson, Arkansas; age not stated) were mated with individual F₂ males. Mated females were given diazinon at doses of 0 (control), 0.18 or 9.0 mg/kg bw/day until parturition or day 22. There were two concurrent control groups. The test material was mixed with peanut butter to give a daily dose volume of 1 mL. No information was provided on the stability of diazinon in the feed mixture. Animals were weighed daily during gestation. The uterus was removed from dams that failed to deliver by day 22 and examined microscopically. At birth, pups were examined for viability and gross physical defects, and their sex recorded. Within 6 h of birth, pups were randomised within treatment groups to give each treated or control dam four male and four female pups from a like-treated dam. Offspring were weighed daily and examined for morbidity and mortality until weaning on day 28. All animals were killed at 101 days of age.

Levels of plasma corticosterone, *in vitro* adrenal corticosterone production, and hepatic corticosterone metabolising capacity *in vitro* were determined in mice decapitated within 15 sec of being removed from a rested state in their cages. Plasma corticosterone levels were also measured in animals stressed by swimming in ice water for 20 seconds. Animals were decapitated 15 min after the swimming, and immediately after sacrifice, liver and adrenal glands were excised and trunk blood was collected for analysis of plasma corticosterone. The *in vivo* reductive capacity of corticosterone by the intact livers was estimated, and the adrenal corticosterone production in the adrenals and the plasma corticosterone levels was also estimated.

Results

Statistically significant increases in plasma corticosterone levels (males and females), bodyweight (females), liver weight (females), total liver corticosterone reduction (males and females), and liver reduction of side-chain corticosterone (males) were observed at 0.18 mg/kg bw/day only. At 9.0 mg/kg bw/day, a reduction in adrenal weight was observed in females only. In the absence of any dose-response relationship, the above findings were not considered to be treatment-related.

This study was not adequate for regulatory purposes.

Summary

Groups of six mated adult female F₂ hybrid mice given diazinon at doses of 0 (control), 0.18 or 9.0 mg/kg bw/day until parturition or day 22. There were two concurrent control groups. Within 6 h of birth, pups were randomised within treatment groups to give each treated or control dam four male and four female pups from a like-treated dam. Offspring were weighed daily and examined for morbidity and mortality until weaning on day 28. All animals were killed at 101 days of age. Statistically significant increases in plasma corticosterone levels (males and females), bodyweight (females), liver weight (females), total liver corticosterone reduction (males and females), and liver reduction of side-chain corticosterone (males) were observed at 0.18 mg/kg bw/day only. At 9.0 mg/kg bw/day, a reduction in adrenal weight was observed in females only. In the absence of any dose-response relationship, the above findings were not considered to be treatment-related. This study was not adequate for regulatory purposes (Spyker Cranmer et al, 1978).

7.2 Rat

Ginkis MLA (1989) A two generation reproductive study in albino rats. EPA Guidelines No. 83-4 Report no. 88128/MIN 852218. Lab: Research Department, Toxicology/Pathology Division, Ciba-Geigy Corporation, Summit, NJ, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 2 Dec, 1985 - 15 Sep, 1986. Report date: 9 Feb, 1989. (US GLP statement provided)

Sprague-Dawley rats (CR CD; 146 males/145 females) from the Charles River Laboratory, USA were assigned to acclimatisation colonies one week after arrival (males aged 38 days; females aged 37 days). One hundred and twenty rats of each sex were then allocated to treatment groups (30/sex/group). Diazinon technical (Ciba-Geigy Agricultural Division; Batch FL 841650; 94.9% purity) was given to the rats at dietary levels of 0 (control), 10, 100 or 500 ppm, for two parental generations of animals and their offspring throughout all phases of this study.

Stability, homogeneity and test material concentration: Control animals received Purina #5002 Certified Rodent Chow (pulverised). Acetone was used as a solvent to prepare the feed and was subsequently removed by evaporation. For the test groups, homogeneous blends of technical diazinon in the diet were prepared. A stability study confirmed the stability of admixtures of diazinon technical in rodent feed at concentrations of 5 ppm and 2000 ppm, for at least 35 days at room temperature. The test material concentrations represented 93 to 101% of target concentrations over this period. The homogeneity of six representative batches of diazinon technical in rodent feed was tested, and three samples from each batch were analysed. For the six batches the range in the relative standard deviation was 1.4% to 2.7%, and the samples ranged from 95 to 100% of the target concentrations at 10, 100 and 500 ppm. The test material concentrations were also analysed periodically (weeks 1, 5, 9, 21, 23, and 41). The concentration of one 10 ppm batch was determined to be 77% of the expected concentration. As animals only received this batch of feed for 2-3 days, it was not considered that this batch would affect the outcomes of the study. However, the batch was replaced after 2-3 days with an acceptable batch. All other batches were within 10% of the target concentrations.

Experimental design: The first day of F₀ (P₁) exposure was designated day 0 of the pre-mating phase.

Summary of experimental design

Event	Period
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Pre-mating exposure F0 (males and females)	10 weeks
F0 (P1) mating: males and females housed 1:1 Animals separated on the day that evidence of mating or pregnancy was observed	3 weeks maximum
Deliveries of F1 pups. Records made of number and sex of viable pups and number of stillborn pups. Newborn examined for gross abnormalities. On postnatal day 4, litters were randomly culled to eight pups (4/sex). Other pups were subject to gross internal examination. Pup bodyweights recorded on lactation days 0, 4, 7, 14, and 21. Pups were observed for changes in appearance and behaviour.	
F0 (P1) males sacrifice	Days 110, 117, 118, and 119 of study.
F0 (P1) females sacrifice	Following weaning of F1 pups on day 133 of study.
Selection of F1 pups for second parental generation (P2): 30 animals/sex/group selected. Wherever possible, one pup of each sex was randomly selected from each available litter.	
The protocol for P2 animals was similar to the P1 phase of the study, except that the pre-mating exposure of P2 animals was 11 weeks.	

In-life observations: All parental animals were observed daily for deaths, changes in appearance and behaviour. Feed consumption for males was recorded weekly during the pre-mating period only. Bodyweight data were recorded weekly for males during pre-mating, mating and at terminal sacrifice. Feed consumption and bodyweight data for females were recorded weekly during the pre-mating period and on days 0, 7, 14 and 20 of gestation. Female bodyweights were also recorded on lactation days 0, 4, 7, 14, and 21. Females that showed evidence of mating but failed to produce a litter were weighed as scheduled during presumed gestation and at termination. Parental animals that died or were sacrificed were necropsied. Apart from recording the pregnancy status, no other reproductive parameters were assessed for females that died before their scheduled sacrifice.

Post-mortem examination: All parental animals were sacrificed and necropsied, and their gonads were removed and weighed. Uterine implantation sites were counted. Any grossly abnormal tissues were collected and fixed, as were samples of vagina, cervix, uterus, ovaries, mammary tissue, epididymides, seminal vesicles, prostate, pituitary and coagulating gland. These tissues were examined microscopically for all parental females. Similar tissues were collected and examined from forty F2 pups following their sacrifice on lactation day 21.

Reproduction parameters: A range of reproductive parameters were recorded and reproductive indices calculated, including fertility and mating index for both sexes, gestation duration in females, pre- and post-culling survival of pups, and pup sex ratio.

Results

F0 (P1) generation

Tremors were seen in 3/30 females at 500 ppm in the post-mating phase of the study. No tremors were seen at other doses. Two females at this dose were also observed with dystocia (difficulty in giving birth), and died or were killed as a result. It is not clear if the dystocia was related to treatment. A single female died at 100 ppm after having been described as unthrifty and suffering from chromodacryorrhea. The cause of death of this female was unknown. Other signs (including alopecia, and soft stool) were not considered to be related to treatment as they were seen at a low incidence and/or were seen in both control and treated animals.

Test article consumption: The ranges of diazinon consumption in F0 animals are outlined in the Table below.

Diazinon intake in F0 rats (range in mg/kg bw/day)

		Dietary concentration (ppm)		
		10	100	500
Pre-mating	Males	0.5-0.89	5.05-8.92	26.08-44.24
	Females	0.67-0.91	6.39-9.09	33.10-48.86
Gestation	Females	0.62-0.75	6.18-7.36	32.89-36.58

Food consumption: Slight (6 to 12%) but significant ($p < 0.05$) increases in food consumption were seen in high-dose F0 females in the pre-mating period and during gestation days 7-14, compared with controls. This effect (increased food consumption) was not considered to be toxicologically significant. No changes in food consumption were seen in F0 males pre-mating.

Bodyweights: Group mean bodyweights were not affected by treatment in the pre-mating (males and females) or gestational (females only) periods in F0 animals. Decreases in bodyweights of about 4 to 5% were seen in high-dose females during lactation. This change in weights reached statistical significance on lactation day 14 only, and was not considered to be biologically significant. On occasions, the group mean bodyweight gains in high-dose animals were significantly ($p < 0.05$) reduced compared with controls. These findings were generally isolated in nature during pre-mating, and were not considered to be related to treatment as the magnitude of the changes was not great. During gestation, decreases in bodyweight gain at 500 ppm were observed for days 0-7 (17% reduction, $p < 0.05$), 7-14 (11%), 14-20 (5%) and 0-20 (10%). These decreases in bodyweight gain in high-dose females during gestation, though significant only for the days 0-7 interval, were considered to be treatment-related. Increases in bodyweight gain in high-dose females during lactation days 14-21 may have resulted from a decrease in pup survival at 500 ppm, and the resultant decrease in viable pups present at lactation day 0. However, bodyweight gains were reduced in high-dose females on lactation days 0-4, 4-7, 7-13, and 0-21 by up to 50% compared with controls.

Pathology: No treatment-related gross observations were noted at the F0 parental necropsy examination, and histopathological examination did not reveal any treatment-related findings.

Reproductive parameters: As shown in the Table below, there were decreases in the survival indices for F1 pups (male and female) for days 0-4 (pre-cull) and days 4-21 (post-cull). All other reproductive parameters were similar in control and treated groups.

Summary of F1 litter survival (mean %)

	Dietary concentration (ppm)			
	0	10	100	500
Pups surviving days 0-4 (pre-cull)	88.7	97.6	95.4	61.3*
Pups surviving days 4-21 (post-cull)	99.0	98.7	93.5*	61.7*
Males surviving days 0-4 (pre-cull)	96.1	98.6	95.1	64.3*
Males surviving days 4-21 (post-cull)	100.0	99.1	93.5	59.5*
Females surviving days 0-4 (pre-cull)	89.0	97.0	95.7	60.5*
Females surviving days 4-21 (post-cull)	97.8	98.2	93.5	67.6*

*p<0.05

F1 pup observations: Gross examination of F1 pups revealed a single high-dose pup with a filamentous tail. No other gross malformations were observed. Lethargy and unthriftiness were observed in pups from single litters at 500 ppm. No milk was found in the stomachs of about 90 pups (6 litters) at the high-dose. Significant (p<0.05) decreases in group mean bodyweights were observed in high-dose male and female F1 pups from day 4 onwards. The reduction in bodyweights reached 28% compared with controls by day 21 postpartum, and this effect was treatment-related. At 100 ppm, significant (p<0.05) but isolated decreases in bodyweights (about 7% compared with controls) were also seen in males at day 14 only and females on day 7 only.

F1 (P2) generation

A range of minor clinical signs was seen in control and treated F1 (P2) animals during the study. No increase in the incidence of clinical signs was observed at any dose in the pre-mating or mating phases of the study. In the post-mating phase, 4/30 high-dose females had tremors, but there were no other clinical signs related to treatment. There were no compound-related deaths in any group.

Test article consumption: The ranges of diazinon consumption in F1 animals are outlined in the Table below.

Diazinon intake in F1 rats (range in mg/kg bw/day)

		Dietary concentration (ppm)		
		10	100	500
Pre-mating	Males	0.51-1.07	5.19-10.73	27.25-59.73
	Females	0.61-1.00	6.18-10.46	31.97-61.12
Gestation	Females	0.59-0.68	6.06-6.60	32.13-33.60

Food consumption: Significant (p<0.05) reductions in group mean food consumption (8-14% compared with controls) were seen in high-dose males at every pre-mating sample interval. On a few occasions, the food consumption was also reduced (5-8%; p<0.05) at 100 ppm, but at this dose the effect was not considered to be biologically significant. Food consumption in F1 females in the pre-mating and gestational phases of the study was similar to control values. The reduction in food consumption in pre-mating high-dose males was exacerbated by treatment, as the F1 males were about 25% smaller than controls at the beginning of the pre-mating period.

Bodyweights: Group mean bodyweights were significantly reduced ($p<0.05$) at 500 ppm in F1 males in the pre-mating period. These reductions ranged from 25% at day 0 to 12% at day 63, and the terminal bodyweights of high-dose males were about 14% lower than controls. In high-dose females, group mean bodyweights were about 16% lower than controls at day 0 pre-mating ($p<0.05$), and this difference was only 6% by the end of the pre-mating phase. Decreases in bodyweights ranged from 6-10 % in high-dose females during gestation and lactation. So while bodyweights in F1 males and females were reduced by about 28% at the end of the 21-day postnatal period, the reductions in bodyweights were not so great by the end of the F1 (P2) pre-mating phase.

At 100 ppm, the mean bodyweight of F1 males was significantly reduced ($p<0.05$) at every pre-mating sample interval from day 56 to day 91. However, the difference in bodyweights was only 5-6% compared with controls during this period, which was consistent with the 5% lower bodyweights in this group at day 0 pre-mating.

Pre-mating, the bodyweight gain in F1 high-dose males was occasionally reduced compared with controls ($p<0.05$), and for the 0-77 day period, the overall reduction in bodyweight gain was 10%. At 100 ppm, there were also occasions when bodyweight gains were statistically significantly reduced compared with controls, but for the 0-77 day pre-mating phase the overall reduction in bodyweight gain was about 6%. In high-dose females, bodyweight gains during pre-mating and lactation were variously increased or decreased at different intervals. During gestation however, bodyweight gains were significantly reduced ($p<0.05$) for the intervals 0-7, 14-20, and 0-20. The bodyweight gain over the whole lactation period was greatest in high-dose females, possibly related to the reduced number of pups being supported due to reduced pup survival at this dose.

Pathological examination: There were no significant treatment-related findings in parental animals following gross or microscopic examination.

Reproductive parameters: As shown in the Table below, there was a decrease in the number of viable F2 newborn, and decreases in the fertility and mating indices in F1 (P2) males and females at 500 ppm, though these findings did not reach statistical significance. Testes weights were reduced in high-dose males, probably related to the decrease in bodyweights at this dose. Reduced survival was also seen in male and female F2 pups at the day 0-4 (pre-cull) and day 4-21 (post-cull) intervals.

Summary of F1 generation reproductive and fertility findings

	Dietary concentration (ppm)			
	0	10	100	500
Mean number of viable newborn	12.96	12.05	12.22	8.74
Male/female fertility ^{§†}	26/29 (89.7%)	22/27 (81.5%)	23/28 (82.1%)	19/26 (73.1%)
Male/female mating index ^{‡¶}	29/30 (96.7%)	27/29 (93.1%)	28/29 (96.6%)	26/30 (86.7%)
Testes weight (g)	5.253	5.543	5.329	4.734*

* $p<0.05$, [§] No. of pregnant females/no. of females with positive evidence of mating, [‡] No. of females with positive evidence of mating/no. of females co-housed, [†] No. of fertile males/no. of males with positive evidence of mating, [¶] No. of males with positive evidence of mating/no. of males co-housed

Summary of F2 litter survival (mean %)

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	Dietary concentration (ppm)			
	0	10	100	500
Pups surviving days 0-4 (pre-cull)	96.2	98.4	97.9	44.0*
Pups surviving days 4-21 (post-cull)	100.0	96.5	98.2	76.7*
Males surviving days 0-4 (pre-cull)	98.2	99.5	98.8	44.6*
Males surviving days 4-21 (post-cull)	100.0	95.3	97.6	78.0*
Females surviving days 0-4 (pre-cull)	95.9	97.4	97.1	42.9*
Females surviving days 4-21 (post-cull)	100.0	98.7	98.8	72.2*

*p<0.05

F2 pup observations: There were no treatment-related clinical signs or malformations in F2 pups. At 500 ppm, no treatment-related findings were observed at necropsy or following histopathological examination of F2 pups. Significant (p<0.05) decreases in group mean bodyweights were observed in male and female F2 pups at days 4, 7, 14, and 21 postnatal. The bodyweights were reduced by up to 40% on day 21, in a time-related manner. The effect on bodyweights was somewhat greater in this generation than in the F1 pups.

Summary

In a 2-generation reproduction study, technical diazinon (94.9% purity) was given to Sprague-Dawley rats at dietary concentrations of 0, 10, 100, or 500 ppm. Clinical signs were limited to tremors in a few animals (F0 and F1) at the high dose. There was a low incidence of parental mortality (F0) at 500 ppm, possibly related to difficulty in delivering offspring, which may have been related to treatment. There were no treatment-related gross or histopathological findings associated with treatment at any dose. Decreases in bodyweight and/or bodyweight gains were seen in high-dose F0 (P1) females and F1 (P2) males and females. Pup bodyweights at 500 ppm were markedly reduced in both generations at delivery and during lactation, as was pup survival. The decrease in pup survival may have been due to starvation, as milk was absent from the stomachs of a number of F1 pups sacrificed on day 4. Reproductive parameters were generally unaffected by treatment at concentrations of 100 ppm and below, but there were reductions in fertility and mating indices in the F1 (P2) generation at the high-dose level. The NOEL for this study was 100 ppm (approximately 5 mg/kg bw/day) based on reduced parental and pup bodyweights, parental mortality and clinical signs, and reduced pup survival at 500 ppm (approximately 25 mg/kg bw/day) (Ginkis, 1989).

Weatherholz WM (1982) Two-generation reproduction study of diazinon (technical) in rats. Report no. 2132-102. Lab: Hazleton Laboratories America, INC. Virginia USA. Sponsor: Nippon Kayaku Co. Ltd. Tokyo Japan. Study duration: 14 Jul, 1980 – 12 Jun, 1981. Report dated: 16 Feb, 1982. (US GLP statement provided)

Technical diazinon (purity 97.36%; Nippon Kayaku Japan; Batch 5046) was given to albino rats (Fischer 344; Charles River Breeding Laboratories, USA) continuously in the diet (Purina Rodent Laboratory Chow) through two successive generations. Dose levels (based on weekly bodyweight) were 0 (control), 0.1, 1.0, or 10.0 mg/kg bw/day through both generations. All female groups inadvertently received feed intended for males for four days during week 43 of the study. Consequently the intake of test material was approximately 10% greater than intended during that period, but this slight increase in intake was not expected to have any significant effect

on the outcome of the study. The study was conducted in accordance with US EPA Guideline 83-4.

Animals were acclimatised to laboratory conditions for approximately 3 weeks prior to initiation of the study. Fifty-two males and 104 females were selected to form the first parental generation (F0). At the initiation of treatment, bodyweights ranged from 145-196 g for males and 109-143 g for females. The parental animals for the second generation (F1) were selected from the weanling offspring (F1a) of the preceding filial generation. After mating (1 male and 2 females in each mating box), animals were housed individually.

Summary of experimental design

Event	Period
First Generation (F0) (13 males and 26 females/group)	
Growth period	15 weeks
Mating period	2 weeks
F0 (P1) males sacrificed (without necropsy)	At the end of the mating period.
F0 females deliver naturally	
All F0 females sacrificed (without necropsy)	Day 21 of lactation
F1a weanlings.	
F1a pups (15 males and 30 females/group) selected to grow to maturity.	
5 pups/sex/group selected for gross necropsy. Remaining weanlings discarded.	Day 21 of lactation
Second generation (F1) (15 males and 30 females/group)	
Growth period	15 weeks
Mating period	2 weeks
F1 females deliver naturally	
F1 males (10/group) selected for gross necropsy	Day 21 of lactation
F1 females (25/group) selected for gross necropsy. Remaining females discarded without necropsy	Day 21 of lactation
F2a weanlings	
5 pups/sex/group selected for gross necropsy. Remaining weanlings discarded without necropsy.	Day 21 of lactation

Dietary analysis: Diets were prepared weekly and analysed for stability (pretest and week 8), homogeneity (pretest) and concentration of test material (pretest, weekly for 4 weeks and then 4-weekly for the remainder of the study). The diets were homogeneous at the pretest sample interval. At the highest concentration, the diazinon concentration for males and females was generally lower than the target level (ie. 86-87% of target), and on occasion was as low as 60% of the target concentration. At the low and middle concentrations, the mean diazinon concentration was 90-100% of target concentrations for males and females. Stability of the test material was similar at pretest and 4-week intervals.

Parental observations: All animals were observed twice daily for mortality and moribundity, and individual bodyweights, food consumption and clinical signs were recorded weekly during the growth period. Maternal bodyweights and clinical signs were recorded at days 0 and 20 of

gestation, and days 1, 4, 7, 14, and 21 of lactation. Pregnancy was determined by the presence of sperm in the daily vaginal smears or by the observation of vaginal plug.

Breeding F0 and F1: The experimental design of this study is summarised in the Table above. All F0 males were killed following the completion of the mating phase. Pregnant females were allowed to deliver their pups. The total number of pups per litter, number of each sex, and individual bodyweights and clinical signs were recorded on days 1, 4, 7, 14, and 21 post-parturition. On day 7, all litters were reduced to ten pups. Selection of pups was by random culling, with equal numbers of males and females selected when possible. After the lactation period, 15 males and 30 females per group were selected at random to grow to maturity for the next generation of the study. Animals not selected were killed and discarded.

Reproduction indices: The following calculations were made using parental and offspring data: parental indices, pregnancy rates, male fertility rates, gestation index, lactation index, offspring indices, viability (percent survival), sex ratio, numbers alive at birth, and offspring body weight.

Sacrifice and gross pathology: Five F1 and F2 pups/sex/group were selected at random at weaning (day 21 of lactation) for necropsy. Those pups not selected for gross necropsy or to continue onto the next generation were killed and discarded. Ten F1 males and twenty-five F1 females per group were selected at random for gross necropsy following day 21 of lactation. Those F1 animals not selected for necropsy, and all F0 animals, were killed and discarded.

The following tissues (and any gross lesions) from each necropsied F1 and F2 animal were preserved in 10% neutral buffered saline, and sectioned, stained with haematoxylin and eosin and examined microscopically: brain, pituitary, spinal cord (thoracic), eyes, salivary glands, oesophagus, thyroid, lung, heart, unusual lesion (if any), aorta, liver, spleen, kidneys, adrenals, pancreas, stomach, mesenteric lymph nodes, small intestine, large intestine, urinary bladder, testes with epididymides, seminal vesicles, prostate, ovaries, uterus, nerve with muscle, skin, mammary gland, rib junction, bone marrow.

Results

Mortality and clinical signs: No parental animals died or were sacrificed during the study. The report did not include any details on the incidence or severity of clinical signs, but it was stated that there were no compound-related clinical signs evident in treated animals during the study. A range of clinical signs was reported in both control and treated groups. These signs included eye problems (redness, lacrimation, discharge, bloody encrustation), alopecia, rough haircoat, urine stains, soft faeces, and thin or hunched appearance. Clinical signs were stated to occur at similar incidences in both generations, but the incidence of clinical signs was higher in females than males.

Bodyweight and food consumption: Group mean bodyweights were not affected by treatment in either generation during the growth phase of the study, and bodyweight changes were similar in control and treated groups. Group mean food consumption was also similar in control and treated groups. Statistically-significant changes in food consumption were seen in some treated groups, but these findings were isolated, variable, and not related to dose, and were therefore not considered to be treatment-related. Group mean bodyweights were unaffected by treatment during gestation and lactation. During the F1 lactation phase there was a decrease in bodyweight gain (days 0-21) of about 18% in high-dose females compared with controls, but this finding was not

considered to be toxicologically significant, as the mean bodyweight was only about 3% lower than controls.

Reproduction and litter data: There were no significant treatment-related effects on reproduction data in either generation. Pregnancy rates, mean gestation lengths, and gestation and lactation indices were generally comparable between control and treated groups. High-dose females had slight decreases in pregnancy rate and lactation index (number of females with litters surviving to weaning/the number of females that delivered viable offspring) compared with controls during the first generation. There was a reduction in the survival index at the high dose in the first generation, mainly due to a decrease in survival in the day 1-4 period. The relationship between these findings and treatment was not clear.

Summary of F0 reproduction and F1 survival indices

	Dose (mg/kg bw/day)			
	0	0.1	1.0	10.0
Number of females	26	26	26	26
Number of pregnancies	21	23	22	20
Pregnancy rate (%)	80.8	88.5	84.6	76.9
Lactation index (%)	100	95.7	95.2	90.0
Mean number of offspring alive at birth	10.0	9.8	9.4	10.2
Percent survival at day:				
1	100	100	95.5	100
4	99.6	99.3	95.2	90.7
7 (pre-cull)	100	95.7	100	92.8
14	98.2	99.5	99.0	98.3
21	100	100	100	98.9

Summary of F1 reproduction and F2 survival indices

	Dose (mg/kg bw/day)			
	0	0.1	1.0	10.0
Number of females	30	30	30	30
Number of pregnancies	28	27	28	27
Pregnancy rate (%)	93.3	90.0	93.3	90.0
Lactation index (%)	100	100	96.4	100
Mean number of offspring alive at birth	10.4	10.7	10.9	10.6
Percent survival at day:				
1	99.8	98.0	100	99.7
4	99.8	99.6	99.7	98.9
7 (pre-cull)	96.4	99.1	95.0	95.4
14	92.5	98.3	99.6	91.0
21	98.2	100	99.6	99.6

Clinical observations: A range of clinical signs were noted in control and treated animals, including small size, thin appearance, bloody encrustation around eyes, and shortened or stubby tails. There was no consistent dose-response relationship for these effects in either generation, and the findings were not considered to be treatment-related.

Pathology: Gross pathological examination did not reveal any effects that were considered to be treatment-related. Similarly, histopathological examination revealed a variety of common findings in both control and treated groups, but there was no relationship with dose and these findings were not considered to be treatment-related.

The NOEL was 10 mg/kg bw/day, as no treatment-related adverse effects were observed at any dose in parental animals or offspring in either generation. However, as there was no toxicity demonstrated at the high dose, the adequacy of the study for regulatory purposes is questionable.

Summary

Technical diazinon (97.36% purity) was given to Fischer 344 rats (13 males/26 females first generation; 15 males/30 females second generation) continuously in the diet through two successive generations at dose levels of 0 (control), 0.1, 1.0, or 10.0 mg/kg bw/day. No parental animals died or were sacrificed in a moribund condition during the study, and there were no compound-related clinical signs evident in treated animals. Group mean bodyweights were not affected by treatment in either generation during the growth phase of the study nor during gestation or lactation, and group mean food consumption was also similar in control and treated groups. There were no significant treatment-related effects on reproduction data in either generation. Pregnancy rate, mean gestation duration, and gestation and lactation indices were generally comparable between control and treated groups. High-dose females had slight decreases in pregnancy rate and lactation index (number of females with litters surviving to weaning/the number of females that delivered viable offspring) compared with controls during the first generation, and there was a slight reduction in the survival index at the high dose in the first generation, mainly due to a decrease in survival in the day 1-4 period. The relationship between these findings and treatment was not clear. Gross and histopathological examinations did not reveal any effects that were considered to be treatment-related. The NOEL for this study was 10 mg/kg bw/day. The adequacy of this study for regulatory purposes was limited, as no treatment-related toxicity was observed at any dose (Weatherholz, 1982).

Johnston CD (1965) Diazinon: Three-generation reproduction study in the rat. Report not numbered. Lab: Woodard Research Corporation. Sponsor: Ciba-Geigy Corp. Report dated 1965 (Pre-GLP)

The reproductive toxicity potential of the wettable powder formulation 50W (Ciba-Geigy; lot FL 519; containing 50% technical diazinon; purity not stated) was tested in albino rats (Charles River Laboratories) for three generations. Diazinon 50W was incorporated into the diet (Purina Laboratory Chow) with a mechanical mixer. No information was provided on the stability, homogeneity or test material concentration in the diet. In the F0 generation, 20 males were mated with 20 females (10-day mating period) when the animals were 100 days old, and had been receiving the test diet containing 4 ppm of the active ingredient for 70 days. A similar group received the test diet only, and served as controls. In subsequent generations, animals received 0, 4, or 8 ppm diazinon in the diet. Each rat was weighed fortnightly, except during mating, and females were not weighed during gestation and lactation. The experimental protocol is summarised below.

Summary of experimental design

Event	Period
First Generation F0 (P1) (20 males and 20 females/group)	0 and 4 ppm groups
Growth period. Animals received test diets.	100 days
Mating period.	10 days
F0 females deliver naturally (F1a litters).	
Observations made for number and weight of live young, number of stillborn, physical condition of dams and pups.	
Pups weaned. Observations made at weaning: number and mean weight of survivors, physical condition of dams, pup malformations. Pups killed and necropsied.	
F0 (P1) animals re-mated (different pairings)	
F0 females deliver naturally (F1b litters). Same observations made as for F1a litters.	
F1b offspring weighed and examined at weaning. Representatives from each pup group used as second generation parental animals ie. F1 (P2). Remainder of pups killed and necropsied. All F0 parental animals sacrificed (without necropsy)	
F1b weanlings.	
F1b pups (Controls: 10 males and 20 females; 4 ppm: 20 males and 40 females) selected to grow to maturity.	
Second generation (F1/P2) (10 males and 20 females/group)	0, 4, and 8 ppm
All F1b (P2) animals received control diets for up to 16 days after weaning.	
Animals from F1b control litters used as P2 controls. Pups from F1b test group (4 ppm diazinon) receive 4 or 8 ppm diazinon.	Test diets given for 81 days before mating
Observations and necropsy conducted as for first generation.	
P2 animals re-mated.	
Representative pups from the second litter (F2b) used as parental animals (P3) for the third generation.	
Third generation F2 (P3) (10 males and 20 females/group)	0, 4, and 8 ppm
F2b (P3) rats received diets containing 0, 4, or 8 ppm diazinon	Test diets given for 74 diets before mating
Matings, observations at birth and at weaning, and sacrifice of F3a litters followed the procedures used in previous generations.	
F3b weanlings	
At weaning, all F3b pups were killed and the heart, liver, kidney and bodyweights of 2 animals/sex/litter were recorded. Portions of these tissues and portions of spleen, adrenal, thyroid, gonad and bone marrow were preserved in 10% formalin.	
Preserved tissues from 1 animal/sex/litter/group were examined microscopically.	
Parental (P3) animals were killed after weaning the F3b pups. Females that failed to bear two litters were examined post mortem for uterine implantation sites.	

Results

F0 generation: Two control animals died during the study, and one control animals was killed in poor condition. All animals that received 4 ppm diazinon were in good condition throughout the study. Bodyweights were not affected by treatment. No treatment-related adverse effects on litter data were observed, and no pup abnormalities were reported.

Summary of F0 reproduction parameters and F1 survival

	Dietary concentration (ppm)			
	0	0	4	4
Litters	F1a	F1b	F1a	F1b
Number of litters per group	11/20	15/19	18/20	18/20
Total number of stillbirths	3	10	3	3
Total number of live young	117	144	195	216
Number of young per litter	10.6	9.6	10.8	12.0
Mean birth weight (g)	5.9	6.3	5.7	6.3
Young alive at weaning (%)	68	97	84	95
Mean weaning weight (g)	29.3	33.7	31.0	35.3

F1 generation: One animal of each sex in each group died during the study. Bodyweights in control and treated groups were similar. Litter and survival parameters were generally similar between control and treated groups. At 8 ppm there was an increase in the number of stillbirths in both litters, but as the mean litter size was unaffected by treatment, and 10/18 of these stillbirths from the second mating resulted from two litters, the relationship of this finding with treatment is not clear.

Summary of F1 reproduction parameters and F2 survival

	Dietary concentration (ppm)					
	0	0	4	4	8	8
Litters	F2a	F2b	F2a	F2b	F2a	F2b
Number of litters per group	18/20	19/19	18/20	11/19	17/20	17/19
Total number of stillbirths	3	6	3	0	8	18
Total number of live young	158	189	160	110	150	163
Number of young per litter	8.8	9.9	8.9	10.0	8.8	9.6
Mean birth weight (g)	6.3	6.1	6.4	5.9	6.3	5.8
Young alive at weaning (%)	89	90	89	82	93	91
Mean weaning weight (g)	42.1	38.8	39.3	36.5	40.1	38.7

F2 generation: One male from each group and 5 females that received 8 ppm diazinon died during the study. The causes of death were not determined, and necropsies were not performed. Bodyweights were similar in control and treated groups. The reproduction parameters were similar in controls and treated groups. There was an increase in the incidence of stillbirths in the first litter of animals treated with 4 ppm diazinon. However, as there was no dose-response relationship for this effect, and the increase in stillbirths was attributed to a single litter, this finding was not considered to be treatment-related.

Summary of F2 indices of reproduction and F3 indices of survival

	Dietary concentration (ppm)					
	0	0	4	4	8	8
Litters	F3a	F3b	F3a	F3b	F3a	F3b
Number of litters per group	16/20	18/20	18/20	19/19	16/18	14/16
Total number of stillbirths	1	5	10	2	2	6
Total number of live young	135	172	149	203	154	125
Number of young per litter	8.4	9.6	10.7	8.3	9.6	8.9
Mean birth weight (g)	6.3	6.8	6.1	6.6	6.1	6.9
Young alive at weaning (%)	88	86	87	86	86	90
Mean weaning weight (g)	34.0	39.1	31.4	35.2	33.2	42.2

Organ weights in selected F3b offspring were not affected by treatment, and histopathological examination (limited in number) did not reveal any treatment-related findings.

The NOEL for this study was 8 ppm (estimated to be 0.8 mg/kg bw/day), as no adverse, treatment-related effects were observed in parental animals or offspring at any dose. Due to the study design, animals did not receive 8 ppm diazinon for each of the 3 generations, and as no toxicity was demonstrated at the high dose, the adequacy of this study for regulatory purposes is limited.

Summary

The reproductive toxicity potential of the diazinon wettable powder formulation 50W (containing 50% diazinon) was tested in albino rats for three generations. In the F0 generation, animals received a diet containing 0 or 4 ppm of the active ingredient. In the second and third generations, animals received diets containing 0, 4, or 8 mg/kg bw/day diazinon. No adverse, treatment-related effects were observed in parental animals or offspring during the study. The NOEL for reproductive toxicity was 8 ppm (approximately 0.8 mg/kg bw/day) diazinon, but as no toxicity was demonstrated at the high dose, the adequacy of this study for regulatory purposes is limited (Johnston, 1965).

Abd El-Aziz MI, Sahlab AM & Abd El-Khalik M (1994) Influence of diazinon and deltamethrine on reproductive organs and fertility of male rats. Pharmacology Department, College of Veterinary Medicine and Animal Health Research Institute, Cairo, Egypt. Dtsch tierärztl Wschr 101: 213-248

Diazinon (Neocidal[®]; 50% diazinon; described as an oily solution) was given to mature male and female albino rats (Laboratory Animal Colonies, Egypt) by oral gavage at doses of 1.5 and 3.0 mg/kg bw/day. It is not clear if these doses are based on the active ingredient or the formulated product. The animals were fed a diet of milk, barley and water *ad libitum*. No information was provided on the stability or purity of the test material. Males were divided into groups of 15. One group served as controls and received distilled water (0.5 mg/kg bw/day), and groups 2 and 3 received diazinon for 65 consecutive days. Animals were allowed to recover for at least 21 days after cessation of treatment. Blood samples were obtained from each group prior to treatment and at 14, 28, 42 and 65 days, and after the recovery period. Serum was used to determine testosterone concentrations by radioimmunoassay. At the end of the recovery period, rats were killed by decapitation, and their testes, epididymides and seminal vesicles and prostate

glands weighed. Sperm count, percentage of live cells, progressive motility and total head abnormalities were determined from epididymal contents.

At the end of the diazinon administration period, 5 rats from each group were isolated. The remaining males in each group were mated individually with females (8/group) for 48 h to determine the conception rate. The males that had been separated from the groups were maintained on control diets for 60 days, and then mated with females to determine if any fertility effects were reversible.

Results

Dose-related, statistically significant decreases in sex organ weight (testicles, seminal vesicles, prostate gland), plasma testosterone concentration, and number and motility of sperm were observed. Increases in sperm head deformity incidence was also noted after 65 days of treatment at 1.5 and 3.0 mg/kg bw/day. After the 21-day recovery period, these effects were still significant, although there was generally a slight recovery in most parameters compared with the end of the treatment period. Fertility was decreased at both diazinon doses, and this effect persisted after a 60-day recovery period.

Summary of sex organ weight (g) (mean $\pm$ SD)

Organ		Dose (mg/kg bw/day)		
		0	1.5	3.0
Testicles	65-day treatment	1.72 $\pm$ 0.05	1.38 $\pm$ 0.05**	1.16 $\pm$ 0.032***
	21-day recovery	1.68 $\pm$ 0.025	1.47 $\pm$ 0.04**	1.20 $\pm$ 0.033***
Seminal vesicles	65-day treatment	0.23 $\pm$ 0.014	0.16 $\pm$ 0.008**	0.12 $\pm$ 0.014***
	21-day recovery	0.24 $\pm$ 0.015	0.17 $\pm$ 0.006**	0.13 $\pm$ 0.013***
Prostate gland	65-day treatment	0.19 $\pm$ 0.014	0.12 $\pm$ 0.008**	0.09 $\pm$ 0.002***
	21-day recovery	0.19 $\pm$ 0.016	0.14 $\pm$ 0.009*	0.12 $\pm$ 0.003**

(n = 5) *p<0.05, **p<0.01, ***p<0.001

Semen quality (mean $\pm$ SD)

	Dose (mg/kg bw/day)		
	0	1.5	3.0
Sperm cell concentration after 65-day treatment (units not given)	1.90 $\pm$ 0.18	1.30 $\pm$ 0.064*	0.92 $\pm$ 0.08**
Sperm cell concentration after 21-day recovery	1.91 $\pm$ 0.19	1.44 $\pm$ 0.068*	1.22 $\pm$ 0.09**
Live cell (%) -after 65-day treatment	97.89 $\pm$ 1.84	83.74 $\pm$ 1.42***	80.22 $\pm$ 1.04***
Live cell (%) -after 21-day recovery	98.22 $\pm$ 1.96	86.42 $\pm$ 1.84**	86.47 $\pm$ 1.79**
Motility (%) -after 65-day treatment	95.82 $\pm$ 0.72	17.24 $\pm$ 0.85***	7.41 $\pm$ 0.64***
Motility (%) -after 21-day recovery	96.46 $\pm$ 0.85	27.96 $\pm$ 2.14***	17.22 $\pm$ 1.98***
Total sperm abnormalities (%) after 65-day treatment	7.68 $\pm$ 0.32	22.86 $\pm$ 1.17***	42.28 $\pm$ 1.68***
Total sperm abnormalities (%) after 21-day recovery	8.24 $\pm$ 0.42	18.37 $\pm$ 0.62***	30.02 $\pm$ 0.79***

(n = 5) *p<0.05, **p<0.01, ***p<0.001

Serum testosterone concentration (ng/mL, mean $\pm$ SD)

Time of sampling (days)	Dose (mg/kg bw/day)		
	0	1.5	3.0
0	3.73 $\pm$ 0.09	3.48 $\pm$ 0.082	3.56 $\pm$ 0.06
14	3.66 $\pm$ 0.07	3.20 $\pm$ 0.09**	3.08 $\pm$ 0.06***
28	3.58 $\pm$ 0.07	3.16 $\pm$ 0.08**	2.76 $\pm$ 0.07***
42	3.67 $\pm$ 0.05	2.48 $\pm$ 0.10***	2.22 $\pm$ 0.09***
65	3.68 $\pm$ 0.04	2.40 $\pm$ 0.09***	1.83 $\pm$ 0.09***
After 21-day recovery	3.56 $\pm$ 0.06	2.34 $\pm$ 0.07***	1.98 $\pm$ 0.08***

(n = 5) **p<0.01, ***p<0.001

Summary of male fertility

	Dose (mg/kg bw/day)				
	0	1.5		3.0	
		65-day treatment	60-day recovery	65-day treatment	60-day recovery
Number of female rats	8	8	8	8	8
Number of pregnant rats	6	2	3	1	1
Pregnancy (%)	75	25	37.5	12.5	12.5

Summary

When male rats were given diazinon by oral gavage at doses of 1.5 and 3.0 mg/kg bw/day for 65 consecutive days, there were statistically significant, dose-related decreases in sex organ weight,

sperm cell count, percentage of live sperm cells, sperm motility, and serum testosterone concentration, and an increase in the total sperm head deformity incidence. These effects persisted after a 21-day recovery period that followed the treatment period. When treated males were mated with untreated females, there was a decrease in the male fertility, even after removal of the males from the test diets for 60 days (Abd El-Aziz et al, 1994).

8. DEVELOPMENTAL TOXICITY

8.1 Rat

Fritz H (1974) Reproduction study - G24480 (Diazinon techn.). Rat. Segment II (Test for teratogenic or embryotoxic effects). Report not numbered. Lab: Ciba-Geigy Ltd, Pharmaceuticals Division, Toxicology/Pathology, Switzerland. Sponsor: Ciba-Geigy, Basle, Switzerland. Study duration: not stated. Report date: 16 May, 1974. (Pre-GLP)

Diazinon technical (purity and source not stated) was given to groups of pregnant Sprague-Dawley rats (28 to 30 per group; source not stated) by gavage on days 6 through 15 of gestation at doses of 0 (control), 15, 50, or 100 mg/kg bw/day. The dose volume was 10 mL/kg bw, and the vehicle was carboxymethylcellulose (CMC; concentration not reported). Control animals received CMC only. Autopsies were performed on the dams at gestation day 21, and fetuses were removed by caesarean section at this time. General condition, weight gain and signs of treatment were recorded daily. Food consumption was determined on days 6, 11, 16 and 21. The maternal organs (ovaries and uterus, including mucosa and contents) were examined and the fetuses were inspected and weighed. The situs of the body cavities (thorax, abdomen, pelvis) was assessed. One third of the fetuses were fixed (alcohol, Formalin and acetic acid) and examined. The remaining two thirds of fetuses were placed in 70% alcohol, cleared in 1% potassium hydroxide, and stained with Alizarin Red S for skeletal examination.

Results

Bodyweights were reduced in high-dose dams throughout the treatment period, with animals losing about 5-10 % of their bodyweight between days 6 and 8 of gestation. The rate of bodyweight gain in high-dose animals was similar to other groups from day 9 to 15 of gestation, and by the end of the study, the group mean bodyweights were similar at all doses. Food consumption was almost halved in high-dose animals between days 6 and 11 of gestation, but was similar in all groups between days 16 and 21 of gestation.

No significant, treatment-related effects on reproductive parameters were seen. Isolated instances of malformations were confined to a single finding of hypognathia inferior and median cleft palate at 50 mg/kg bw/day. At 100 mg/kg bw/day the incidence of delayed ossification was occasionally greater than at 15 or 50 mg/kg bw/day, but was generally similar to that seen in the CMC controls.

Summary of Effects on Reproductive Indices and Fetal Development

Reproductive Indices	Dose (mg/kg bw/day)			
	0	15	50	100
Number of dams	30	30	28	28
Number of embryonic resorptions-mean	0.70 (5.8%)	0.93 (7.6%)	0.79 (6.8%)	0.85 (6.7%)
Number of fetal resorptions	0	0	0	1

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Number of dead fetuses	1	0	0	0
Number of live fetuses with malformations	0	0	1	0
Mean weight of live fetuses	5.19	5.23	5.26	5.18
Skeletal Abnormalities				
Number of skeletons examined	227	228	201	214
Phalangeal nuclei – Forelimb ^{1,2}	12 (5.3%)	2 (0.9%)	4 (2.0%)	16 (7.5%)
Phalangeal nuclei – Hindlimb ^{1,3}	67 (29.5%)	41 (18.0%)	32 (15.9%)	72 (33.6%)
Calcaneus ¹	38 (16.7%)	10 (4.4%)	17 (8.5%)	45 (21.0%)
Sternebrae ⁴	54 (23.7%)	31 (13.6%)	30 (14.9%)	37 (17.3%)
Vertebrae ⁵	1 (0.4%)	1 (0.4%)	1 (0.5%)	0
Vertebrae ⁶	1 (0.4%)	2 (0.9%)	4 (2.0%)	0
Ribs ⁷	0	1 (0.4%)	0	0

¹ Ossification still absent; ² Proximal phalanges V; ³ Proximal phalanges (presumably phalanges V); ⁴ Centres incompletely ossified, still bipartite; ⁵ Some centres of thoracic vertebrae still dumbbell-shaped; ⁶ Some centres of thoracic vertebrae bipartite; ⁷ Unilateral fusion of the base of 2 ribs.

Summary

Diazinon technical was given to groups of pregnant Sprague-Dawley derived rats by oral gavage at doses on 0, 15, 50, or 100 mg/kg bw/day on days 6 to 15 of gestation. Decreases in food consumption and bodyweight were observed at the high dose. No treatment-related effects were observed on reproductive parameters or on the incidence of malformations. No major developmental effects were seen at any dose. The NOEL for this study was 50 mg/kg bw/day, based on maternal bodyweight loss at 100 mg/kg bw/day. The NOEL for developmental toxicity was 100 mg/kg bw/day.

Tauchi K (1979) Teratological study of diazinon in the rat. Report not numbered. Lab: Toxicology Division, Institute for Animal Reproduction, Japan. Study duration: May - Nov, 1979. Report date: 1 Nov, 1984. (No GLP statements provided)

Range-finding study: Prior to the commencement of the main study, a preliminary dose range-finding study was conducted in pregnant Wistar-Imamichi rats (SPF, Institute for Animal Reproduction, Japan; age and weight not stated). In the preliminary study, diazinon (purity and source not stated) was given to groups of 5 rats by oral gavage on pregnancy days 7 to 17, at doses of 0 (control), 1.25 (4 animals only), 2.5, 5.0, or 12.5 mg/kg bw/day. Ethylenethiourea (ETU) was similarly given to groups of 5 rats at doses of 0, 10, 20, 40, or 60 mg/kg bw/day as a positive control. Prior to administration, diazinon was emulsified in a small amount of Tween 80. ETU was mixed with water. The dose volume for diazinon was 2 mL/kg bodyweight (as determined on day 7), and for ETU was 4 mL/kg bodyweight. Observations for maternal health status and bodyweight measurements were conducted every day. Maternal animals were sacrificed on day 21 of gestation, and fetuses were examined for abnormalities. ChE activity in the brain homogenate of maternal animals was determined by the Δ pH/h method.

Piloerection and/or tremors were observed in some animals at 5 mg/kg bw/day during the treatment period. At 12.5 mg/kg bw/day, piloerection, salivation, urination, loss of activity, depression and bodyweight decreases were observed from the first day of treatment. The severity of effects increased with time to include chromodacryorrhea, nasal bleeding, convulsions and death at this dose level. The high incidence of maternal mortality (4/5 animals) at 12.5 mg/kg bw/day made it difficult to determine the effect of treatment on fetal development. Mean fetal bodyweights were significantly decreased at 5 mg/kg bw/day, but as there was also an increase in

the number of fetuses at this dose level, the decrease in fetal weight was not directly attributable to treatment.

Brain ChE activity was inhibited by about 40% compared with controls at 1.25 mg/kg bw/day, and by 50-60% compared with controls at the other doses. No fetal abnormalities were reported in the diazinon groups. In the ETU groups, 3/5 dams died at 60 mg/kg bw/day, bodyweights were decreased at 40 mg/kg bw/day, and various types of gross malformations were reported at 40 mg/kg bw/day. No further information on the incidence or type of malformations was provided.

Based on the mortality observed in this preliminary study, the doses selected for the main study were 0.53, 1.45, and 4.0 mg/kg bw/day diazinon, and 50 mg/kg bw/day ETU.

Main study: Diazinon (source and purity not stated) was given by gavage to groups of female Wistar-Imamichi rats (30/dose; 10 weeks old at beginning of study; Institute for Animal Reproduction, Japan) at doses of 0 (controls), 0.53, 1.45, or 4.0 mg/kg bw/day on days 7 to 17 of gestation. Similarly, ETU (positive control; 50 mg/kg bw/day) was given to a group of 20 animals. The preparation of the test and control materials, including dose volumes, was identical to that used in the preliminary study. No information was provided on the stability or homogeneity of the test material formulation. After the treatment period, 20 animals from each group were selected at random for sacrifice at day 21 of gestation, at which time the fetuses were obtained for examination. The remaining 10 animals in each group were maintained through to day 3 post-partum. At this time, the litters were adjusted to 8 pups each (4 males and 4 females), and these remaining pups were weaned until day 21 post-partum. After weaning, two pups (1 male/1 female) from each of these litters were then used for further examinations ([†]see below), namely autopsy at weaning, autopsy at 10 weeks of age, fertility study, and behaviour and learning ability study. In the ETU group, all maternal animals were autopsied on day 21 of gestation, and fetuses were used for teratological examinations only.

The health of all animals was checked daily. Maternal animals were weighed on days 0, 7, 14, 17, and 21 of gestation, and days 0, 3, 7, 14, and 21 post-partum. Offspring were weighed weekly. Food intakes were estimated at the same intervals as bodyweights. At caesarean section, the following parameters were recorded: number of implantation sites, number of corpora lutea, number of live fetuses, number of dead fetuses (residual implants, placental residue, resorbed or dead fetuses), placental weight, weight of live fetuses, sex, gross malformations, and crown-rump length.

Half of the live fetuses per litter were used for skeletal examination, and abnormalities, variations and ossification status were recorded. The remaining half of the fetuses were used for visceral examination. Histological examinations were conducted on livers, kidneys and hearts of 5 litters from the control and 4 mg/kg bw/day groups.

At parturition, the following parameters were recorded: pregnancy period, number of live-born young, weight of newborn, sex of newborn, and gross malformations. During weaning, pup bodyweights were recorded on days 3, 7, 14, and 21 post-partum, and the number of deaths occurring between days 0 and 3 post-partum were used to calculate the perinatal survival rate. The following indexes were also calculated: parturition rate for live young (number of live-born young/number of implantation sites); perinatal survival index (number of surviving young/number of live-born young); weanling survival index (number of survivors at weaning/number of pups selected on day 3 post-partum).

A number of other parameters were also measured in this study[†]. These included morphological differentiation (auricular standing on day 3 post-partum, incisor extrusion on day 10, hair growth around nasal area on day 10, eyelid opening on day 16), developmental parameters at weaning (righting reflex, Preyer response to Galton whistle, pain response, grip strength, posture and abnormal gait), fertility (copulation ability and nursing performance), behaviour and learning ability (light-dark discrimination and spontaneous activity). Pregnant rats from the fertility study were allowed to give birth and nurse their pups. Gross pathological examinations were conducted on all animals in this study.

Results

It was reported that there were no maternal deaths or signs of intoxication reported at any diazinon dose. Animals exposed to ETU displayed alopecia by day 17, but no other signs of intoxication were reported.

Bodyweights and food consumption: Group mean bodyweights were similar in control and treated animals. A slight but significant ($p < 0.05$) decrease in bodyweight was observed in ETU-treated animals on day 17 of gestation, but as the decrease only represented a 2-3% decrease compared with controls, this effect was not considered to be treatment-related. No statistically-significant decreases in bodyweights were observed during lactation. A statistically-significant decrease in group mean food consumption (reduced by 10% compared with controls) was observed in animals at 4 mg/kg bw/day during the 7-14 day interval only. Over the 21-day gestation period, the food consumption at 4 mg/kg bw/day was about 8% lower than controls. This decrease in food consumption may have been related to treatment, but the toxicological significance of such an effect is unclear. Similar reductions in food consumption were also observed in ETU-treated rats for the 7-14 and 14-17 day intervals. Food consumption during lactation was similar in control and treated groups.

Caesarean section and post-partum findings: No statistically-significant, treatment-related adverse effects were seen at the caesarean section examination. Gross fetal abnormalities were confined to a single stunted control fetus, and a single tailless fetus with anal atresia and club foot at 0.53 mg/kg bw/day. By comparison, in the ETU-treated group there were significant ($p < 0.001$) decreases in fetal weight and crown-rump lengths (males and females), and a total of 249/252 fetuses with gross abnormalities. At day 3 post-partum, there were no adverse, treatment-related findings in diazinon-treated groups, and the incidence of all parameters was similar in control and treated groups. Group mean bodyweight changes and the pup survival index were similar in control and treated groups at day 21 post-partum.

Skeletal examination: As shown in the Table below, skeletal examination of fetuses (20 litters/dose) did not reveal any treatment-related increase in the incidence of skeletal variations or abnormalities. Examination of the ossification status revealed statistically-significant decreases in the incidence of fetuses with unossified odontoid process. This effect was not dose-related and not considered to be toxicologically significant. There were significant ($p < 0.05$) increases in the incidence of fetuses with poorly ossified sternebra, and in the incidence of fetuses with five ossified metatarsal centres at 4 mg/kg bw/day.

Summary of Fetal Skeletal Abnormalities

	Dose (mg/kg bw/day)
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	0	0.53	1.45	4.0
No. of litters examined	20	20	20	20
No. of fetuses examined	140	131	135	137
<i>Variants</i>				
Total number of fetuses with abnormalities (litters)	1 (1)	2 (2)	2 (2)	0
Total number of fetuses with variations (litters)	0	6 (5)	3 (3)	2 (2)
Sternum: Fetuses with poorly ossified sternebra (litters)	3 (2)	4 (2)	7 (4)	21* (9)
Vertebra: Fetuses with unossified odontoid process (litters)	63 (18)	37* (13)	40* (17)	37* (15)
Hind limb: Fetuses with five ossified metatarsal centres	131 (93.6%)	125 (95.4%)	129 (95.6%)	136* (99.3%)

* p<0.05

Visceral examination: As shown in the Table below, visceral abnormalities were confined to the kidney (enlargement of the renal pelvis) and the ureter (hydroureter). An increase in the incidence of dilated renal pelvis was seen at 4 mg/kg bw/day, but as the magnitude of this change was not great and there was no dose-response relationship, this effect was not considered to be treatment-related. The incidence of hydroureter was higher in controls than in the treated groups, again with no dose-response relationship, and this effect was not considered to be toxicologically significant.

Summary of Fetal Visceral Abnormalities

	Dose (mg/kg bw/day)			
	0	0.53	1.45	4.0
No. of litters examined	20	20	20	20
No. of fetuses examined	130	124	126	125
<i>Variants</i>				
Kidney: Enlargement of renal pelvis (litters)	0	2 (2) (1.6%)	0	6 (4) (4.8%)
Ureter: hydroureter (litters)	21 (10) (16.2%)	6 (3) (4.8%)	6 (5) (4.8%)	5 (3)* (4.0%)

* p<0.05

Morphological differentiation: There were no consistent dose-related effects that were considered to be toxicologically significant in this portion of the study. Significant (p<0.001) decreases in the number of animals displaying incisor eruption at 10 days were seen in all treated groups, and there was an increase in the incidence of auricular standing at 3 days, but the biological significance of these findings was not clear.

Other findings: In this study, small numbers of animals (up to 10/group) were allowed to mate (as a test for fertility) and give birth to their offspring (F2). Fertility was similar in control and treated groups. Slight, but significant (p<0.05) decreases in the total number of pups and the number of implants were seen at 4 mg/kg bw/day. However, due to the small group sizes in this segment of the study, the toxicological significance of these findings is unclear. Bodyweight in weanlings was generally similar in control and treated groups. Gross pathological examination of maternal animals at day 21 of gestation, and of F1 offspring at 3 and 10 weeks post-partum did not reveal any consistent findings which could be attributed to treatment. On occasions, group mean organ weights were statistically-significantly different to controls, but in the absence of any dose-response relationship, these effects were not considered to be treatment-related.

Summary

When diazinon was given by gavage to groups of female Wistar-Imamichi rats at doses of 0 (controls), 0.53, 1.45, or 4.0 mg/kg bw/day on days 7 to 17 of gestation, the NOEL was 1.45 mg/kg bw/day, based on the reduction in maternal food consumption and the increased incidence of delayed ossification of sternebra in fetuses at 4.0 mg/kg bw/day. No other signs of developmental toxicity were observed and no major malformations were seen. Brain ChE activity was not assessed in the main study.

Infurna RN (1985) A teratology study of diazinon technical in Charles River rats. Toxicology/Pathology Report no. 52-83. Master Index no. 82-2-96. Lab: Ciba-Geigy Corp., Safety Evaluation Facility, Reproductive and Genetic Toxicology Subdivision, Summit, NJ, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 20 Dec, 1982 - 6 Jan, 1983. Report date: 19 Apr, 1985. (US GLP statement provided)

Diazinon technical (Ciba-Geigy, batch FL-821568, stability and purity not stated) was given to groups of pregnant rats (CrI. COBS CD (SD) (BR); 27/group; 200-350 g; Charles River, Kingston New Jersey, USA) by gastric intubation at doses of 0 (control), 10, 20, or 100 mg/kg bw/day on gestation days 6 through 15. Control animals received 10 mL/kg bw/day of 0.20% carboxymethyl cellulose (CMC) containing 0.5% Tween 80. Animals in the 10, 20 and 100 mg/kg bw/day groups received the test material as a 0.10%, 0.20%, or 1.00% suspension, respectively, in 0.2% CMC containing 0.5% Tween 80. The volume of suspension given to each animal was determined by the bodyweights on gestation days 6, 10, and 14.

Dams were observed daily for changes in appearance and behaviour, and were weighed on days 0, 6, 10, 14, 18, and 20 of gestation. Food consumption calculations were made for gestational days 0 through 5; daily for days 6 through 15; days 16 through 17; and days 18 through 19. Dams were killed on day 20 of gestation and the ovaries were examined and corpora lutea counted. The uteri (including contents) were weighed and live fetuses, dead fetuses, and intrauterine resorption sites were counted. Viable fetuses and their placenta were weighed and the fetuses were examined for gross abnormalities. Approximately one third of the fetuses were fixed in Bouin's solution for about one week and then sectioned by hand and examined for soft tissue abnormalities. The following tissues and organs were examined: brain, eyes, spinal cord, heart and major blood vessels, nasal passages, trachea, lungs, diaphragm, oral cavity, tongue, oesophagus, stomach, intestines, liver, pancreas, thymus, spleen, kidneys, ureters, bladder, adrenals, ovaries, uterus or testicles. The remaining two thirds of the fetuses from each litter were cleared, stained with Alizarin Red S and examined for skeletal abnormalities. All dams were killed and examined for gross pathology, and all gross lesions were excised and submitted for microscopic evaluation. ChE activity was not measured.

Results

There were no unscheduled deaths during this study. Clinical signs consisted of alopecia, bleeding from nasal and/or oral mucosa and encrusting about the eye, but these effects were seen at similar low incidences in control and treated groups, and were not related to treatment. All internal organs appeared normal at the scheduled necropsy of dams.

Bodyweights and food consumption: Significant ($p < 0.01$) decreases in mean food consumption (up to 35% compared with controls) were seen at 100 mg/kg bw/day on days 6 to 9 inclusive. Food consumption at all other doses and at other intervals was similar to controls. Significant ($p < 0.01$ or $p < 0.05$) decreases in mean bodyweights were observed at 100 mg/kg bw/day during the study, with reductions of 10% (compared with controls) at day 10, 7% at day 14, 5% at day 18 (not statistically significant) and 5% at day 20. Mean body weights of dams without the gravid uterus on day 20 were similar in all control and treated groups. Statistically significant decreases in mean bodyweight gains were also seen at 100 mg/kg bw/day during treatment, and for the day 0 to day 20 period, the mean bodyweight gain in high dose animals (minus the gravid uterus) was reduced by about 25% compared with controls. Bodyweight gain was most affected during the 6-10 day interval at the high dose, with animals losing weight over this period.

Summary of Mean Maternal Bodyweight (g)

Gestation (days)	Dose (mg/kg bw/day)			
	0	10	20	100
0	248	246	243	249
6	279	278	271	276
10	293	293	284	265*
14	311	310	303	288*
18	349	357	341	331
20	381	386	380	360**
20 (minus uterus, placenta and fetuses)	306	313	303	294

* $p < 0.01$, ** $p < 0.05$

Summary of Mean Maternal Bodyweight Gain (g)

Gestation (days)	Dose (mg/kg bw/day)			
	0	10	20	100
0-6	31	32	28	30
6-10	14	14	13	-11*
10-14	18	18	18	23**
14-20	70	75	78	72
0-20 (minus uterus, placenta and fetuses)	58	67	60	44*

* $p < 0.01$, ** $p < 0.05$

Reproductive parameters: As shown in the Table below, there were no statistically-significant, treatment-related effects on reproductive indices in this study. At 20 mg/kg bw/day the mean number of resorptions and the post-implantation loss were significantly decreased compared with controls ($p < 0.05$), but these findings were considered to be incidental to treatment. There were slight increases in both the pre-implantation and post-implantation losses and in the mean number of resorptions, and a consequent small decrease in the mean number of live fetuses, at 100 mg/kg bw/day but these findings were not significantly different to controls. Fetal sex ratios in treated groups were not significantly different to controls. Slight increases in the mean fetal weight at 100 mg/kg bw/day were considered to be incidental to treatment and may have resulted from the slight decrease in the number of fetuses at that dose.

The increase in resorptions in the 100 mg/kg bw/day group was attributable to high resorption incidences in two dams. The number of resorptions in these dams was 12 and 9, respectively, while the range for the remainder of the high dose group was 0-2. Without these two dams the

mean number of resorptions at the high dose was 0.95. The high rate of resorptions in these two dams also contributed to the increase in post-implantation loss percentage at the high dose.

Summary of Reproductive Indices

Reproductive Indices	Dose (mg/kg bw/day)			
	0	10	20	100
Mean number of implantations/litter	15.2	14.4	14.6	13.8
Number of litters examined	24	21	25	22
Mean number of resorptions	1.0	1.0	0.4*	1.8
Pre-implantation loss (%)	6.9	10.0	9.5	12.8
Post-implantation loss (%)	6.9	7.7	2.8*	13.4
Mean number of live fetuses	14.2	13.4	14.2	11.9
Fetal weight-male (g)	3.4	3.5	3.5	3.6*
Fetal weight-female (g)	3.2	3.4	3.3	3.4*

*p<0.05

Malformations: A single incidence of exencephaly was observed in a fetus at 20 mg/kg bw/day, and single instances of umbilical hernia, filament tail and sublingual extraneous soft tissue were seen in separate fetuses at 100 mg/kg bw/day. Because of the isolated nature of these findings they were not considered to be treatment-related. Similarly, isolated instances of visceral (situs inversus) and skeletal (bowed humerus, bowed scapula) malformations were considered to be incidental to treatment. A significant ($p<0.05$) increase in the incidence of T-13 rudimentary ribs was also seen at 20 mg/kg bw/day, but as there was no dose-response relationship for this finding, it was not considered to be treatment-related. The total number of external malformations was significantly different to controls ($p<0.05$) at 100 mg/kg bw/day only, but as the incidence of findings was low, and the malformations were morphologically unrelated, this finding was considered to be incidental to treatment.

Summary of Fetal Malformations (by fetus)

Variant	Dose (mg/kg bw/day)			
	0	10	20	100
<i>External</i>				
Fetuses examined	340	282	355	262
Litters examined	24	21	25	22
Exencephaly	0	0	1	0
Umbilical hernia	0	0	0	1
Filament tail	0	0	0	1
Sublingual extraneous soft tissue	0	0	0	1
<i>Visceral</i>				
Fetuses examined	105	82	108	81
Litters examined	24	20	24	21
Situs inversus	0	0	1	0
<i>Skeletal</i>				
Fetuses examined	235	198	247	181
Litters examined	24	21	25	22
Humerus-bowed	0	0	0	1
T-13 rudimentary rib	2	2	5*	1
Scapula-bowed	0	0	1	1

*p<0.05

Variations: A number of visceral variations were reported, including short or absent renal papilla(e) and dilated ureter(s), but as these findings were seen in control and treated groups, with no relationship to dose, they were not considered to be treatment-related. A significant increase ($p<0.005$) in the incidence of mottled liver (by fetus and litter) was observed at 100 mg/kg bw/day. Samples of the mottled high dose livers and the apparently normal control livers were used for histopathological examination. The pathology report indicated that all specimens were within normal limits and no between-group differences were observed. On this basis, the liver mottling at the high-dose level was considered to be an artifact and not related to treatment.

Significant ($p<0.007$) increases in the incidence of rudimentary T-14 ribs (by fetus and by litter) were observed at 100 mg/kg bw/day. The control incidence of this finding (0%) was low, and the high-dose incidence (5%) was within the historical control range for the testing facility. However, this effect was consistent with fetotoxicity, and was attributed to the maternotoxicity seen at this dose. The incidence of other skeletal variations was similar in control and treated groups.

Statistical trend analysis revealed a significant dose-related increase for the incidence of mottled liver and rudimentary ribs at T-14, and for total external malformations, but the trend for each of these findings was only significant due to the increased incidence in the high-dose group. No significant dose-related effects were observed for any of the other parameters examined.

Summary of Fetal Viseral and Skeletal Abnormalities

Variant	Dose (mg/kg bw/day)			
	0	10	20	100
<i>Visceral</i>				
Number of fetuses examined	105	84	108	81
Number of fetuses with variations	42	32	28	25
Mottled liver	0	0	0	6*
<i>Skeletal</i>				
Number of fetuses examined	235	198	247	181
Number of fetuses with variations	228	195	246	178
Number of fetuses with variations excluding metacarpals and forepaws	183	158	190	139
Rudimentary T-14 ribs	0	6	6	9**
Wavy/angularated ribs	1	0	2	3

*p<0.005, **p<0.007

Summary

Groups of pregnant Charles River SD rats were given technical diazinon by oral gavage at doses of 0, 10, 20, or 100 mg/kg bw/day from days 6 through 15 of gestation. No unscheduled deaths or treatment-related clinical signs were reported at any dose. At 100 mg/kg bw/day, decreases in mean food consumption, mean bodyweights (5-10%; days 10, 14, and 20), and mean bodyweight gains were observed. There were no treatment-related effects on reproductive indices. At the high dose, slight increases in both the pre-implantation and post-implantation losses and in the mean number of resorptions, and a consequent small decrease in the mean number of live fetuses, were not significantly different to controls. An increase in the incidence of rudimentary T-14 ribs at the high dose was considered to be to treatment-related, and attributed to the maternotoxicity seen at this dose. No other treatment-related variations or malformations were observed at 100 mg/kg bw/day, or at 10 and 20 mg/kg bw/day. The NOEL for this study was 20 mg/kg bw/day, based on the maternotoxicity (decreased food consumption, bodyweight and bodyweight gains), and fetotoxicity (increased incidence of rudimentary ribs) at 100 mg/kg bw/day. The NOEL for teratogenicity was 100 mg/kg bw/day.

8.2 Hamster

Robens JF (1969) Teratologic studies of carbaryl, diazinon, norea, disulfiram, and thiram in small laboratory animals. Division of Pharmacology and Toxicology, Food and Drug Administration, Washington DC, USA. Toxicol Appl Pharmacol 15: 152-163

The teratogenic potential of technical diazinon (Ciba-Geigy, USA; purity not stated) was tested in pregnant Golden Syrian hamsters and New Zealand White rabbits. The sources and ages of the animals were not provided. All fetuses were examined for gross defects when delivered by caesarean section. All fetuses with sufficient developmental form for determination of structural defects were counted in the number of fetuses/litter, even though some of these fetuses were dead when delivered. Resorption sites and fetuses which died in late embryonic life were counted as dead fetuses.

Hamsters were given diazinon by oral gavage as a suspension in corn oil (dose volume 10 mL/kg bw) at doses of 0.125 mg/kg bw/day (on gestation days 6 through 8; 8 dams) or 0.25 mg/kg bw/day (on gestation day 7 or 8; 5 dams). Controls (8 dams) were similarly treated

with the vehicle alone. Dams were sacrificed on gestation day 14. Four fetuses were chosen at random from each litter for staining and skeletal examination.

Results

Cholinergic signs (diarrhoea, salivation, uncoordination) were reported in all dams, but no hamsters died after treatment with diazinon. No major malformations or increase in fetal mortality were seen at either dose. No test for fetal viability was made, as the dams were killed on day 14 of gestation.

Summary of Effects on Reproductive Indices

Reproductive Indices	Dose (mg/kg bw/day)		
	0 (CMC control)	0.125	0.25
Days of gestation treated	NS	6, 7, 8	7 or 8
No. of dams that died/No. treated	0/8	0/8	0/5
Mean number of fetuses/litter	12.9	14.3	14.4
Fetal mortality (%)	5.7	5.8	2.7
Mean fetal weight (g)	1.7	1.7	1.5

NS = not stated

Summary

Diazinon was given to Syrian Golden hamsters (0.125 or 0.25 mg/kg bw/day) during gestation. Cholinergic signs were seen, but no signs of developmental toxicity or fetal malformations were reported at any dose. The level of reporting in this paper was not adequate for the establishment of a developmental toxicity NOEL for regulatory purposes.

8.3 Rabbit

Robens JF (1969) Teratologic studies of carbaryl, diazinon, norea, disulfiram, and thiram in small laboratory animals. Division of Pharmacology and Toxicology, Food and Drug Administration, Washington DC, USA. Toxicol Appl Pharmacol 15: 152-163

The teratogenic potential of technical diazinon (Ciba-Geigy Chemical Corp, USA; purity not stated) was tested in pregnant Golden Syrian hamsters and New Zealand White rabbits. The sources and ages of the animals were not provided. All fetuses were examined for gross defects when delivered by caesarean section. The viability of rabbit fetuses was determined by placing them in a chicken-hatching incubator for 6 h. All fetuses with sufficient developmental form for determination of structural defects were counted in the number of fetuses/litter, even though some of these fetuses were dead when delivered. Resorption sites and fetuses which died in late embryonic life were counted as dead fetuses.

Rabbits were given diazinon by gelatin capsule orally with the aid of a balling gun on gestation days 5 through 15, at doses of 30 mg/kg bw/day (8 does) or 7 mg/kg bw/day (9 does). Control animals (21 does) received empty gelatin capsules. The does were sacrificed on gestation day 28. The internal organs of rabbit fetuses were examined and fixed, and the skeletons stained with Alizarin red and examined for bone abnormalities.

Results

No treatment-related malformations and no fetotoxicity were observed in rabbits given diazinon. At 30 mg/kg bw/day, there was significant mortality in the does (6/8 died) and severe cholinergic signs (no further details provided).

Summary of Effects on Reproductive Indices

Reproductive Indices	Dose (mg/kg bw/day)		
	0 (CMC control)	7	30
No. of dams that died/No. treated	0/21	0/9	6/8
Mean number of fetuses/litter	7.5	9.3	9.0
Fetal mortality (%)	16.5	2.3	0
Mean fetal weight (g)	36.2	36.3	29.6

Summary

Diazinon was given to New Zealand White rabbits (7 or 30 mg/kg bw/day) during gestation. Cholinergic signs and high maternal mortality was seen in rabbits at the highest dose. No signs of developmental toxicity or fetal malformations were reported at any. The level of reporting in this paper was not adequate for the establishment of a developmental toxicity NOEL for regulatory purposes.

Harris SB (1981) A teratology study of diazinon (CAS number 333-41-5) in New Zealand White rabbits. Report no: CGA/SAI 281005. Lab: Science Applications Inc, Division of Toxicology, La Jolla, California USA. Sponsor: Ciba-Geigy Corporation, Greensboro, NC, USA. Study duration: 24 Feb, 1981 - 2 Apr, 1981. Report date: 28 Jul, 1981. (US FDA GLP Compliance Statement provided)

Diazinon (Ciba-Geigy Corp.; 89.2% purity; Lot no. 88093) in epoxidized soybean oil was given to pregnant New Zealand White rabbits (Hills of Home Ranch, California, USA; aged 4-5 months, starting weight 3-4.1 kg) by oral gavage at doses of 0 (control), 7, 25, or 100 mg/kg bw/day (group sizes; 19, 18, 19, and 22, respectively) on gestation days 6 through 18. The test material was formulated in a 0.2% aqueous solution of carboxymethylcellulose (CMC), and the dose volume was 1 mL/kg bw. Control animals received CMC suspension only. The CMC was prepared weekly, and refrigerated after mixing. The test material suspensions were prepared daily and discarded after use. To ensure that the suspensions were homogeneous, they were continuously mixed with a magnetic stirrer. The test material concentration in formulations was not determined analytically.

Bodyweights were measured on gestation days 0, 6 through 18, 25, and prior to caesarean section on day 30. The animals were observed twice daily for clinical signs of intoxication. Any animals that died during the study were necropsied shortly after discovery. On day 30, does were necropsied and the thoracic and abdominal cavities were examined in situ for the presence of gross lesions. The reproductive organs were examined in situ and abnormalities recorded. Other parameters recorded at this time were the gravid uterus weight, number of corpora lutea, location and distribution of live and dead fetuses, the number and type of resorption sites, individual fetal bodyweights, and fetal external anomalies.

Visceral examination of the fetuses included the gross observation of abdominal cavity tissues and organs (umbilical blood vessels, stomach, spleen, pancreas, liver, gall bladder, diaphragm), determination of the sex of the fetus, examination of the kidneys, adrenal glands, ureters, and urinary bladder. The heart, lungs and brain were also dissected and examined. After the visceral examination, the fetuses were processed with Alizarin Red S for skeletal examination.

Results

Maternal findings: An increase in mortality was seen at 100 mg/kg bw/day, with 9/22 does dying at this dose. No mortality occurred at other doses. Maternal bodyweight gain was reduced by about 40% compared with controls at 100 mg/kg bw/day. When the bodyweight changes were corrected for the weight of the gravid uteri, does in treated groups generally lost more weight than control animals. However, statistical analysis did not reveal any significant differences between control and treated groups with respect to bodyweight changes during the study. There was an increase in the incidence of a range of clinical signs at 100 mg/kg bw/day, and these effects included emesis, oral discharge, nasal discharge, soft stool, diarrhoea, anorexia, tremors, convulsions, hypoactivity, and unthriftiness. A doe in the 25 mg/kg bw/day group aborted (day 27 of gestation), with all pups found dead.

Summary of Maternal Indices

Maternal Indices	Dose (mg/kg bw/day)			
	0	7	25	100
Mortalities (%)	0	0	0	9/22 (40.9%)
Actual mean bodyweight change (g)	317	318	360	192
Corrected mean maternal bodyweight change* (g)	-142	-208	-155	-220
Mean number of corpora lutea	8.6	9.5	9.1	8.3

*Day 30 weight – (day 6 weight – gravid uterus weight)

Fetal findings: There were no statistically-significant, treatment-related effects on reproductive parameters, including the mean number of implantation sites, proportions of live, dead, or resorbed fetuses per litter, fetal weight, and sex ratio.

Summary of Effects on Reproductive Indices

Reproductive Indices	Dose (mg/kg bw/day)			
	0	7	25	100
Number of litters examined	18	17	15	11
Number of fetuses examined	135	146	118	81
Number of live fetuses (%)	135 (95.1%)	146 (95.4%)	118 (95.9%)	81 (95.3%)
Mean number of live fetuses/litter	7.5	8.6	7.9	6.8
Mean live fetal weight	49.5	45.6	48.6	47.3
Number of resorbed fetuses	6	6	3	4
Number of dead fetuses	1	1	2	0
Sex ratio (M:F)	1.5:1.0	1.0:1.0	1.0:1.0	1.0:1.0

Skeletal findings: There were no statistically-significant, treatment-related increases in the incidence of fetal malformations or variations. A small number of various skeletal malformations were observed at a low incidence in control and/or treated groups. Such findings included fusing

of sternebrae, small clavicle, fused ribs, and vertebral column scoliosis. There was no increase in the incidence of these effects at the high dose, and these effects were not considered to be treatment-related due to the isolated nature of the findings, and the lack of a dose-response relationship. A wide range of skeletal variations was reported at all doses including controls. The incidence of a number of these variations was occasionally increased at 100 mg/kg bw/day, including absent sternebra 5, and incomplete ossification of sternebra 6. The incidence of these skeletal variations was not significantly different to controls, but may be an indication of delayed development in the presence of frank maternotoxicity.

Summary of Fetal Skeletal Abnormalities

Skeletal Abnormalities	Dose (mg/kg bw/day)			
	0	7	25	100
No. of fetuses examined	135	146	118	81
<i>Malformations</i>				
Left clavicle small	1	0	0	0
Ribs fused	1	0	0	1
Sternebrae 3,4,5 fused	1	0	1	0
Sternebrae 4,5 fused	2	1	4	0
Both clavicles small	0	1	0	0
Vertebral column scoliosis	1	0	0	0
<i>Variations</i>				
Sternebra 5 absent	0	0	6 (5.1%)	11 (13.6%)
Sternebra 6 incomplete ossification	21 (13.5%)	8 (5.5%)	12 (10.2%)	24 (29.6%)

Visceral findings: Isolated single instances of visceral malformations were noted in control or 25 mg/kg bw/day animals, and these effects included common truncus arteriosus and ductus arteriosus arising from the left pulmonary artery, scoliosis, and an interventricular septal defect. These findings were not related to dose, and were not considered to be treatment-related.

Summary of Fetal Visceral Abnormalities

Visceral Abnormalities	Dose (mg/kg bw/day)			
	0	7	25	100
No. of fetuses examined	135	146	118	81
<i>Malformations</i>				
Common truncus arteriosus	0	0	1	0
IV septal defect	1	0	0	0
Gall bladder duplicated	1	0	0	0
Ductus arteriosus left pulmonary artery	1	0	0	0
Heart. Common truncus arteriosus	1	0	0	0

Summary

Diazinon was given by oral gavage to groups of pregnant New Zealand White rabbits on gestation days 6 through 18 at doses of 0 (CMC control), 7, 25, or 100 mg/kg bw/day. Frank maternotoxicity was observed at the high dose, with significant mortality (40%), an increase in the incidence of a range of clinical signs of intoxication, and decreases in bodyweight gains. There were no significant effects on reproductive parameters at any dose, and no major treatment-related malformations were observed. At 100 mg/kg bw/day there was a slight increase

in a number of skeletal variations, including delayed ossification. These effects were not significantly increased compared with controls, but were consistent with delayed development in the presence of frank maternotoxicity. The NOEL for this study was 25 mg/kg bw/day, based on maternotoxicity and delayed fetal development at 100 mg/kg bw/day. The NOEL for teratogenicity was 100 mg/kg bw/day.

Edwards JA, Falconer DM, Masters RE, Anderson A & Dawe IS (1987) The effect of diazinon technical on pregnancy of the rabbit. Report no. NKU 103/87328. Lab: Huntingdon Research Centre Ltd., Huntingdon UK. Sponsor: Nippon Kayaku, Agrochemical Division, Tokyo, Japan. Study completed: 11 Nov, 1986. Report date: 3 Jul, 1987. (US EPA, OECD and Japan MAFF GLP statements provided; EPA FIFRA 83-3)

This study was conducted to investigate the developmental toxicity potential of diazinon (Nippon Kayaku, Japan; stated purity 96.02%; batch 86022) given by intragastric intubation to pregnant New Zealand White rabbits (13-16 weeks old; Cheshire Rabbit Farms and/or Ranch Rabbits and/or Buxted Rabbit Company Ltd., UK) on days 6-18 of gestation. The doses used in the main study were selected following a pilot study and then a preliminary study in non-pregnant animals. The test material was given as a 5% aqueous solution in gum arabic, with an initial dose volume of 5 mL/kg bw, in all phases of this study.

Pilot study: Diazinon was given to sexually mature, non-pregnant NZW female rabbits (one or two per group) at doses of 20, 30, 60, or 100 mg/kg bw/day. Periods of dosing were seven, twelve, thirteen and three days, respectively, at the above dose levels.

Preliminary study: Female NZW rabbits (6/group) were given diazinon at doses of 0 (control), 10, 30, or 50 mg/kg bw/day for 13 consecutive days, with treatment beginning on day 1 of the study. Dose volumes were adjusted for individual animals before doses 3, 5, 8, and 11. Animals were killed eight days after the last dose, then dissected and examined for macroscopic pathological changes. Body weights were measured prior to treatment, and on days 1, 4, 6, 8, 14, and 21, and food consumption was calculated for days 1-2, 3-5, 6-8, 9-11, 12-14, 15-17, and 18-20. Blood samples were obtained 2 h after dosing on the last day of treatment, and used for ChE determinations.

Main study: Female NZW rabbits received diazinon at doses of 0 (control), 2.5, 10, or 40 mg/kg bw/day on days 6 to 18 of gestation. Animals were dosed within 2 h of test material formulation, and dosage volumes were adjusted according to body weight on days 8, 10, and 14. There were two phases of mating in this study, a main phase, and a supplementary phase. In the main phase, most of the mated does were allocated to groups on the day of mating to give roughly equal distribution of animals in each of the groups. The remaining animals in the main phase were not allocated to groups until day 6 of pregnancy. In the supplementary phase of mating, an extra 5 to 12 mated does were allocated to each of the dose groups. At 0, 2.5, 10, and 40 mg/kg bw/day, the groups numbered 17, 17, 20, and 18 does, respectively, in the main mating phase, and 8, 5, 5, and 12 does, respectively, in the supplementary mating phase.

The stability and homogeneity of the test material formulation was tested. The mean concentration of diazinon in the test suspensions were analysed during the study and found to be within 10% of nominal concentrations. Diazinon suspended in 5% gum arabic at nominal concentrations of 0.005% and 5% w/v formed an homogenous suspension, which was maintained by magnetic stirring for up to 2 h after preparation. The chemical stability of diazinon suspended in 5% gum arabic at ambient temperature was confirmed over a 4-h period.

All animals were handled regularly and observed daily for signs associated with treatment. All animals that died or were killed were weighed and subjected to post-mortem examination. All rabbits were weighed on days 1, 6, 8, 10, 19, 23, and 29 of gestation, and food consumption was calculated at these same intervals. Animals were killed on day 29 of gestation, then dissected and examined for congenital abnormalities and macroscopic pathological changes in maternal organs. The uteri and ovaries were examined to determine the number of corpora lutea, the number and distribution of live young, the number and distribution of embryonic/fetal deaths, the individual fetal weights, and fetal abnormalities.

Embryonic/fetal deaths were classified as:

- . early: only placenta visible at termination,
- . late: both placenta and embryonic remnants visible at termination,
- . abortion: only implantation site scars visible at termination.

Live young were examined externally then killed, weighed, dissected to examine for visceral and skeletal abnormalities, and sexed.

Results

Pilot study: At 100 mg/kg bw/day, the animal treated showed anorexia, salivation, body tremors and abnormal posture. The animal was killed on day 3 due to the severity of the intoxication. At 60 mg/kg bw/day, the animal displayed similar effects, but with reduced severity. After a three-day recovery period, the animal showed no obvious treatment-related effects. At 30 mg/kg bw/day, both animals displayed weight loss, and some post-dosing salivation and unsteadiness. One animal died on day thirteen. At 20 mg/kg bw/day, both animals had slight weight reductions, but did not display any addition signs of intoxication.

Preliminary study: No mortalities or signs of treatment-related intoxication were observed at 10 and 30 mg/kg bw/day. At 50 mg/kg bw/day, treatment-related effects included reduced faeces, post-dosing unsteadiness and body tremors. At this dose, one animal also showed post-dosing salivation, piloerection, abnormal movement and posture, and was killed.

A marked decrease in bodyweight gain was seen at 50 mg/kg bw/day, and the group mean bodyweight of animals at this dose did not recover to pre-treatment levels until the day 21 measurement. At 30 mg/kg bw/day, bodyweight gain was reduced until day 4 only. There was an apparent decrease in group mean food consumption at 50 mg/kg bw/day until about day 11 of treatment, but after this time, food consumption was similar at all doses. At 10 and 30 mg/kg bw/day, food consumption was generally slightly elevated compared with controls through most of the study. At all doses there was a marked, dose-related decrease in plasma ChE activity of between 75 and 88% compared with controls. Similarly, erythrocyte ChE activity was inhibited at all doses, by about 70 to 80% compared with controls. No treatment-related macroscopic changes were noted at necropsy.

Main study: At 2.5 and 10 mg/kg bw/day, there was no increase in the incidence of treatment-related signs of intoxication. At 40 mg/kg bw/day, most animals displayed signs of intoxication, including unsteadiness, abnormal movement or posture, body and/or facial tremors, salivation, and piloerection. The number of animals that died or were killed due to loss of condition was similar in control and treated groups.

Bodyweight and food consumption: Food consumption was reduced only in the 40 mg/kg bw/day group during the treatment period, but was similar in all groups after cessation of treatment. This reduction in daily food consumption was about 16% compared with controls for days 14 to 18. Group mean bodyweights were similar in all groups during treatment. There was a slight reduction in bodyweights at 40 mg/kg bw/day, with reductions of up to 6% compared with controls on day 19 of gestation. However, the mean bodyweight for this group was about 3% lower than controls pre-treatment, and so the change in weight at this dose level was probably not biologically significant. There was a decrease in the mean bodyweight gain at 40 mg/kg bw/day, and at this dose animals did not gain weight for the first few days of treatment. After gestation day 8 the rate of mean bodyweight gain at this dose was similar to controls. At 2.5 and 10 mg/kg bw/day, bodyweight and bodyweight gains were similar to controls throughout the study. No treatment-related effects were observed at autopsy examination.

Litter data: As shown in the Table below, no consistent, treatment-related effects were seen on litter size, or on pre- or post-implantation losses. There was a slight increase in the incidence of late embryonic deaths at 10 and 40 mg/kg bw/day, and in total embryonic deaths at 40 mg/kg bw/day, but these increases did not reach statistical significance. These results may be an indicator of the maternotoxicity at the higher doses. Statistically-significant increases in the incidence of implants and in decreases in pre-implantation losses at 10 mg/kg bw/day were considered to be incidental to treatment. There was a statistically-significant decrease in the mean fetal weight at 10 and 40 mg/kg bw/day. This decrease in weight was about 11% compared with controls, but there was no consistent dose-response relationship with this finding. Mean litter weights were also reduced at 40 mg/kg bw/day compared with controls, but again there was an absence of any consistent dose-relationship for this effect. No statistically-significant differences in the litter sex ratios were observed.

Litter Data: Main findings (group mean values)

Litter data	Dose (mg/kg bw/day)			
	0	2.5	10	40
Late embryonic deaths	0.5	0.6	0.9	1.1
Total embryonic deaths	1.1	1.3	1.3	1.6
Implants	8.9	9.6	10.6*	9.7
Pre-implantation loss (%)	17.5	17.3	6.6*	8.7
Litter weight (g)	344.5	335.7	370.7	328.7
Mean fetal weight (g)	44.7	42.4	39.7**	40.9*

*p<0.05, **p<0.01

Malformations and anomalies: There was no statistically-significant increase in the incidence of visceral or skeletal anomalies or malformations at any dose. The percentage of litters with malformations was elevated at 2.5 and 10 mg/kg bw/day, but not at the high dose. Statistically-significant reductions in the incidence of skeletal anomalies were seen at 2.5 and 40 mg/kg bw/day, but the absence of a dose relationship suggested that this finding was not toxicologically significant. No statistically significant increases in skeletal variations were observed at any dose.

Summary of Fetal Malformations and Anomalies

Malformations	Dose (mg/kg bw/day)			
	0	2.5	10	40

Fetuses affected	2/133 (1.5%)	5/133 (3.8%)	3/150 (2%)	1/130 (0.8%)
Visceral anomalies-fetuses affected	11/131 (8.3%)	6/128 (4.7%)	6/147 (4.1%)	16/129 (12.4%)
Skeletal anomalies-fetuses affected	37/131 (28.2%)	13/128 ** (10.1%)	21/147 (14.3%)	10/129** (7.8%)

** p<0.01

Summary

Pregnant New Zealand White rabbits were given diazinon (96.02% purity) at doses of 0, 2.5, 10, or 40 mg/kg bw/day on days 6 through 18 of gestation. At 40 mg/kg bw/day, maternal body weight gain and food consumption were decreased and animals displayed signs of intoxication including unsteadiness, abnormal movement or posture, body and/or facial tremors, salivation, and piloerection. No consistent, treatment-related affects were seen on litter size, or on pre- or post-implantation losses. There was a statistically significant decrease in the mean fetal weight at 10 and 40 mg/kg bw/day, but there was no dose-response relationship associated with this finding. There was no statistically-significant increase in the incidence of visceral or skeletal anomalies or malformations at any dose. The NOEL for this study was 10 mg/kg bw/day, based on the clinical signs, reduced bodyweight gains and reduced food consumption in maternal animals at 40 mg/kg bw/day.

8.4 Dog

Earl FL, Miller E & Van Loon EJ (1973) Reproductive, teratogenic and neonatal effects of some pesticides and related compounds in Beagle dogs and miniature swine. Special Pharmacological Animal Laboratory, Division of Toxicology, Food and Drug Administration, Washington DC, USA. Pestic. Environ. Continuing Controversy, Am. Conf., Toxicol. Occup.Med/. 8: 253-266

The developmental toxicity potential of diazinon (source and purity not stated) was assessed in female purebred Beagle dogs and Hormel-Hanford cross miniature pigs (US FDA, Division of Toxicology). The dogs and the pigs were bred daily on two consecutive days when in oestrus. Post-breeding days were calculated from the first day of breeding. Forty-eight control beagles and twenty-seven control sows were bred and served as test controls for the study.

Diazinon in corn oil was given by capsule to groups of six pregnant Beagle dogs at doses of 1, 2, or 5 mg/kg bw/day from the day of breeding through the due date of 63 days, and dosed through the eighth week of lactation and then necropsied. Pups were necropsied at days 1, 7, 14, 21, 28, 56, and 84 (number of pups/interval not stated) and rib bone marrow smears prepared. A group of six females was similarly bred as untreated controls. Females were re-bred and included in the study.

Results

At 1 mg/kg bw/day, 6/8 females produced pups. In one litter, all seven pups died by day 13 post-partum. The females were described as very nervous at this dose level, and one ate the limbs from one of its pups. At 2 mg/kg bw/day, 6/8 females produced offspring (a total of 33 pups). The number of pups in individual litters was not provided. One female was very weak and died during caesarean section. Three pups in different litters starved because they would not suckle, and two pups had an oedematous thickened duodenal wall similar to that seen in older animals. At

10 mg/kg bw/day, 7/10 bred females produced offspring (a total of 34 pups). At 21 days one pup had a pronounced fontanelle and no teeth, and its head was at a peculiar tilt. At 28 days one pup from another litter had a thickened wall of the duodenum. At day 84 another pup (from a different litter) had fibrous strands attaching the stomach to the liver and kidney. The liver was also attached to the diaphragm and the bladder to the abdominal wall, and the pericardial sac was attached to the diaphragm. No unusual necropsy results were observed in the control animals.

There was a slight but dose-related decrease in the average number of pups/litter, but this finding was not significantly different to in-group controls, and was similar to the test controls for the study and to historical controls cited in this report. The incidence of stillborn pups was elevated at 1 and 5 mg/kg bw/day compared with concurrent, test group and historical controls, but there was no consistent dose-response relationship. The author stated that the females were very nervous during birth and would not lie still, and pups in treated groups were lacking in normal suckling abilities, and would not feed.

Summary of Effects on Reproductive Indices

Reproductive Indices	Dose (mg/kg bw/day)					
	Test controls (0)	Historical controls* (0)	Concurrent controls (0)	1	2	5
Number bred	48	199	6	8	8	10
% Pregnant	87.5	84.5	100	75	75	70
Av. number of pups/litter	4.8	4.9	6.3	6.3	5.5	4.9
% Pups with terata	1.0	0.24	0	0	0	2.9
% Pups stillborn	2.0	5.7	10.5	15.8	9.1	14.7

*Period from Jan 1970 to Oct 1972

Summary

When diazinon was given to pregnant Beagle dogs at doses up to 5 mg/kg bw/day, there were no significant increases in the incidence of malformations, or on reproductive performance. The maternal animals were described as nervous, but the level of reporting in this study was inadequate to determine if the high dose produced frank maternotoxicity. This study was inadequate for the establishment of an NOEL for regulatory purposes.

8.5 Pig

Earl FL, Miller E & Van Loon EJ (1973) Reproductive, teratogenic and neonatal effects of some pesticides and related compounds in Beagle dogs and miniature swine. Special Pharmacological Animal Laboratory, Division of Toxicology, Food and Drug Administration, Washington DC, USA. Pestic. Environ. Continuing Controversy, Am. Conf., Toxicol, Occup.Med/. 8: 253-266

The developmental toxicity potential of diazinon (source and purity not stated) was assessed in female purebred Beagle dogs and Hormel-Hanford cross miniature pigs (US FDA, Division of Toxicology). The dogs and the pigs were bred daily on two consecutive days when in oestrus. Post-breeding days were calculated from the first day of breeding. Forty-eight control beagles and twenty-seven control sows were bred and served as test controls for the study.

Swine: Two groups of seven sows were given diazinon at doses of 5 or 10 mg/kg bw/day from day 1 of breeding. The period of gestation was not described. Sows were allowed to farrow and the pigs were examined. The examination intervals were not reported.

Results

Swine: At 5 mg/kg bw/day all seven sows farrowed. A single pig in each of three different litters displayed abnormalities. One pig had a dome-shaped head, another had marked brachygnathia with the condyles of the mandible being fused to the temporal condyles of the skull, and teeth were absent in the lower jaw and in the upper jaw immediately above the mandible. The other abnormal pig had a lateral curvature of the left front limb involving the soft tissue with the distal end of the leg being twisted medially. The right front leg had an extra set of metacarpal bones on the medial side. The author stated that such a condition is not normally seen in swine. At 10 mg/kg bw/day, 2/6 sows produced normal offspring, and the remaining sows died as a result of diazinon intoxication.

Summary of Effects on Reproductive Indices

Reproductive Indices	Dose (mg/kg bw/day)		
	Test controls	5	10
Number bred	27	7	7
% Pregnant	92.6	100	28.6
Average number of pups/litter	6.2	6.9	8.5
% Pups with terata	0	6.3	0
% Pups stillborn	1.9	0	0

Summary

In pregnant swine given diazinon at up to 10 mg/kg bw/day, there was significant maternal mortality at the high dose unaccompanied by any increase in the incidence of malformations. Several instances of abnormalities were reported at 5 mg/kg bw/day, but because of the small number of animals in this study, and the isolated nature of the findings, it was not possible to attribute the abnormalities to diazinon administration. This study was inadequate for the establishment of an NOEL for regulatory purposes.

Cameron DM (1995) Target animal tolerance study in gestating sows. Report no: VRB 10/951862. Lab: Huntingdon Life Sciences Ltd, UK. Sponsor: Virbac, France. Study date: 30 Nov, 1994 - 1 Feb, 1995. Report date: 11 Dec, 1995. (US FDA, OECD, Japan MAFF, UK Health, EC Council GLP Compliance Statements provided)

This study was conducted to determine the effects of topical diazinon application to pregnant sows. The test material was a 19.9% (w/v) solution of diazinon (Virbac; batch number BD-4068; code 586.01) applied as a single topical dose to the dorsal midline of pregnant female Large White hybrid pigs (R Beedles, UK; 2-4 years old on delivery; 160-285 kg bw on the day prior to dosing). Groups of 4 animals received doses of 0 (control), 20, 60, or 100 mg/kg bw (of the active ingredient), on day 85 of gestation, with dose volumes of 0, 0.1, 0.3, or 0.5 mL/kg bw, respectively.

All animals were observed several times daily for any clinical signs of toxicity or other abnormalities, including examination of the application sites for signs associated with treatment.

Sows were weighed on arrival, and at days -8, 1, 14, and within one day of parturition and at termination (ten days post-farrowing). Piglets were weighed within one day of parturition and at termination. Food consumption was recorded twice daily from the time of allocation to termination, and all pigs were offered 4 kg of feed per day, increasing to 5 kg/day at parturition.

Venous blood samples were obtained from all sows prior to the morning feed on days -5, 2, and 7 and within 48 h of farrowing, for haematological and biochemical determinations. Haematology parameters were PCV, Hb, erythrocyte count, MCHC, MCV, reticulocyte count, WBC, differential count, platelet count, prothrombin time (PT), and activated partial thromboplastin time (APTT). Biochemistry parameters were glucose, total protein, albumin, globulin, urea, creatinine, AP, ALT, AST, LDH, glutamate dehydrogenase (GDH), total bilirubin, electrolytes, cholesterol, plasma ChE, erythrocyte ChE. Venous blood samples were obtained from all piglets ten days after parturition for determination of plasma and erythrocyte ChE only. The following parameters were recorded for each sow: date and time of farrowing, observations on parturition and indication of milk production, litter size (alive and dead), individual piglet bodyweights within 24 h of birth and 10 days post-partum, morphological or functional abnormalities in piglets, and clinical observations on litters and piglet mortality.

At the study termination, sows were killed by IV injection of sodium pentobarbitone. Samples of skin from the dose application site were preserved. Uteri, ovaries and skin were examined microscopically. Piglets were sacrificed by IV injection of sodium pentobarbitone, but post-mortem examinations were not conducted at termination.

Results

No treatment-related clinical signs were reported in sows or piglets at any dose. Bodyweights and food consumption were not affected by treatment. There were no dose-related changes in the incidence of piglet survival, bodyweights or viability. No significant, dose-related changes in haematology parameters were observed in sows.

ChE inhibition: Significant ($p < 0.05$) decreases in plasma and erythrocyte ChE activity were observed at 60 and 100 mg/kg bw at day 2 after treatment. The decrease in activity was not dose-dependent, and the inhibition at 100 mg/kg bw was similar to that observed at 60 mg/kg bw. At day 7, significant ($p < 0.01$), dose-related decreases in plasma ChE inhibition were observed at all dose. Erythrocyte ChE activity was decreased at all doses, but the inhibition was not dose-related or statistically significant, and inhibition ranged from 13-21% compared with controls. At parturition, no significant, dose-related decreases in plasma or erythrocyte ChE inhibition were seen at any dose. No other dose- or time-dependent effects on other haematological parameters were seen during the study. In piglets, plasma and erythrocyte ChE activity was not significantly affected by treatment.

ChE Inhibition (mean percentage reduction)

Dose (mg/kg bw)	Plasma ChE			Erythrocyte ChE		
	Day 2	Day 7	Parturition	Day 2	Day 7	Parturition
20	5	22**	0	9	13	15
60	50**	44**	22	21*	21	14
100	45*	50**	5	15*	14	12

* p<0.05, **p<0.01

Pathology examination did not reveal any effects that were considered to be treatment related.

Summary

Treating pregnant sows on gestation day 85 with a single topical application of diazinon (19.9% formulation) at doses of 20, 60, or 100 mg/kg bw (active ingredient) did not result in any treatment-related mortality or clinical signs of intoxication. Plasma ChE activity was inhibited at all doses on day 7 and at 60 and 100 mg/kg bw on day 2 after treatment. This effect was reversible. No adverse effects were observed on other biochemical or haematological parameters, or on reproductive parameters.

**9. GENOTOXICITY.
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A summary of submitted and published findings of genotoxicity studies with diazinon is shown in the Table below.

Summary of Mutagenicity Testing with Diazinon

Assay	Bacterial strain or Cell type	Concentration (or Dose)	Metabolic activation	Results	Reference
Gene Mutation					
<i>S. typhimurium</i>	TA1535 TA1536 TA1537 TA1538	50-1000 µg/plate	+, - +, - +, - +, -	-, - -, - -, - -, -	Marshall et al, 1976
	TA98 TA100 TA1535 TA1537 TA1538 <i>E.coli</i> WP2 hcr^-	200-5000 µg/plate	+, - +, - +, - +, - +, - +, -	-, - -, - -, - -, - -, - -, -	Shirasu et al, 1976
	TA98 TA100 TA1535 TA1537 <i>E.coli</i> WP2 $uvrA$	313-5000 µg/plate	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	Geleick & Arni, 1990
	TA98 TA100 TA1535 TA1537 TA1538	200-5000 µg/plate (repeated with 1500, 2500, 5000, 7500 and 10000 µg/plate for TA 1535)	+, - +, - +, - +, - +, -	-, - -, + -, + -, - -, -	Jones & Wilson, 1988
Host-mediated	Mouse (ICR) & <i>S. typhimurium</i> G46	30-70 mg/kg bw		-	Shirasu et al, 1976
Mammalian cells	Mouse lymphoma (L5178Y)	12-120 µg/mL 6-60 µg/mL (with activation)	+, -	-, -	Beilstein et al, 1986
		10-75 µg/mL 5-30 µg/mL (with activation)	+, -	+, - (Only 13% cell viability at the highest dose)	Henderson et al, 1988

Results (+, positive; -, negative) are expressed relative to the presence (+) or absence (-) of metabolic activation.

Summary of Mutagenicity Testing with Diazinon (cont.)

Assay	Bacterial strain or Cell type	Concentration	Metabolic activation	Results	Reference
Chromosomal Effect Assays (<i>in vitro</i>)					
Rec-	<i>B. subtilis</i> H17/M45	Up to 20 µL	+, -	-, -	Shirasu et al, 1976
<i>E. coli</i>	SD-4	50% (v/v)		-	Hurni & Ohder, 1970
	WP67 (<i>uvrA</i> ⁻ , <i>polA</i> ⁻) WP871 (<i>uvrA</i> ⁻ , <i>recA</i> ⁻ , <i>lexA</i> ⁻)	10-10000 µg/mL	+, -	-, -	Bootman & May, 1986
Sister Chromatid Exchange	Chinese hamster lung cells (V79)	100 µg/mL (with activation)	+	+	Matsuoka et al, 1979
		10-40 µg/mL 10-80 µg/mL (with activation)	+, -	-, -	Chen et al, 1981 & Chen et al, 1982
	Human lymphoid cells (LAZ-007)	0.02-20 µg/mL	+, -	+, -	Sobti et al, 1982
	Human lymphocytes (CCL 156)	0.12-100 µg/mL 0.12-200 µg/mL (with activation)	+, -	-, -	Strasser & Arni, 1988
	Human lymphocytes (PHA stimulated)	0.0668-20 µg/mL 0.0668-66.8 µg/mL (with activation)	+, -	-, -	Murli, 1990a

Results (+, positive; -, negative) are expressed relative to the presence (+) or absence (-) of metabolic activation.

Assay	Species	Dose	Result	Reference
Chromosomal Effect Assays				
Sister Chromatid Exchange (marrow cells)	Mouse (ICR) [MTD in males]	0, 10, 50, or 100 mg/kg bw; PO	-	Murli, 1990b
	Mouse (ICR) [MTD in females]	0, 150, 160, or 175 mg/kg bw; PO	-	Murli, 1993
	Hamster (Chinese)	0, 6.5, 13, or 26 mg/kg bw; PO	-	Hool & Müller, 1981a
Micronucleus (marrow cells)	Mouse (Tif:MAGF)	0, 30, 60, or 120 mg/kg bw; PO	-	Ceresa et al, 1988
Dominant Lethal	Mouse (NMRI)	0, 15, or 45 mg/kg bw; PO	-	Fritz, 1975
Nucleus Anomaly (marrow cells)	Hamster (Chinese)	0, 6.5, 13, or 26 mg/kg bw; PO	-	Hool et al, 1981
Clastogenicity	Human lymphocytes	5-20 µg/mL	-	Bootman et al, 1986
Spermatogonia	Mouse (NMRI)	0, 10.5, or 21 mg/kg bw; PO	-	Hool & Müller, 1981b
Spermatocytes	Mouse (NMRI)	0, 10.5, or 21 mg/kg bw; PO	-	Hool & Müller, 1981c
Other Assays				
DNA Repair	Rat (Tif:RAIf)	10.2-1044 µg/mL	-	Hertner & Arni, 1990

9.1 Gene Mutation Assays.1**Assays.1****Assays.1****Assays****Gene Mutation Assays.1****Gene Mutation Assays.1****Gene Mutation****Gene Mutation****Gene Mutation**

Marshall TC, Dorough HW & Swim HE (1976) Screening of pesticides for mutagenic potential using Salmonella typhimurium mutants. Department of Entomology, University of Kentucky, Kentucky, USA. J Agr Food Chem 24: 560-563

Technical diazinon (source, purity and lot no. not stated) did not increase the number of histidine revertants in *S. typhimurium* strains TA 1535, TA 1536, TA 1537, or TA 1538 at concentrations up to 1 mg/plate in the absence or presence of an hepatic S9 fraction from a male phenobarbitone-treated Sprague-Dawley rat. Higher concentrations (≥ 1 mg/plate) were associated with precipitate formation. The positive control used in all experiments was nitrosocarbaryl. Hence, there was no evidence of direct or indirect mutagenic activity in these experiments.

Shirasu Y, Moriya M & Kato K (1976) Mutagenicity testing on diazinon in microbial systems. Lab: The Institute of Environmental Toxicology, Tokyo, Japan. Study duration: Not given. Report date: 11 June, 1976. (Pre-GLP)

Technical diazinon (batch and source not stated; purity 99.7%) in DMSO did not increase the number of histidine revertants in *S. typhimurium* strains TA 1538, TA 1537, TA 1535, TA 100, or TA 98, or tryptophan revertants in *E. coli* strain WP2 hcr^- , at 0.2, 1, or 5 mg/plate, in the absence or presence of hepatic S9 fraction from a Aroclor 1254-treated male Sprague-Dawley rat. Solvent (DMSO) and positive controls (β -propiolactone, 2-nitrofluorene, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide, 9(5)-aminoacridine, 2-aminoanthracene) gave the expected increase in number of revertants. In a Rec-assay with *B. subtilis* strains H17 and M45, diazinon in DMSO did not cause any growth inhibition when applied undiluted to filter paper disks.

Similarly, although oral gavage administration of dimethylnitrosamine (50 mg/kg bw; positive control) to male ICR mice (Clea Japan Inc.) followed by an IP inoculum of *S. typhimurium* G46 bacteria caused the expected significant increase in the number of histidine revertants in a host-mediated assay, diazinon at either 30 or 70 mg/kg bw did not.

Jones E & Wilson LA (1988) Ames metabolic activation test to assess the potential mutagenic effect of diazinon. Report no. PAM 29/871638. Lab: Huntingdon Research Centre Ltd, Cambridgeshire, England. Sponsor: Pan Medica, Carros Cedex, France. Study duration: 14 Oct - 16 Nov, 1987. Report date: 12 Feb, 1988. (OECD, Japan and US GLP compliant)

Technical diazinon (batch no. 121539; purity 97.14%; source not reported) in ethanol did not increase the number of histidine revertants in *S. typhimurium* strains TA 1537, TA 1538, or TA 98, at 50, 150, 500, 1500, or 5000 μ g/plate in the absence or presence of a hepatic S9 fraction from Aroclor 1254-pretreated CD (Sprague-Dawley derived) rats. However, for strains TA 100 and TA 1535, a statistical ($p < 0.001$) dose-related increase in revertant number were observed over the same concentration range, in the absence of S9. A repeat assay at a higher and over a more closely spaced concentration range (1500, 2500, 5000, 7500 and 10000 μ g/plate), confirmed the result for TA 1535 but cultures of TA 100 were contaminated. At the maximum tested concentration (10000 μ g/plate), the mean number of TA 1535 revertants was increased but this was only 13% of that observed for the control (N-ethyl-N'-nitro-N-nitrosoguanidine, 5

µg/plate). Solvent (ethanol) and positive controls (2-nitrofluorene, N-ethyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine and 2-aminoanthracene) gave the expected results.

Geleick D & Arni P (1990) *Salmonella and Escherichia/liver-microsome test. Study no. 891346. Lab: Ciba-Geigy Ltd, Genetic Toxicology Laboratories, Laboratories of Residue Analysis Unit, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Agricultural Division, Basle, Switzerland. Study duration: 21 Sep, 1989 - 10 Jan, 1990. Report date: 8 Feb, 1990. (OECD, EEC, Japan and US GLP compliant)*

Tests were performed according to EPA Guideline 40 and OECD Guideline 471. Technical diazinon (Ciba-Geigy batch FL 880045; purity 88.0%) in DMSO did not increase the number of histidine revertants in *S. typhimurium* strains TA 1537, TA 1535, TA 100, or TA 98, or tryptophan revertants in *E. coli* strain WP2uvrA, at 313, 625, 1250, 2500, or 5000 µg/plate in the absence or presence of a hepatic S9 fraction from Aroclor 1254-pretreated Tif:RAIf (SPF) rats. Dose selection was determined from preliminary toxicity testing that showed colony number to be unchanged over the range tested in the main study. Solvent (DMSO) and positive controls (4-nitroquinoline-N-oxide, 2-nitrofluorene, cyclophosphamide, sodium azide, 9(5)-aminoacridine, 2-aminoanthracene) gave the expected results. Thus, these experiments confirm the results of earlier studies showing the lack of mutagenic activity in bacterial cells.

Henderson LM, Davies SE, Ransome SJ, Brabbs CE, Tinner AJ & Bottoms MA (1988) *An assessment of the mutagenic potential of diazinon using the mouse lymphoma TK locus assay. Report no. PAM 30/88419. Lab: Huntingdon Research Centre Ltd, Cambridgeshire, England. Sponsor: Pan Medica, Carros Cedex, France. Study duration: 13 Jan - 22 Mar, 1988. Report date: 6 Jul, 1988. (OECD, Japan and US GLP compliant)*

Technical diazinon (batch no. 121539; purity 97.14%; source not reported) in DMSO at concentrations up to 75 µg/mL (10-75 µg/mL) in the absence of metabolic activation increased the mutation frequency at the thymidine kinase locus in mouse lymphoma L5178Y cells. However, this increase despite being significantly increased was discounted because it did not achieve the required 2-fold increase, in mutation frequency over vehicle controls. In the presence of an hepatic S9 fraction from Aroclor 1254-treated CD (Sprague-Dawley derived) rats, the increase in mutation frequency over the range of 5-30 µg/mL was dose-related, significant, and twice the vehicle control frequency at the highest concentration tested. However, since cell viability at the highest concentration was only 13%, the biological relevance of this increased mutation frequency is questionable. The positive controls, ie. ethyl methanesulfonate in the absence of metabolic activation, and 20-methyl-cholanthrene in the presence of S9, increased the mutation frequency relative to vehicle controls by 14-fold and 4-fold respectively. Thus, there was limited evidence of some possible indirect mutagenic activity in these experiments in mouse lymphoma L5178Y cells *in vitro*.

Beilstein P, Dollenmeier P & Müller D (1986) *L5178Y/TK⁺ Mouse lymphoma mutagenicity test. Study no. 840396. Lab: Ciba-Geigy Ltd, Experimental Pathology, Tissue Culture Laboratories, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Agricultural Division, Basle, Switzerland. Study duration: 21 Oct, 1985 - 7 Feb, 1986. Report date: 31 Jul, 1986. (US GLP compliant)*

Technical diazinon (Ciba-Geigy batch P203008; purity 97.2%) did not affect mutation frequency at the thymidine kinase locus in mouse lymphoma L5178Y cells at concentrations up to 120 µg/mL (12-120 µg/mL) in the absence of metabolic activation, and up to 60 µg/mL

(6-60 µg/mL) in the presence of hepatic S9 fraction from Aroclor 1254-treated male Tif:RAIf (SPF) rats. The highest concentrations tested were selected on the basis of an 85% cell survival rate. The positive controls, ethyl methanesulfonate in the absence of metabolic activation, and dimethylnitrosamine in the presence of S9, increased the mutation frequency by 32-fold and 15-fold respectively. Thus, there was no evidence of direct or indirect mutagenic activity in these experiments in mammalian cells *in vitro*.

9.2 Chromosomal Aberration Tests.2

Aberration Tests.2

Aberration Tests.2

Aberration Tests

Chromosomal

Chromosomal Aberration Tests.2 Chromosomal

Chromosomal Aberration Tests.2 Chromosomal

Bootman J & May K (1986) Diazinon: Assessment of its ability to cause lethal DNA damage in strains of *Escherichia coli*. Study no. 86/NKL041/322. Lab: Cell Biology Laboratory, Life Science Research Ltd, Suffolk, England. Sponsor: Nippon Kayaku Co Ltd, Tokyo, Japan. Study duration: 3-12 Jun, 1986. Report date: 19 Aug, 1986. (Company QA only)

To assess possible direct DNA action resulting in lethality of bacterial strains having deficient DNA repair mechanisms, a comparison between the survival of normal (WP2) and DNA repair-enzyme deficient (WP67, *uvrA*⁻, *polA*⁻; CM871, *uvrA*⁻, *recA*⁻, *lexA*⁻) *E. coli* strains in the presence of diazinon (Nippon Kayaku Co Ltd, Tokyo, Japan; batch no. 86032; purity 96.16%), mitomycin C (0.05 µg/mL; without S9 activation), 2-aminoanthracene (5 µg/mL; with S9 activation) or ampicillin (25 µg/mL) was performed. In preliminary testing, technical diazinon was shown to be devoid of any bacteriocidal effects after 2 or 18 h at the maximum concentration tested, i.e. 10 mg/mL. Comparing survival for WP2 with WP67 or CM871 indicated that only for the treble-enzyme deficient strain, CM871 at a concentration in excess of 1 mg/mL without the hepatic S9 fraction was there any evidence for DNA damage. Positive controls gave the expected results.

Bootman J, Hodson-Walker H & Dance C (1986) In vitro assessment of the clastogenic activity of diazinon on cultured lymphocytes. Study no. 86/NKL040/473. Lab: Cell Biology Laboratory, Life Science Research Ltd, Suffolk, England. Sponsor: Nippon Kayaku Co Ltd, Tokyo, Japan. Study duration: 20 May - 16 Jul, 1986. Report date: 5 Nov, 1986. (OECD, Japan and US GLP compliant)

Exposing phytohaemagglutinin-stimulated human lymphocyte cultures separately to 2 known clastogens, i.e. chlorambucil (2.5 µg/mL) in the absence of S9 or cyclophosphamide (6 µg/mL) in the presence of S9 metabolic activation respectively, caused a marked increase in the number of cells with aberrant metaphases. However, such cell cultures in the presence of technical diazinon (Nippon Kayaku Co Ltd, Tokyo, Japan; batch no. 86032; purity 96.16%) at concentrations of 5, 10, or 20 µg/mL, showed no significant changes relative to the vehicle control (DMSO) incidences, irrespective of the presence or absence of S9 metabolic activation. The maximum diazinon concentration selected for testing was dictated by precipitation at 5000 µg/mL, cell death at 1000 and 200 µg/mL, and attenuated mitotic activity at 40 µg/mL.

Hurni H & Ohder H (1970) Report on the mutagenic effect of technical diazinon. Project no. Tif 402 (Study no. not stated). Lab: Biomedical Research, Tierfarm AG, Sisseln, Switzerland. Study duration: not stated. Report date: 11 Sep, 1970. (Pre-GLP)

Technical diazinon (batch, source and purity not stated) in acetone did not increase the number of revertants in the streptomycin-dependent *E. coli* strain SD-4 at concentrations up to 50% (v/v). Solvent (acetone) and positive control (β -propiolactone) gave the expected results.

Matsuoka A, Hayashi M & Ishidate Jr M (1979) Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro. Biological Safety Research Center, National Institute of Hygienic Sciences, Tokyo, Japan. Mutation Res 66: 277-290

Technical diazinon (source, batch and purity not stated) in DMSO at a concentration of 100 $\mu\text{g/mL}$ was lethal for Chinese hamster lung cells (V79) in culture after 3 h, however, in the presence of S9 metabolic activation the frequency of sister-chromatid exchanges observed after 24 h was significantly ($p < 0.05$) increased (27%). The only concentration tested was chosen on the basis of cytotoxicity (lethality) to the V79 cells in the presence of PCB-induced S9 metabolic activation, primarily because the focus of the study was to examine some of the variables associated with induction and incubation co-factors of the S9 activation system. Hence, there were no negative controls but there was a selection of known carcinogens (eg. 2-acetylaminofluorene, benzo(a)pyrene), most of which gave the expected increase in SCEs but there were also some which did not (eg. 3-methylcholanthrene, 4-*o*-tolylazo-*o*-toluidine).

Chen HH, Hsueh JL, Sirianni SR & Huang CC (1981) Induction of sister chromatid exchanges and cell cycle delay in cultured mammalian cells treated with eight organophosphorus pesticides. Mutation Res 88: 307-316
and

Chen HH, Sirianni SR & Huang CC (1982) Sister chromatid exchanges in Chinese hamster cells treated with seventeen organophosphorus compounds in the presence of a metabolic activation system. Department of Experimental Biology, Roswell Park Memorial Institute, Buffalo, New York, USA. Environ Mutagenesis 4: 621-624

Technical diazinon (Ciba-Geigy; batch not stated; purity 99.2%) in DMSO at 0, 0 (vehicle), 10, 20, or 40 $\mu\text{g/mL}$ did not significantly increase the cell generation time (ie. calculated by assessing the percentage of mitoses at M1, M2, or M3) or the frequency of sister-chromatid exchange (ie. 5.97, 5.63 or 5.30/cell respectively in 3 replicate expts) in Chinese hamster lung (V79) cells relative to negative (5.96/cell) or vehicle controls (5.8/cell) after exposure for 29 h and 2 rounds (approx.) of replication in culture.

In a second study (short communication by Chen et al, 1982), the experimental protocol was essentially unchanged except that it was extended to examine the effects of S9 activation in the culture medium. At concentrations up to 80 $\mu\text{g/mL}$ there was no significant increase in the frequency of sister-chromatid exchange (ie. 6.3, 6.4, 6.0, or 6.6/cell at 10, 20, 40, or 80 $\mu\text{g/mL}$ respectively) in V79 lung cells relative to negative (vehicle) controls (5.7/cell). The positive control (cyclophosphamide in DMSO, 5 $\mu\text{g/mL}$) gave the expected significant ($p < 0.01$) increase in frequency of SCE (21.0/cell).

Sobti RC, Krishan A & Pfaffenberger CD (1982) Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Comprehensive Cancer Center for the State of Florida and University of Miami Medical School, Miami, USA. Mutation Res 102: 89-102

Technical diazinon (Chemical Service Inc., Westchester, PA, USA; batch and purity not stated) in ethyl alcohol at 0 (vehicle), 0.02, 0.2, 2, or 20 $\mu\text{g/mL}$ did not significantly increase the

frequency of sister-chromatid exchanges (ie. 7.85, 7.2, 7.8, 8.6/cell) in human lymphoid (LAZ-007) cells of B-cell origin relative to vehicle control (6.96/cell) after exposure for 48 h in culture. However, in the presence of liver microsomal S9 activation (derived from phenobarbitone-treated rats), significantly ($p < 0.01$) increased numbers of SCEs were observed at the highest concentration of 20 $\mu\text{g/mL}$ (13.50/cell) relative to vehicle controls (6.79/cell). Positive controls (cyclophosphamide, 0.1 $\mu\text{g/mL}$) in the presence of S9 gave the expected statistically significant increase ($p < 0.01$) in SCE (11.9/cell).

Strasser F & Arni P (1988) Sister chromatid exchange test on human lymphocytes cell line CCL 156 in vitro. Study no. 871697. Lab: Ciba-Geigy Ltd, Genetic Toxicology Laboratories, Laboratories of Residue Analysis Unit, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Agricultural Division, Basle, Switzerland. Study duration: 1 Feb - 14 Apr, 1988. Report date: 16 May, 1988. (US GLP compliant)

Human lymphocytes (cell line, CCL 156) were obtained from the American Type Collection Centre (ATCC) and maintained in culture. Preliminary cytotoxicity testing of technical diazinon (Ciba-Geigy batch FL 872049; purity 87.5%) in DMSO over a concentration range of 0.12 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$ indicated that at 200 $\mu\text{g/mL}$, approximately 50% suppression of mitotic activity occurred after a 3-h exposure and a 24-h recovery period. The observed cytotoxicity was independent of the presence or absence of a liver microsomal S9 fraction from Aroclor 1254-treated male Tif:RAIf (SPF) rats.

Over a concentration range of 12.5 to 100 $\mu\text{g/mL}$ and in the absence of microsomal activation, a statistical increase ($p < 0.01$) in the incidence of SCE was observed among 80 scored cells in metaphase after exposure for 3 h (and 43.5 h recovery) at each of the 2 lowest concentrations tested, ie. 12.34/cell and 12.64/cell at 12.5 and 25 $\mu\text{g/mL}$. However, at 50 and 100 $\mu\text{g/mL}$ the incidence of SCE was not significant, ie. 11.6/cell and 11.2/cell respectively. Excessive cytotoxicity at 200 $\mu\text{g/mL}$ prevented any meaningful results and the positive control, mitomycin C (0.2 $\mu\text{g/mL}$), gave the expected statistically significant increase ($p < 0.01$) in SCEs (44.93/cell) relative to negative controls (10.76/cell).

In the presence of microsomal S9 activation, significantly ($p < 0.01$) increased numbers of SCEs were observed at 12.5 and 50 $\mu\text{g/mL}$ (13.68 and 12.63/cell respectively) but not at 25, 100, or 200 $\mu\text{g/mL}$ (9.61, 11.08 and 11.18/cell respectively). Positive controls (cyclophosphamide, 3.0 $\mu\text{g/mL}$) gave the expected statistically significant increase ($p < 0.01$) in SCEs (48.06/cell) relative to negative controls (9.81/cell).

Although there were small but significant increases in the incidence of SCEs in the presence or absence of activation, a comparison with the respective positive controls suggests that these small increases from the negative controls are not toxicologically important. Hence, diazinon at concentrations up to 100 $\mu\text{g/mL}$ did not cause an increase in the frequency of sister chromatid exchanges in a human lymphocyte cell line (CCL 156).

Murli H (1990a) Mutagenicity test on diazinon MG8 in an in vitro cytogenetic assay measuring sister chromatid exchange frequencies in cultured whole human lymphocytes. Study no. HLA 12226-0-448. Lab: Hazleton Laboratories America Inc., Kensington, Maryland, USA. Sponsor: Ciba-Geigy Corp., Agricultural Products Division, Greensboro, North Carolina, USA. Study duration: 11 May - 7 Jun, 1990. Report date: 25 Jun, 1990. (US GLP statement provided)

Technical diazinon (Ciba-Geigy batch 790701-ML5755; purity 88.0%) in DMSO at concentrations of 0.668, 2.00, 6.68, 20.0 µg/mL (or 66.8 µg/mL with metabolic activation) did not increase the frequency of sister-chromatid exchanges in phytohaemagglutinin-stimulated human lymphocytes in the absence or presence of S9 metabolic activation. Concentrations tested were chosen on the basis of cytotoxicity (incl. prolonged cell division) and precipitate formation (0.0668 to 5170 µg/mL). The highest usable concentration in the absence or presence of metabolic activation was 20 and 66.8 µg/mL respectively. Negative (culture medium only), solvent (DMSO) and positive controls with and without S9 activation respectively (ie. cyclophosphamide, 2.5 µg/mL and mitomycin C, 0.02 µg/mL respectively) gave the expected results.

Hool G & Müller D (1981a) Sister chromatid exchange study - Chinese hamster (Test for mutagenic effects on bone marrow cells). Study no. 801504. Lab: Ciba-Geigy Ltd, Genetic Toxicology Laboratories, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Protection of Health and Environment, Basle, Switzerland. Study duration: not stated. Report date: 13 Oct, 1981. (Pre-GLP)

Technical diazinon (Ciba-Geigy batch EN 30554; purity 96.1%) in PEG 400 administered by oral gavage at 0, 6.5, 13, or 26 mg/kg bw to 5-bromodeoxyuridine-treated Chinese hamsters (4/sex/dose; Tierfarm, Sisseln, Switzerland) did not increase the frequency of chromatid exchange in bone marrow cells isolated 24 h after dosing. Dose selection was based on the results of an acute median oral lethality study in which the combined sex LD₅₀ was 76 mg/kg bw. Marrow cells harvested from each group (2/sex except for the vehicle control, 1 female and 3 males) had similar SCE frequencies (ie. 3.89, 3.70, 4.41, or 4.06/cell for the 0, 6.5, 13 and 26 mg/kg bw groups respectively) except for positive controls (7,12-dimethylbenz(a)anthracene, 100 mg/kg bw in sodium carboxymethylcellulose) which had the expected significant (p<0.01) increase (10.13/cell).

Murli H (1990b) Mutagenicity test on diazinon MG8: In vitro sister chromatid exchange assay. Study no. HLA 12226-0-458. Lab: Hazleton Laboratories America Inc., Kensington, Maryland, USA. Sponsor: Ciba-Geigy Corp., Agricultural Products Division, Greensboro, North Carolina, USA. Study duration: 18 - 19 Jun, 1990. Report date: 10 Oct, 1990. (US GLP statement provided)

Technical diazinon (Ciba-Geigy batch 790701-ML5755; purity 88.0%) in PEG 400 administered by oral gavage at 0, 10, 50, or 100 mg/kg bw to 5-bromodeoxyuridine-treated ICR mice (5/sex/dose; Harlan Sprague-Dawley, Frederick, Maryland, USA) did not increase the frequency of SCE in bone marrow cells isolated 24 h after dosing. Dose selection was based on results from dose-ranging studies and the maximum dose used (100 mg/kg bw) caused systemic toxicity (listlessness) in males. Marrow cells harvested from males at 100 mg/kg bw had significantly (p<0.05) prolonged cell division cycles (average generation time, 18.48 h compared with 13.18 h for vehicle controls). SCE frequencies for vehicle controls (M, 3.22/cell; F, 4.70/cell) were within the mean historical control range (M, 2.77-3.82/cell; F, 3.1- 4.73/cell) and positive controls (cyclophosphamide, 10 mg/kg bw, IP) gave the expected increase (M, 12.35/cell; F, 16.74/cell), while for treated groups none were significantly different from controls (M, 3.12-3.69/cell; F, 4.54-5.00/cell).

Murli H (1993) Mutagenicity test on diazinon MG87%: In vitro sister chromatid exchange assay in female mice. Study no. HWA 15802-0-458. Lab: Hazleton Washington Inc., Vienna, Virginia, USA. Sponsor: Ciba-Geigy Corp., Crop Protection Division, Greensboro, North

Carolina, USA. Study duration: 25 Aug - 14 Oct, 1993. Report date: 10 Nov, 1993. (US GLP statement provided)

Technical diazinon (Ciba-Geigy batch 790701-ML5755; purity 88.0%) in PEG 400 administered by oral gavage at 0, 150, 160, or 175 mg/kg bw to 5-bromodeoxyuridine-treated female CD-1 (ICR) mice (5/group; Charles River Laboratories, Raleigh, Virginia, USA) did not increase the frequency of sister-chromatid exchange in bone marrow cells isolated 30 h after dosing. Although a similar study (HLA 12226-0-458) had been performed 3 years earlier (see Murli, 1990b), the maximum dose selected (100 mg/kg bw) was based on systemic toxicity (listlessness) observed in male mice. Two dose-ranging studies were performed in this study to ascertain the maximum tolerated dose in female mice. At the maximum selected dose of 175 mg/kg bw, 1 died and the other 4 were prostrate with a foamy discharge from the eyes. Marrow cells harvested from mice at all doses had significantly ($p < 0.05$) prolonged cell division cycles, ie. 17.15, 23.88 and 20.39 h respectively relative to the positive (12.10 h) and vehicle control (12.24 h) groups. SCE frequencies for the vehicle (PEG 400) and positive (cyclophosphamide, 10 mg/kg bw, IP) controls were 3.79/cell and 15.9/cell respectively whereas treated groups ranged between 4.24 to 6.35/cell and sufficient cells in metaphase were obtained from only 3/5 mice at 160 mg/kg bw and 2/5 at 175 mg/kg bw because of cytotoxicity.

Hool G & Müller D (1981b) Chromosome studies in male germinal epithelium (Test for mutagenic effects on spermatogonia). Study no. 801501. Lab: Ciba-Geigy Ltd, Genetic Toxicology Laboratories, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Protection of Health and Environment, Basle, Switzerland. Study duration: not stated. Report date: 6 Nov, 1981. (Pre-GLP)

In a mammalian cytogenetics test in male mice, germinal epithelia were examined for chromosomal aberrations following diazinon administration. Technical diazinon (Ciba-Geigy batch EN 30554; purity 96.1%) in PEG 400 at 0, 21, or 63 mg/kg/day was administered by oral gavage to NMRI-derived male mice (15/group except for 12 in the control group) for 5 days. Dose selection to achieve the maximum tolerated dose was based on the results of an acute oral median lethality study in which the combined sex LD_{50} was 187 mg/kg bw (although all deaths contributing to this calculation were among males, cf. Bathe, 1972a; Section 3.1). An excessive death rate, ie. 7/15 at 21 mg/kg bw/day and 15/15 at 63 mg/kg bw/day, necessitated a second study that omitted the highest dose and included a lower dose of 10.5 mg/kg bw/day. Mice were killed 24 h after the last dose and 3 h after an IP injection of colchicine (10 mg/kg bw). Drop preparations of testicular parenchyma from the testes of 10/group and 9 in the control group were prepared and a 100 metaphase figures from each mouse were examined from 8/group for chromatid aberrations, chromosomal aberrations, chromatid gaps and chromosomal pulverisation. No specific chromosome abnormalities were observed at 10.5 or 21 mg/kg bw/day.

Hool G & Müller D (1981c) Chromosome studies in male germinal epithelium (Test for mutagenic effects on spermatocytes). Study no. 801502. Lab: Ciba-Geigy Ltd, Genetic Toxicology Laboratories, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Protection of Health and Environment, Basle, Switzerland. Study duration: not stated. Report date: 20 Oct, 1981. (Pre-GLP)

In a related mammalian cytogenetics test in male mice (see Hool & Müller, 1981b), spermatocytes in germinal epithelia were examined for chromosomal aberrations following diazinon administration. Technical diazinon (Ciba-Geigy batch EN 30554; purity 96.1%) in PEG 400 at 0, 21, or 63 mg/kg/day was administered by oral gavage to NMRI-derived male mice

(15/group except for 12 in the control group) for 4 days (day 0, 2, 3, 5). Dose selection to achieve the maximum tolerated dose was based on the results of an acute oral median lethality study in which the combined sex LD₅₀ was 187 mg/kg bw (although all deaths contributing to this calculation were among males, cf. Bathe, 1972a; Section 3.1). An excessive death rate, ie. 4/15 at 21 mg/kg bw/day and 15/15 at 63 mg/kg bw/day, necessitated a second study that omitted the highest dose and included a lower dose of 10.5 mg/kg bw/day. In the second study, treatment at 10.5 (15/group) and 21 (21/group) mg/kg bw/day was administered intermittently on days 0, 2, 3, 5 and 9, and sacrifice was delayed until day 12, 3 h after an IP injection of colchicine (10 mg/kg bw). Only 1 mouse, at 10.5 mg/kg bw/day, died after the third administration in the second study. Drop preparations of testicular parenchyma from the testes of 10/group and 12 in the control group were prepared and a 100 primary and secondary spermatocytes in metaphase (I and II) from each mouse were examined from 8/group for breaks, fragments, chromosome exchanges (in I), atypical aberrations. Although a fragment was observed in a primary spermatocyte in a mouse from the control group, and another in a secondary spermatocyte from a mouse in the control and 10.5 mg/kg bw groups respectively, no chromosomal abnormalities could be attributed to treatment at 10.5 or 21 mg/kg bw/day.

Fritz H (1975) Dominant lethal study on G 24480 (diazinon techn.) - Mouse. Study no. 327507. Lab: Ciba-Geigy Ltd, Pharmaceuticals Division, Toxicology/Pathology Laboratories, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Protection of Health and Environment, Basle, Switzerland. Study duration: not stated. Report date: 20 Mar, 1975. (Pre-GLP)

Technical diazinon (Ciba-Geigy batch no. 810; purity not stated) in carboxymethylcellulose was administered once by oral gavage to mature male NMRI-derived albino mice (12/group) at 0, 15, or 45 mg/kg bw. Dose selection to achieve the maximum tolerated dose was based on the results of an acute oral median lethality study in which the combined sex LD₅₀ was 187 mg/kg bw (although all deaths contributing to this calculation were among males, cf. Bathe, 1972a; Section 3.1). Each treated male was allowed to mate with different groups of 3 untreated females at weekly intervals for 6 weeks. Females were examined daily for the presence of a vaginal plug and its presence was deemed to be day 0 of gestation.

Treated males observed daily during the first week after dosing showed signs of somnolence and dyspnea at 45 mg/kg bw. Bodyweight gain in males, measured before and a week after treatment, was similar in all groups. There were no treatment-related effects observed on the pregnancy rate, number of live and dead fetuses or the number of resorptions in pregnant females sacrificed on day 14 of gestation.

Hool G, Langauer M & Müller D (1981) Nucleus anomaly test on somatic interphase nuclei - Chinese hamster (Test for mutagenic effects on bone marrow cells). Study no. 801503. Lab: Ciba-Geigy Ltd, Genetic Toxicology Laboratories, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Protection of Health and Environment, Basle, Switzerland. Study duration: not stated. Report date: 5 Nov, 1981. (Pre-GLP)

Chinese hamsters (6/sex/group; Tierfarm, Sisseln, Switzerland) received a daily oral gavage dose of PEG 400 or technical diazinon (Ciba-Geigy batch, EN 30554; purity 96.1%) in PEG 400 at 0, 6.5, 13, or 26 mg/kg bw on 2 consecutive days. Dose selection was based on the results of an acute oral gavage lethality study in which the combined sex LD₅₀ was 76 mg/kg bw. Six hamsters (3/sex) from each treatment group were killed for preparation of bone marrow smears 24 h after the second dose. A positive control group of hamsters (6/sex) was dosed similarly with cyclophosphamide (128 mg/kg bw/day) in PEG 400 and sacrificed 24 h after the second dose.

One control female, and a male and female at 26 mg/kg bw died after the second treatment. However, there were no significant increases in the frequency of cells with nuclei anomalies, ie. single 'Jolly' bodies (Howell-Jolly bodies), micronucleated erythroblasts, micronucleated leucopoietic cells or polyploid cells. Marrow cells from cyclophosphamide-treated hamsters had the expected significant increase in the frequency of these nuclear anomalies.

Ceresa C, Langauer M & Puri E (1988) Micronucleus test, mouse (OECD conform). Study no. 871696. Lab: Ciba-Geigy Ltd, Genetic Toxicology Laboratories, Laboratories of Residue Analysis Unit, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Agricultural Division, Basle, Switzerland. Study duration: 25 Jan - 9 Apr, 1988. Report date: 24 May, 1988. (US GLP compliant, OECD guideline 474)

In the preliminary experiment, Tif:MAGF mice (24/sex/group; Ciba-Geigy Tierfarm, Sisseln, Switzerland) received a single oral gavage dose of water (vehicle) or technical diazinon (Ciba-Geigy batch FL 872049; purity 87.5%; 120 mg/kg bw). Dose selection was based on the results of acute oral gavage toxicity studies (2/sex/dose), which indicated that 2 of 4 mice died at 200 mg/kg bw and none at 120 mg/kg bw. Eight mice from each treatment group were killed for preparation of bone marrow smears 16, 24, or 48 h after dosing. A positive control group of mice (8/sex) was dosed similarly with cyclophosphamide (64 mg/kg bw) and sacrificed 24 h later. The protocol for the definitive study was similar, except that two additional doses (30 and 60 mg/kg bw) were included and all the mice (8/sex/group) were killed at the same time (24 h after dosing). In neither experiment were there any deaths, significant increases in the frequency of micronucleated polychromatic erythrocytes, or changes in the ratio of polychromatic to normochromatic cells.

9.3 Other Genotoxic Effects.3 Effects.3 Other Genotoxic Effects.3 Effects

Other Genotoxic Effects.3 Other Genotoxic Other Genotoxic Effects.3 Other Genotoxic

Hertner T & Arni P (1990) Autoradiographic DNA repair test on rat hepatocytes (OECD conform). Study no. 891345. Lab: Ciba-Geigy Ltd, Genetic Toxicology Laboratories, Laboratories of Residue Analysis Unit, Basle, Switzerland. Sponsor: Ciba-Geigy Ltd, Agricultural Division, Basle, Switzerland. Study duration: 10 Aug - 14 Dec, 1989. Report date: 6 Feb, 1990. (OECD, EEC, Japan and US GLP compliant)

Technical diazinon (Ciba-Geigy batch FL 880045; purity 88.0%) was negative in an *in vitro* unscheduled DNA repair test in primary hepatocytes freshly isolated from male Tif:RAIf (SPF) rats (Tierfarm, Sisseln, Switzerland) over a concentration range of 1.1 to 120 µg/mL (preliminary and duplicate tests; 4 cultures per group in each independent test). Concentrations tested were chosen on the basis of a preliminary cytotoxicity test (10.2 to 1044 µg/mL) in which 120 µg/mL was shown to be the highest usable concentration. Negative (DMSO solvent) and positive controls (2-acetylaminofluorene at 45 µM) gave the expected results.

10. SPECIAL STUDIES

10.1 Neurotoxicity Studies

10.1.1 Hen

Krinke G, Ullmann L & Sachsse K (1973) Acute oral LD₅₀ and neurotoxicity study of technical diazinon in the domestic fowl (*Gallus domesticus*). Report no. PH 2.635. Lab: Ciba-Geigy Corp., Toxicology/Pathology, Stein, Switzerland. Sponsor: Ciba-Geigy Corp., Basle, Switzerland. Study duration: not stated. Report date: 12 Nov, 1973. (Pre-GLP)

Diazinon (Ciba-Geigy Corp.; purity and batch no. not given) in polyethylene glycol was administered orally by gavage to White Leghorn fowls (2/sex/group; source was not reported) at 1, 2.15, 3.59, or 10 mg/kg bw on two occasions, 21 days apart. Doses selected for investigation were based on a preliminary study where the median lethal dose was calculated to be 3.6 mg/kg bw. Although not implicitly stated, the high mortality observed after the first dose, ie. 3 of 4 dead at 3.59 and 10 mg/kg bw (not surprising given the doses were equal or in excess of the LD₅₀), appears to have necessitated the use of an antidote (10 mg/kg bw atropine, IM, 30-60 min before treatment) prior to the second treatment. For the positive control, tri-orthocresyl phosphate (1000 or 2150 mg/kg bw) was administered once by oral gavage to a group with 2/sex. Signs of delayed neurotoxicity (eg. leg paralysis) were monitored daily in all treatment groups for a total of 6 weeks. Surviving fowls at 2.15 mg/kg bw (1 M and 2 F) and 3.59 mg/kg bw (1 F) were killed at the end of the observation period and their right ischiadicus, fibularis and tibularis nerves and samples of their spinal cord (cervical, thoracic and lumbar) removed and processed for histopathology under light microscopy. No histopathology was performed on the 4 surviving fowls at 1 mg/kg bw.

There were no delayed neurotoxic symptoms observed and the histopathological examination did not reveal any nerve degeneration in diazinon-treated fowls at 2.15 or 3.59 mg/kg bw but was apparent in all positive control birds (though none of these results were reported in any detail).

Jenkins LJ & Jones LP (1988) Acute delayed neurotoxicity of diazinon MG-8 in domestic fowl. Report no. 5152-87. Lab: Stillmeadow Inc., Houston, Texas, USA. Sponsor: Ciba-Geigy Corp., Agricultural Division, Greensboro, North Carolina, USA. Study duration: 19 Jan – 1 Mar, 1988. Report date: 23 Apr, 1988. (US GLP statement provided)

A preliminary range-finding study performed in 5 hens in order to establish appropriate doses to ascertain the median lethal oral dose of technical diazinon (Ciba-Geigy Corp.; purity 87%; Lot no. FL 872049) in 'Production Red Heavy Breed' hens (Texas Animal Specialities Humble, Texas, USA) indicated that doses of 5, 10, 12.5, or 15 mg/kg bw were appropriate. Using these doses and 4 hens/group, the LD₅₀ of diazinon in corn oil was calculated to be 12.49 mg/kg bw. Protection from cholinergic symptoms (induced by treatment at doses in excess of the LD₅₀ in the definitive study) was afforded when the hens were pretreated with atropine (10 mg/kg bw, IM) 1 h before dosing and with 2-PAM (50 mg/kg bw, IM) concurrently at the time of diazinon treatment. Additional protection was gained by administering atropine and 2-PAM (in combination) approximately 1 and 5 h after diazinon treatment.

In the definitive study, diazinon (18/group) or tri-orthocresyl phosphate (positive control; 8/group) at 13.78 and 500 mg/kg bw respectively was administered to hens by gastric intubation. A negative control group of 10 hens was treated with vehicle only (corn oil) at 1 mL/kg bw. However, owing to a preparation error, the actual diazinon dose administered in the first treatment was approximately twice the target dose, ie. 28.09 mg/kg bw (determined by GC). All hens (incl. controls) received the atropine/2-PAM therapy before and after treatment and 2 hens

in the diazinon-treated group required 1 or 2 additional antidote treatments respectively on the second day after treatment because of persistent cholinergic signs. Signs of delayed neurotoxicity (ie. gait and locomotor changes) were monitored 3 times weekly starting 3 days after treatment. Since there were no observed changes in locomotion for the diazinon-treated hens after 21 days of observation, treatment (as for day 0) was repeated. The second treatment (with the lower dose) required no additional atropine/2-PAM therapy after the second dose (ie. after 5 h).

After the second 3-week observation period, segments from the upper cervical bulb, mid-thoracic and lumbo-sacral regions of the spinal cord, the medulla oblongata of the brain, the sciatic nerve (distal 2 cm above the branch to the peroneal and tibial nerves) and the left tibial nerve (distal 1.5 cm) were taken at necropsy from all hens except for those that died following treatment (ie. 1 vehicle control hen after the first treatment and 1 diazinon-treated hen after the first and second treatments respectively). These tissues were then fixed and processed for light microscopy.

Reduced activity that persisted for 2-4 days after the first treatment was observed in 8/17 diazinon-treated, 5/8 positive control and 2/9 vehicle control hens. Ataxia occurred in 5/8 positive control hens and in 3/17 hens from the diazinon group. After 2 weeks, hens in the positive control group had reduced activity accompanied by progressive ataxia and bodyweight loss (in association with inappetence). Body weight or food consumption for diazinon-treated hens were not significantly different to vehicle controls. Similarly, no delayed ataxia or histopathological lesions were observed, whereas the positive controls had the anticipated axonal swelling and degeneration in both the peripheral and central nerves.

Classen W (1996) Delayed neurotoxicity in hens following acute exposure. Report no. 952030. Lab: Ciba-Geigy Corp., Short/Long Term Toxicology, Stein, Switzerland. Sponsor: Ciba-Geigy Corp., Crop Protection Division, Basle, Switzerland. Study duration: 12 Sep - 11 Oct, 1995. Report date: 29 Apr, 1996. (OECD, Japan and US GLP compliant)

To increase the likelihood of detecting a potential for diazinon to cause organophosphorus induced delayed neuropathy (OPIDN), the maximum dose selected was one that was greater than the median lethal dose. To reduce the incidence of death at this normally lethal dose, the diazinon-induced cholinergic stimulation (leading to death) was antagonised by IM administration of atropine (20 mg/kg bw) or with atropine in combination with physostigmine (0.15 mg/kg bw). Atropine therapy was administered 2, 4, 8, 10, 14, 16, 20, 23, 27, 30, 33, 38, and 48 h after diazinon administration whereas for the combination therapy it was 0, 6, 12 and 18 h after dosing. The median lethal oral dose for LSL-Lohmann hens (Animalco AG., Stetten, Switzerland) was calculated in a preliminary study to be 50 mg/kg bw. Only slight cholinergic signs (ie. reduced activity, diarrhoea, mild dyspnea, ataxia, abnormal posture, recumbency) were observed for 4 h after a dose of 10 or 15 mg/kg bw.

In the definitive study, diazinon (Ciba-Geigy Corp.; purity 96.3%; Lot no. MC305130) in peanut oil was administered by oral gavage to hens (6/group except 10/group at the highest dose) at 10, 30, or 100 mg/kg bw. Together with a positive control group that was dosed with tri-orthocresyl phosphate (500 mg/kg bw) by the same route and negative controls treated with and without the antidote therapies, signs of delayed neurotoxicity (ie. postural and gait abnormalities) and any other clinical signs were monitored daily for 21 days. An additional assessment to monitor the degree of ataxia, which involved forced ladder climbing, was performed at 3-day intervals.

After the 3-week observation period, tissues (cerebellum, medulla oblongata, cervical, thoracic and lumbar segments of the spinal cord, and, sciatic and tibial nerves) were taken from *in situ*

formalin perfused hens following deep pentobarbitone-induced anaesthesia and examined for macroscopic changes. These tissues were then processed for light and electron microscopy. The degree of ChE inhibition in plasma, erythrocytes, brain and spinal cord was determined (by colorimetric assay) in satellite groups, 24 and 48 h after dosing. Neurotoxic esterase activity in the brain and spinal cord was also measured.

Although 2 hens (2/10) died 48-72 h after treatment at 100 mg/kg bw, another at 30 mg/kg bw that died unexpectedly a few hours after dosing was replaced. None of the dead hens were necrosed because any delayed neurotoxicity generally takes 10 days to develop. All hens in all groups had reduced activity for 24 h and ataxia was only observed in the positive control group (6/6) for 4 days whereas impaired gait was observed in most diazinon-treated hens (18/19). The mean time to onset of the impaired gait did not appear to be related to dose, ie. 1, 1.8 and 1.1 days at 10, 30 and 100 mg/kg bw respectively, however, the duration did appear to be related to dose, ie. 1, 1.3 and 5.1 days, respectively. Diarrhoea was observed in most hens at 30 and 100 mg/kg bw (5/6 and 6/6 respectively) with the duration being related to dose, ie. 1.4 and 5 days respectively, whereas recumbency was observed only in all surviving hens (7/7) at 100 mg/kg bw. Increased ataxia scores (from ladder climbing) were only found among hens in the positive control group from day 18 onwards and bodyweight loss occurred in the 100 mg/kg bw diazinon group where the group mean was significantly reduced on days 7, 10, 14 and 21 (all by 11-12%) after dosing. As shown in the Table below, ChE inhibition in plasma was almost complete at all tested doses after 24 and 48 h whereas marked inhibition of ChE in the spinal cord and brain over the same duration was only observed at 30 and 100 mg/kg bw. Very little inhibition of ChE activity in erythrocytes was observed at any dose after either 24 or 48 h. As anticipated there was a substantial reduction in the neurotoxic esterase activity in the brain and spinal cord at 24 and 48 h for the tri-orthocresyl phosphate-treated group (positive control), however, none was observed among any of the diazinon-treated groups.

ChE Inhibition (mean percentage reduction)

Dose (mg/kg bw)	Plasma		Erythrocyte		Brain		Spinal Cord	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
10	93	97	4	[1]	9	[3]	[2]	1
30	90	79	3	[2]	64	29	62	20
100	88	97	15	[3]	83	66	81	59

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls;

* p≤0.05; ** p≤0.01

As anticipated, the positive control hens had moderate to marked neuropathy with degenerative lesions being apparent throughout the entire nervous system except for thoracic spinal cord and the sciatic nerve. No histopathological lesions were observed for any of the diazinon-treated groups.

10.1.2 Rat

Chow E & Richter AG (1994) Acute neurotoxicity study with D-Z-N® Diazinon MG87% in rats. Report no. F-00175. Lab: Plant Protection Division, Environmental Health Center, Farmington, Connecticut, USA. Sponsor: Ciba-Geigy Corp., Plant Protection Division, Greensboro, North Carolina, USA. Study duration: 14 - 18 Feb, 1993. Report date: 20 Jan, 1994. (US GLP statement provided)

To investigate neurotoxicity after a single dose, technical diazinon (Ciba-Geigy Corp.; purity 88%; Lot no. FL-880045) in corn oil was administered to groups of 12-hour fasted Sprague-Dawley rats (Hsd:Sprague Dawley® SD™; Harlan Sprague Dawley, Frederick, MD, USA; 15/sex/group) by gavage at 0, 2.5, 150, 300, or 600 mg/kg bw. Dose selection was based on the results of 3 median lethal dose studies (Study nos. 7679-90, 7680-90 and F-00174) that revealed the lowest lethal dose was 750 mg/kg bw and the highest non-lethal dose was 500 mg/kg bw. Hence, at the highest selected dose of 600 mg/kg bw, significant neurotoxic effects were anticipated. The selected mid-high and mid-low doses (150 and 300 mg/kg bw) were reducing multiples of the highest dose and so were anticipated to give intermediate or minimal effects whereas at the lowest dose of 2.5 mg/kg bw a previous investigation (Study no. HWI 6117-221) had indicated that no reduction in ChE activity was evident. Triadimefon (150 mg/kg bw), a fungicide, in corn oil was administered to another group of rats (10/sex) as a positive control.

Clinical monitoring was performed twice daily and food consumption and bodyweight were recorded before treatment and at weekly intervals for 2 weeks. Five rats in each group, not scheduled for the functional observation battery (FOB) tests, were used exclusively as the source of blood to assess the extent of ChE inhibition (by colorimetric assay) at the estimated time of maximal effect on day 1 (ie. approximately 9-11 h after dosing) and on day 15 after dosing.

Neurological tests, namely FOB and figure-8 maze motor activity, were performed in the presence of 'white noise' on 10 rats in each group 1 week before treatment, at the estimated time of maximal ChE inhibition on day 1 (ie. approximately 9-11 h after dosing), and on days 8 and 15 after treatment. The FOB tests comprised home cage observations, manipulative measurements, open field and reflex responses, neuromuscular tests and physiological functions. These tests examined the following parameters;

Home cage observations; posture, tremors, convulsions, stereotypy, bizarre behaviour, faecal colour and composition, and gait.

Manipulative measurements; ease of removal from cage, respiration character, position of hindlimbs when held by tail, pupillary size, lacrimation, staining, eye prominence, palpebral closure, piloerection, fur appearance, salivation, vocalization, and ease of handling.

Open field tests; arousal, circling, convulsions, gait, stereotypy, tremors, number of defecations, numbers of rears, number of urine pools, bizzare behaviour and head position.

Neuromuscular tests; fore and hindlimb grip strength, hindlimb foot splay and hindlimb extensor strength.

Physiological measurements; body tone, rectal temperature and muscle tone.

After each FOB test series, 6 rats in each group were individually tested in a figure-8 maze for spontaneous activity (measured by light-beam interruption). After the day 15 FOB test, 5 rats/group were anaesthetised, then sacrificed by whole-body perfusion (with glutaraldehyde) and a gross necropsy performed. The only tissues removed for histopathological examination were brain, spinal cord with ganglia (at each level, ie. cervical, thoracic, lumbar and sacral), peripheral nerves (left and right sciatic, fibular, tibial, lateral cutaneous sural), Gasserian ganglion, eyes with associated optic nerves, skeletal muscle and any other gross lesions detected.

Two males and a female died on day 2, 4 and 5 respectively after treatment at the highest dose of 600 mg/kg bw. Although these deaths were attributed to treatment, a male in the ChE satellite group at 300 mg/kg bw that died after a blood collection was deemed to be accidental. Similarly, a control group male that displayed clinical signs consistent with OP intoxication was assumed to have been misdosed and removed from the study. ChE activities in plasma and erythrocytes that were 6-7 standard deviations from the mean appeared to confirm the assertion.

Clinical signs that appeared to be treatment-related were reduced activity (3/15 and 13/15 males at 300 and 600 mg/kg bw respectively, and 9/15 females at 600 mg/kg bw), tremors (1/15 and 3/15 males at 300 and 600 mg/kg bw respectively, and 4/15 females at 600 mg/kg bw), chromodacryorrhoea (2/15 and 5/15 males at 300 and 600 mg/kg bw respectively, and 4/15 females at 600 mg/kg bw) and diarrhoea (2/15 males at 600 mg/kg bw). Reduced bodyweight gain that was significant ($p \leq 0.01$) for males at 300 and 600 mg/kg bw (by 25 % and 30% respectively) at week 1 was accompanied by a corresponding significant ($p \leq 0.01$) reduction in food consumption (ie. by 17% and 16% respectively). A reduced food consumption that achieved significance ($p \leq 0.05$) at 150 and 600 mg/kg bw (8% and 10% respectively) but not (quite) at 300 mg/kg bw (8%) did not cause a significant reduction in bodyweight gain in females at any dose.

Clear treatment-related FOB findings were observed at the time of maximal ChE inhibition (ie. approximately 9-11 h after dosing) and at doses ≥ 150 mg/kg bw, but not at any dose on day 8 or 15. In addition to the incidence of FOB effects shown in the Table below, males and females at 600 mg/kg bw had reduced forelimb grip strength (by 24% and 27% respectively); males had reduced hindlimb foot splay at 300 and 600 mg/kg bw (by 19% and 22% respectively); and females at 600 mg/kg bw had reduced hindlimb grip strength (by 27%). Males at 600 mg/kg bw and females at 300 and 600 mg/kg bw had impaired arousal and were easier to handle. Tail pinch response at 600 mg/kg bw and rectal temperature at 300 and 600 mg/kg bw (by 3.4% and 11% respectively in males and 4.7% and 12% respectively in females) were also reduced in both sexes. Maze activity among males at doses ≥ 300 mg/kg bw and females at doses ≥ 150 mg/kg bw appeared to be reduced. Rats in the positive control group had the expected increase in excitability.

Incidence of FOB Effects (9 h after dosing)

	Male			Female		
	Dose (mg/kg bw)					
	150	300	600	150	300	600
No. tested	10	10	10	10	10	10
<i>Autonomic Effects</i>						
Impaired respiration	0	0	10	0	0	6
Altered faecal consistency	3	9	10	0	3	3
Lacrimation	0	0	9	0	0	5
Salivation	0	2	5	0	0	0
Soiled fur	3	9	9	0	3	2
Nose stain	0	6	4	0	3	9
Mouth stain	0	0	3	0	5	5
Involuntary mouth opening (open field)	0	5	5	3	9	10

Involuntary mouth opening (home cage)	0	0	0	1	4	0
<i>Neuromuscular Effects</i>						
Ataxic gait	0	9	9	3	10	10
Abnormal gait	2	9	10	7	7	10
Impaired righting reflex	0	2	8	0	2	9
Impaired hindlimb extensor reflex	0	3	10	0	0	6
<i>CNS Excitability Effects</i>						
Tremors (home cage)	0	1	3	0	0	1
Tremors (open field)	0	6	10	0	5	10
Muscle fasciculations (open field)	0	5	10	0	0	3

As shown in the Table below, ChE activities in plasma and erythrocytes at the time of maximal inhibition (ie. approximately 9-11 h after dosing) on day 1 were significantly reduced ($p < 0.01$) at doses ≥ 150 mg/kg bw in both sexes. However, the significant ChE inhibition observed in the plasma of males at the lowest tested dose of 2.5 mg/kg bw was unexpected.

ChE Inhibition‡ (mean percentage reduction)

Dose (mg/kg bw)	Plasma ChE		Erythrocyte ChE	
	male	female	male	female
2.5	27**	53**	7	4
150	76**	84**	82**	76**
300	76**	85**	83**	77**
600	78**	84**	85**	76**

‡ 9-11 h after dosing; ** $p \leq 0.01$.

A small uterine mass in a female at 300 mg/kg bw, which was considered to be incidental to treatment since it did not involve the nervous system, was the only finding observed during macroscopic examination. This lesion was not examined microscopically.

In summary, diazinon administered to rats once by gavage at 2.5, 150, 300, or 600 mg/kg bw caused reduced activity, tremors, chromodacryorrhoea and diarrhoea 9-12 h after dosing together with reduced food consumption and bodyweight gain for a week at doses ≥ 300 mg/kg bw. FOB tests performed on day 1, 8 and 15 revealed significant effects only on day 1, when the acute OP poisoning effects (clinical signs) were maximal. No histopathology related to treatment was observed, however, significant plasma ChE inhibition was observed 9-11 h after dosing in both sexes at the the lowest tested dose of 2.5 mg/kg bw.

Pettersen JC & Morrissey RL (1994) 90-Day subchronic neurotoxicity study with D-Z-N® Diazinon MG87% in rats. Report no. F-00176. Lab: Ciba-Geigy Corp., Crop Protection Division, Environmental Health Center, Farmington, Connecticut, USA. Sponsor: Ciba-Geigy Corp., Crop Protection Division, Greensboro, North Carolina, USA. Study duration: 22 Mar - 25 Jun, 1993. Report date: 26 Aug, 1994. (US GLP statement provided)

Diazinon was fed to Sprague-Dawley Crl:CD®(SD)BR rats (15/sex/group; Charles River, Raleigh, NC, USA) in the diet at concentrations of 0, 0.3, 30, 300, or 3000 ppm for 13 weeks (equal to 0.017, 1.7, 17, or 177 mg/kg bw/day in males and 0.019, 1.9, 19, or 196 mg/kg bw/day in females). Basal diets admixed with technical diazinon (Ciba-Geigy Corp.; purity 88%; Lot no. FL-880045) were prepared monthly and stored at 4°C. Diets not used within 35 days were discarded and 8/13 prepared blends that were tested were found to be suitable with respect to homogeneity, stability and concentration ($SD \pm 4.2\%$ of targets). Doses selected were based on the results of a 3-month (Singh et al, 1988, see section 5.1.3) and a 1-2 year study (Kirchner et al, 1991, see section 6.2), so that it was anticipated that there would be no deaths at the highest dose and no inhibition of ChE activities at the lowest. Food consumption and bodyweight were measured weekly during treatment, and clinical monitoring was performed twice daily. All rats were palpated weekly (though the justification for this in a 3-month study was not given) and ophthalmoscopy was performed prior to and at the completion of treatment (though not with the satellite group). Five rats in each group, not scheduled for the functional observation battery (FOB) tests, were used exclusively as the source of blood to assess the extent of ChE inhibition (by colorimetric assay) during weeks 4, 8, and 13. Regional brain ChE activity (ie. cerebellum, cerebral cortex plus hippocampus, and striatum) in these 5 rats was then measured at week 13. Neurological tests, namely FOB and figure-8 maze motor activity, were performed in the presence of 'white noise' on 10 rats in each group 1 week before and then again on the 4th, 8th and 13th week of treatment. The FOB tests comprised home cage observations, manipulative measurements, open field and reflex responses, neuromuscular tests and physiological functions. These tests examined the following parameters;

Home cage observations; posture, tremors, convulsions, stereotypy, bizarre behaviour, faecal colour and composition, and gait.

Manipulative measurements; ease of removal from cage, respiration character, position of hindlimbs when held by tail, pupillary size, lacrimation, staining, eye prominence, palpebral closure, piloerection, fur appearance, salivation, vocalization, and ease of handling.

Open field tests; arousal, circling, convulsions, gait, stereotypy, tremors, number of defecations, numbers of rears, number of urine pools, bizzare behaviour and head position.

Neuromuscular tests; fore and hindlimb grip strength, hindlimb foot splay and hindlimb extensor strength.

Physiological measurements; body tone, rectal temperature and muscle tone.

After each FOB test series, rats in each group were individually tested in a figure-8 maze for spontaneous activity (measured by light-beam interruption). After 13-weeks of treatment the FOB-tested rats were anaesthetised, then sacrificed by whole-body perfusion (with glutaraldehyde) and a gross necropsy performed. The only tissues removed for histopathological examination were brain, spinal cord with ganglia (at each level, ie. cervical, thoracic, lumbar and sacral), peripheral nerves (left and right sciatic, fibular, tibial, lateral cutaneous sural), Gasserian ganglion, eyes with associated optic nerves, skeletal muscle and any other gross lesions detected. Since a preliminary histopathological assessment of sections from the control and 3000 ppm rats revealed no significant differences other than for some lesions in the nerve roots of the sacral spinal cord, this tissue was the only one examined in detail from rats at 0.3 and 300 ppm.

All rats survived treatment, though clinical signs consistent with OP toxicity were observed in males (ie. hypersensitivity to touch and sound) and females (ie. muscle fasciculations and tremors) at 3000 ppm. At this concentration bodyweight gain was significantly ($p<0.01$) reduced for the first 6 weeks in males and 12 weeks in females. This reduced weight gain, associated with a reduction in food consumption that achieved significance ($p<0.01$) during weeks 1 and 2 in males (av. 15%) and weeks 1, 2 and 4 (av. 14%) in females, resulted in a generally reduced bodyweight throughout treatment but which only achieved significance during weeks 1 to 6 (av. 11%) in males and weeks 1-9 (av. 11%) and 11 in females. Significant changes in bodyweight and food consumption that occurred at other concentrations were probably unrelated to treatment because they were transient, lasting no more than a week, and had no apparent trend. There were no treatment-related ophthalmoscopic findings.

Clear treatment-related FOB test findings were observed for males and females at 3000 ppm. For males there was reduced fore and hindlimb grip strength (av. for both was 16%) throughout treatment (ie. weeks 4, 8 and 13) that did not achieve significance. However, these reductions in limb grip strength which averaged 25% among females, achieved significance ($p<0.01$) for forelimb grip strength at weeks 4, 8 and 13 and at week 4 for hindlimb grip strength ($p<0.05$). Hindlimb foot splay was also significantly reduced ($p<0.05$) by 32% at week 4 and by 26% and 23% (both not sig.) at week 8 and 13 respectively. Rectal temperature among females was significantly reduced ($p<0.05$) at week 13 and 4/10 had signs of dehydration at week 4. FOB changes observed at other concentrations were probably unrelated to treatment because they did not appear to be dose related. Similarly, maze activity among all groups appeared to be unchanged by treatment.

As shown in the Table below, ChE activities in plasma and erythrocytes were significantly reduced ($p<0.01$) as a function of dose so that, as anticipated, no inhibition was observed at the lowest tested concentration of 0.3 ppm. Relative to erythrocytes, inhibition of plasma ChE activity was generally more pronounced at 30, 300 and 3000 ppm in both sexes except for plasma ChE activity in males at 30 and 300 ppm. ChE activity in all regions of the brain was significantly ($p<0.01$) reduced at 300 and 3000 ppm in females and at 3000 ppm in males. The spurious values observed in the striatum of males at 30 and 300 ppm were attributed to an inconsistent dissection technique for this small brain region.

ChE Inhibition after 4, 8 and 13 weeks of treatment (mean percentage reduction)

Concentration (ppm)	Plasma		Erythrocyte		Cerebellum†		Cortex/hippocampus†		Striatum†	
	male	female	male	female	male	female	male	female	male	female
0.3	[5], [2], [5]	[12], [3], [5]	16, [11], 11*	19, [7], [9]	0	0	[2]	[10]	4	26
Mean	[4]	[7]	5	1						
30	37**, 39**, 45**	79**, 83**, 86**	60**, 37**, 75**	60**, 53**, 59**	10	[2]	[21]	25*	[89]	18
Mean	40	83	57	57						
300	72**, 78**, 79**	91**, 94**, 95**	71**, 86**, 84**	84**, 81**, 75**	14	55**	6	75**	[138]	74**
Mean	76	93	80	80						
3000	81**, 85**, 85**	94**, 96**, 97**	72**, 80**, 86**	85**, 88**, 79**	64**	89**	77**	92**	62**	96**
Mean	84	96	79	84						

Values in square brackets indicate the extent (%) to which the measured activity was greater than controls; † At week 13; * $p \leq 0.05$; ** $p \leq 0.01$

No treatment-related findings were observed following gross necropsy and no additional histopathological lesions apart from the mild focal degeneration of the sacral spinal cord nerve root axons in 2/10 females at 3000 ppm that were detected in the preliminary screen.

In conclusion, rats fed diazinon in the diet at 0.3, 30, 300, or 3000 ppm for 13 weeks lost bodyweight and had characteristic clinical signs of OP poisoning at the highest concentration tested. FOB studies revealed reduced fore and hindlimb grip strength throughout treatment among all rats at 3000 ppm with additional findings, ie. reduced hindlimb foot splay and rectal temperature, among females. However, the NOEL for the study was established at 0.3 ppm (equal to 0.017 mg/kg bw/day in males and 0.019 mg/kg bw/day in females) based on significant plasma and erythrocyte ChE inhibition at 30 ppm in both sexes

10.2 Porphyrin Biosynthesis Studies

Bleakley P, Nichol AW & Collins AG (1979) Diazinon and porphoria cutanea tarda. School of Applied Sciences, Riverina College of Advanced Education, Wagga Wagga, NSW, Australia. Med J Aust 1: 314-315

Prompted by the observation that chlorinated hydrocarbons can cause experimental porphoria similar to the human disease, porphoria cutanea tarda, and a published report implicating diazinon exposure as a possible cause, a research group from an inland city of Australia investigated the effect of diazinon on the porphyrin biosynthesis in rats. This published report is the first of a series.

Diazinon (purity 85%; source and batch no. not given) administered either orally in the diet (equivalent to 47 mg/kg bw/day) or topically (20 or 40 mg/rat/day, ie. approx. 114 or

228 mg/kg bw/day) to female Dark Agouti rats (6/group; source was not reported) for 12 weeks resulted in an increased concentration of porphyrin in the faeces but not in urine. Relative to an untreated control group, the porphyrin concentration in faeces was 2.4-fold greater after 8 weeks ($p < 0.025$) and 4.9-fold after 12 weeks ($p < 0.005$) of daily dermal application at 114 mg/kg bw/day. Though somewhat surprisingly, excretion at the higher topical concentration of 228 mg/kg bw/day was not significantly elevated after 8 weeks but was after 12 weeks (3.8 fold, $p < 0.005$). No significant elevation in porphyrin excretion in urine was evident after 4, 8, or 12 weeks of treatment, irrespective of dose or administration route. Electrophoresis of the pooled fecal porphyrins from treated rats revealed a number of decarboxylated intermediates in the conversion of uroporphyrinogen to protoporphyrinogen; a feature consistent with a disturbed porphyrin biosynthesis pathway and diagnostic of porphyria. However, the absence of a concomitant elevation of porphyrin concentration in urine was difficult to explain (as was the lack of any dose relationship).

Nichol AW, Elsbury S, Elder GH, Jackson AH & Nagaraja Rao KR (1982) Separation of impurities in diazinon preparations and their effect on porphyrin biosynthesis in tissue culture. Biochem Pharmacol 31: 1033-1038

Impurities present in technical diazinon (Ciba-Geigy Corp.; purity 90%, batch no. not given) were separated by HPLC and incubated with *in vitro* cultures of chicken embryo liver cells to investigate their potential to induce porphyrin accumulation. Diazinon, isodiazinon and sulfo-TEPP were found to increase the porphyrin concentration in these cultures, ie. 900, 1700 and 540 pmoles/mg/24 h respectively in the presence of insulin or 730, 1600 and 220 pmoles/mg/24 h in the absence of insulin. Solvent controls had porphyrin concentrations of 18 and 15 pmoles/mg/24 h in the presence or absence of insulin respectively. It was speculated that the presence of isodiazinon in technical diazinon preparations may be a major contributor in the disturbance of the porphyrin biosynthesis pathway observed following topical administration in rats (see Bleakley et al, 1979, this section).

Collins AG, Nichol AW & Elsbury S (1982) Porphyria cutanea tarda and agricultural pesticides. Department of Applied Sciences, Riverina College of Advanced Education, Wagga Wagga, NSW, Australia. Aust J Derm 23: 70-75

To investigate the contention that the presence of isodiazinon in technical preparations of diazinon is a major cause of the porphyrin biosynthesis disturbance, purified diazinon (from Ciba-Geigy Corp.) at 20 mg/rat/day (approx. 148 mg/kg bw/day) in xylene was topically applied (on a dorsal area) daily to female Dark Agouti rats (6/group) for 100 days. Other groups were treated similarly with isodiazinon (synthesised *in house*) at 2 mg/rat/day (or approx. 15 mg/kg bw/day), a 1:9 mixture with purified diazinon or with xylene (vehicle control). A (provided) graph revealed that the excretion of porphyrin in faeces was elevated after about 60 days and continued to rise for the duration of the treatment with the 1:9 isodiazinon mixture (no other data were shown). An activity assay of the porphyrin biosynthesis enzymes in the liver indicated that only the ferrochelatase activity was reduced by approximately half relative to the control group. However, neither purified diazinon nor isodiazinon alone caused any marked changes in activity. In view of the differing characteristics of diazinon-induced porphyrin accumulation both *in vivo* and *in vitro*, ie. the absence of a concomitant increase in urinary excretion of porphyrin and the accumulation of coproporphyrin and protoporphyrin instead of uroporphyrin, its use as model to establish a causal relationship between diazinon exposure and porphyria cutanea tarda in humans seems speculative. Given that it is generally accepted that porphyria cutanea tarda in humans is associated with an intrinsically low concentration of the enzyme uroporphyrin decarboxylase

(because of a pre-existing genetic defect), the investigators postulated an alternate hypothesis that involved an increased demand for haem for the cytochrome P450 system and some undefined inhibition of an enzyme in the porphyrin biosynthesis cascade resulting in porphyrin accumulation in the presence of a diazinon degradation product, isodiazinon. This hypothesis was tested in the next published report (Nichol et al, 1983).

Nichol AW, Elsbury S, Angel LA & Elder GH (1983) *The site of inhibition of porphyrin biosynthesis by an isomer of diazinon in rats. School of Applied Sciences, Riverina College of Advanced Education, Wagga Wagga, NSW, Australia & Department of Medical Biochemistry, Cardiff, Wales, UK. Biochem Pharmacol 32: 2653-2657*

Isodiazinon (synthesised *in house*; 2 mg/rat/day or 15 mg/kg bw/day), purified diazinon (Ciba-Geigy Corp.; 20 mg/rat/day or 148 mg/kg bw/day), a 1:9 mixture of isodiazinon in purified diazinon or xylene (as vehicle control) were separately applied each day onto a dorsal region of female Dark Agouti rats (6/group) for 100 days. As in previous studies of this series, excretion of porphyrin in faeces was increased above control group levels by about day 70 in the isodiazinon and isodiazinon mixture groups and continued to rise for the remainder of treatment. An assessment of the activity for enzymes involved in the porphyrin biosynthesis, namely ALA synthetase, ALA deaminase, uroporphyrinogen synthetase, uroporphyrinogen decarboxylase, coproporphyrinogen oxidase, protoporphyrinogen oxidase and ferrochelatase together with cytochrome P-450, succinate dehydrogenase, glutamate dehydrogenase and kynurenine hydroxylase, revealed that ferrochelatase activity was significantly reduced ($p < 0.01$) by 13% and 42% after isodiazinon and the isodiazinon:diazinon mixture treatment respectively. Coproporphyrinogen oxidase was also significantly reduced ($p < 0.01$) for the diazinon:isodiazinon mixture (by 28%) but not for isodiazinon alone. TLC analysis of the faecal porphyrins after diazinon:isodiazinon treatment indicated that although the total amount of protoporphyrin and coproporphyrin in faeces had increased, the ratio to one another remained unchanged; a result inconsistent with the reduced ferrochelatase and coproporphyrinogen oxidase activity.

10.3 Immunotoxicity Study

Barnett JB, Spyker-Cranmer J, Avery DL & Hoberman AM (1980) *Immunocompetence over the lifespan of mice exposed in utero to carbofuran or diazinon: 1. Changes in serum immunoglobulin concentrations. Departments of Microbiology and Immunology, and Pharmacology, University of Arkansas & Hazeleton Labs, Virginia, USA. J Environ Path Toxicol 4: 53-63*

[Although this report also describes exposure to carbofuran, only results pertaining to diazinon exposure are summarised.]

In order to detect humoral changes in the adult immune system as a result of *in utero* exposure, diazinon in peanut meal at doses of 0, 0.18, or 9 mg/kg bw/day was fed daily to groups of presumed pregnant mice (43, 21 and 19 respectively; strain or source not reported) throughout gestation. The maximum dose selected was 1% of the lowest dose that caused increased terata, morbid or moribund pups on gestation day 18, whereas the lower dose was set at 2% of the higher dose. Pups were weighed, sexed and examined for birth defects at delivery and then 4/sex were randomly returned to dams in the same treatment group for rearing until weaning on day 28. Serum immunoglobulin isotype concentrations (ie. IgG1, G2a, G2b, M and A) were measured in the blood of pups killed (10/sex/group) on day 101, 400 and 800 post partum.

Although the number of pups/litter (at parturition) for dams at 0.18 and 9 mg/kg bw/day was not significantly different from controls (ie. 7.2, 6.3 and 7.8 at 0, 0.18 or 9 mg/kg bw/day respectively), the mortality for the 9 mg/kg bw/day pups was significantly increased ($p < 0.05$) so that at weaning 12% had died compared with 6% in controls and 2% at 0.18 mg/kg bw/day. This increased mortality was attributed to an increased susceptibility to respiratory infections since necropsy confirmed pulmonary congestion and mucosal infiltration consistent with acute bronchitis. However, longevity for post-weaning mice was not significantly different at day 800 (ie. 33%, 21% and 31% at 0, 0.18 or 9 mg/kg bw/day respectively). A confounding factor for the survival of the high-dose pups to weaning was the significantly ($p < 0.05$) reduced bodyweight gain (exact difference not reported); a difference that diminished after weaning.

Prenatal exposure to diazinon had no effect on serum IgG2b, IgM or IgA levels. However, IgG1 and IgG2a concentrations were significantly ($p < 0.05$) different from controls, though not in any dose-related relationship or with respect to time after exposure. Thus, although changes in immunoglobulin subclass concentration were apparent at the 3 time intervals there were no data to indicate that any impairment of immunocompetence had occurred. Longevity or incidence of disease among groups were comparable, so the biological significance of these immunoglobulin changes is speculative.

11. HUMAN STUDIES

11.1 Short-Term Repeat Oral Administration

Sze P & Calandra JC (1965) Report to Geigy Chemicals Corporation. Subacute Oral Toxicity Study on Diazinon 50W - Humans. Report no. IBT D3719. Lab: Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois, USA. Sponsor: Ciba-Geigy Corp., Ardsley, New York, USA. Study duration: not stated. Report date: 24 Sep, 1965. (Validated and Pre-GLP)

Diazinon 50W (50% (w/w) wettable powder from Ciba-Geigy Corp.; assumed to be 100% pure for dose calculations; batch number was not specified)/starch mixture in gelatin capsules was administered orally before meals three times daily (at 0800, 1200 and 1600 h) to 3 adult male volunteers (77-95 kg bw) such that the the total daily ingestion was 0.05 mg/kg bw/day. Originally it was intended that the dosing regimen proceed for 13 weeks with doses being progressively increased from 0.05 to 5.0 mg/kg bw/day, however, because significant plasma ChE inhibition was observed at 0.05 mg/kg bw/day after only 5 days of treatment the dosing regimen was modified so that this 5-day treatment was followed by a 23-day recovery phase. To confirm the observed degree of plasma ChE inhibition, the protocol was repeated except that the recovery period was reduced to 14 days. Plasma and erythrocyte ChE activities were determined (by an electrometric method, $\Delta pH/h$) before the AM dosing (ie. 16 h after the previous dose) on 5 occasions pre-test, then on the first, third and fifth test days of each treatment phase and on days 3, 6, 10, 17, and 21 of the first recovery phase and days 3, 7 and 14 of the second. Bodyweight and clinical signs were determined daily. Haematology (Hb, erythrocyte count, total and differential leucocyte count and prothrombin time), clinical chemistry (BUN, AP and AST) and urinalysis (albumin, protein and microscopic elements) were determined pre-test, one day after dosing and then on days 1, 8 and 15 of the first recovery phase. Two weeks after the end of the second recovery test, each volunteer was tested for skin sensitisation by the application of an arm patch containing 0.5 mL of 1% aqueous diazinon; application sites were assessed 48 h later.

There were no clinical signs or changes in bodyweight during treatment or recovery. Similarly, no evidence of skin sensitisation was observed or changes to any haematological, clinical chemical or urinary parameters occurred other than for plasma ChE activity. As shown in the Table below, plasma ChE activity was significantly inhibited on day 5 of the first ($p<0.01$) and second ($p<0.05$) treatment cycles. Significant inhibition ($p<0.05$) persisted for 6 days after treatment in the first cycle, while substantial inhibition of up to 22% was observed for 6 days after treatment in the second. No concurrent changes in erythrocyte ChE activity were observed during treatment.

Plasma ChE Inhibition (mean percentage reduction)

Volunteer	Treatment (cycle 1)			Recovery (cycle 1)				
	Day 1	Day 3	Day 5	Day 3	Day 6	Day 10	Day 17	Day 21
1	0	12	43	27	29	9	[3]	3
2	[16]	5	38	31	26	16	22	[2]
3	10	2	33	24	26	15	4	0
Mean	[2]	6	38**	27	27*	13	8	0
	Treatment (cycle 2)			Recovery (cycle 2)				
	Day 1	Day 3	Day 5	Day 3	Day 7	Day 14		
1	3	13	37	22	14	[2]		
2	3	2	35	21	21	0		
3	[3]	5	28	4	0	[3]		
Mean	1	7	33*	16	12	[2]		

Values in square brackets indicate the extent (%) to which the measured activity was greater than the mean pretest value;

* $p\leq 0.05$; ** $p\leq 0.01$.

Therefore, based on significant inhibition of plasma ChE activity observed during treatment at 0.05 mg/kg bw/day for five days, a NOEL for this study could not be established.

Payot PH (1966) Subacute oral toxicity study on diazinon AS - Humans. Report no. (not stated). Lab: Pesticide Research Division. JR Geigy, Basle, Switzerland. Study duration: Mar - Jun, 1966. Report date: Oct 31, 1966. (Pre-GLP)

Gelatin capsules containing 0.5 mg of technical diazinon (purity 95.4%; batch Mg 613) were administered PO post-prandially to 4 adult male volunteers aged between 30 and 45 years and weighing 66, 74, 91 and 95 kg respectively. Since the amount of diazinon in each capsule was fixed (without correction for impure a.i.) and volunteers were given either 4 or 5 capsules depending on whether their bodyweight was closer to 75 or 100 kg bw respectively, the actual administered doses varied slightly, ie. 0.03, 0.027, 0.022/0.027 (alternate day treatment with 4 and 5 capsules) and 0.026 mg/kg bw/day respectively. Each dose was divided so that 2 of the 4 capsules were taken between 0800 and 0900 h and then again between 1300 and 1400 h; for the 5 capsule treatment, the fifth was taken between 0600 and 0700 h. To ascertain reversibility of effects, after complete plasma ChE inhibition in 2 of the 4 volunteers was observed after 1 day of treatment, dosing was suspended on days 5 to 10 of the 42-day regimen, whereas the other 2 (lighter) volunteers who commenced treatment a month later were treated uninterrupted for 34 days.

The recovery of diazinon (measured by UV absorption) from two different batches of capsules ranged between 92% and 96% 2 months after preparation and TLC analysis did not reveal any substantial change in the number or concentration of impurities during this time. Plasma and erythrocyte ChE activities were measured by colorimetric assay on 2 or 3 occasions before treatment and then before breakfast (approx. 0730 h) at 3 to 7 day intervals during treatment. Thus the interval between dosing and ChE activity measurements was (approx.) either 13 or 18 h depending on the number of capsules taken. Haematology measurements involving Hb, Hct, erythrocyte count, total and differential leucocyte count and clotting time were performed 6 days before treatment and then again on days 10 and 43 for the 42-day dosing regimen and 16 days before treatment and then again on day 34 for the 34-day regimen. Serum alkaline and acid phosphatase were measured 6 days before treatment and on days 10, 26 and 43 for the 42-day regimen and only twice during treatment for the 34-day regimen, ie. on days 5 and 34. Urinalysis measured albumin, glucose, urobilinogen, pH and microscopic elements.

There were no clinical signs or changes in bodyweight (apparently assessed before and after treatment though no data were shown) and measured haematological parameters during the study. Acid phosphatase activity in plasma was reduced by 50% and 65% relative to their respective pretest levels in the 2 volunteers during the 42-day regimen; no pretest levels were measured for the other 2 volunteers but both were reduced during treatment by a similar degree assuming comparable pretest activities as in series 1. Alkaline phosphatase activities were little changed during treatment. The ChE activity in plasma, collected before treatment, appeared to fluctuate erratically as shown in the Table below, though it was not entirely clear as to what control was used to determine the 'percentage activity' in plasma. Hence, the activities reported and shown in the Table below are not relative to pretest or controls but to some undefined benchmark.

'Percentage ChE Activity' in Plasma

Volunteer (kg bw)	Pre-test	Day 1	Day 5	Day 10	Day 16	Day 22	Day 29	Day 36	Day 43
1 (91)	5, 13	0	1	28	22.5	32.5	12.5	23	9
2 (95)	4, 11	0	1	33	22.5	34	18	26	7.5
3 (66)	32.5, 41, 16	ND	57.5	ND	14*	19¶	§	32†	
4 (74)	27, 28, 15	ND	42.5	ND	12.5*	24¶	§	31†	

* Day 13; ¶ Day 21; § Day 27 but not measured due to a technical failure; † Day 34; ND=Not determined.

Disturbingly, there appears to be a remarkably good agreement in ChE activity between the samples from different volunteers assayed on the same day (intra-assay variability), ie. 5, 4; 13, 11; 0, 0; 1, 1; 28, 33; 22.5, 22.5; 32.5, 34; 12.5, 18; 23, 26; 9, 7.5; for the first series and 32.5, 27; 41, 28; 16, 15; 57.5, 42.5; 19, 24; 32, 31 for the second series but rather poor inter-assay variability, eg. pretest ChE activities for the same volunteer can vary by up to 2-3 fold. Therefore trying to interpret the physiological relevance of a ChE activity ranging between 4 and 13% in pretest plasma (for the first series) is difficult, aside from trying to ascertain whether any (further) inhibition occurred during treatment. However, since the two treatment series overlapped in time and some of the ChE assays were performed on the same day, it seems acceptable (based on intra-assay variability) to compare the degree of plasma ChE inhibition during treatment in series 1 with pretest levels in series 2 (ie. as a surrogate control) for the corresponding days. Three assay dates were common to both series, ie. 25/4, 2/5 and 9/5, and these correspond to day 29, 36 and 43 of the first dosing regimen and all 3 pre-treatment assay days in series 2. The ChE activities measured on those days are shown in the following Table.

Plasma ChE Inhibition

Assay Date	Treatment Day (Series 1)	Mean Pretest Activity (Series 2)	Treatment Activity§ (Series 1)	Inhibition (%)
25/4	29	30	12.5, 18	58, 40
2/5	36	34.5	23, 26	33, 25
9/5	43	15.5	9, 7.5	42, 52

§ Individual values shown.

Using this approach, the mean plasma ChE inhibition was in the order of 40% while ChE activity in erythrocytes was essentially unchanged throughout treatment in both series. Urinalysis showed no consistent changes attributable to treatment.

Therefore in summary, a daily oral administration of 0.0245-0.03 mg/kg bw/day diazinon for 33-34 days as a divided dose resulted in no clinical signs or changes in bodyweight, haematology or urinalysis in 4 volunteers. However, although ChE activity in erythrocytes was apparently unaffected by the 34- or 36-day treatment, ChE activity in plasma in 2 volunteers would appear to have been completely inhibited after a single administration, albeit from a pre-test activity of 5% and 13% respectively. A second administration after a 5-day recovery resulted in activities greater than pre-test levels with no apparent reduction by subsequent daily administration. Based on the observation that the inter-assay ChE variability was quite marked whereas intra-assay appeared relatively consistent, a comparison of results derived on the same day of assay suggests that significant inhibition (~40%) of ChE in plasma occurs. Thus, in the presence of a 60% reduction in acid phosphatase activity and 40% reduction in plasma ChE activity, a NOEL could not be established because only one dose was tested in this study.

Lazanas JC, Fancher OE & Calandra JC (1966) Report to Geigy Chemicals Corporation. Subacute oral toxicity study on diazinon 50W - Humans. Report no. IBT D4321. Lab: Industrial Bio-Test Laboratories, Inc. Northbrook, Illinois, USA. Sponsor: Ciba-Geigy Corp., Ardsley, New York, USA. Study duration: not stated. Report date: 22 Nov, 1966. (Validated and Pre-GLP)

A mixture of diazinon 50W (50% (w/w) wettable powder from Ciba-Giegy Corp.; batch FL-3354 ARS 783/66, purity 49.6%) and corn starch was prepared and placed into gelatin capsules so that following a three times daily oral administration to 3 healthy adult male volunteers (nos. 4, 5 and 6) the final dose was 0.025 mg/kg bw. A concurrent control group of 3 volunteers (nos. 1, 2 and 3) were given capsules containing corn starch only. All prepared capsules were stored in a refrigerator. Capsules were taken daily before meals, ie. at 0800, 1200 and 1800 h for 43 days. In a second dosing regimen, 3 different volunteers (nos. 1, 7 and 8) were treated at 0.020 mg/kg bw/day for 37 days. After treatment at the 0.025 mg/kg bw/day doses volunteers were given corn starch capsules (placebo) for 101 days whereas this recovery phase was reduced to 41 days for the 0.020 mg/kg bw/day treatment group. Plasma and erythrocyte ChE activities in all 8 volunteers were measured on 5 separate occasions before dosing and then at 1-5 day intervals throughout treatment and recovery using an electrometric method (Δ pH/h). Bodyweight and clinical signs were monitored daily. Haematological parameters, including Hb, erythrocyte count, total and differential leucocyte count and prothrombin time were determined at 4-7 day intervals during treatment and at 4-14 day intervals during recovery, as were clinical chemistry parameters,

ie. ChE (erythrocytes and plasma), BUN, AP and ALT, and urinalysis, ie. pH and microscopic elements.

No clinical signs or changes in bodyweight were observed. No significant changes were detected in any of the haematological, urinary or clinical chemistry parameters measured except for plasma ChE. The results for the observed plasma ChE inhibition at 0.025 mg/kg bw/day are shown in the Table below.

Plasma ChE Inhibition (mean percentage reduction)

Volunteer	Day of Treatment at 0.025 mg/kg bw/day																
	5	8	9	12	15	17	19	22	24	26	29	31	33	35	38	40	43
4	13	18	18	20	12	10	8	22	18	21	20	19	18	18	22	19	26
5 [§]	17	13	25	30	26	22	27	36	36	29	29	37	30	32	34	38	35
6 [¶]	12	12	16	30	18	18	17	26	27	20	12	18	21	24	24	23	19
Mean	14	14	20	27	19	17	17	28	27	23	20	25	23	23	27	27	27
Combined Mean	22**																

§ No capsules taken on the evening of test day 8. He took 4 capsules on test day 9; ¶ No capsules taken on the morning of day 5 and evening of test day 18. He did not take any capsules on test day 22; ** $p \leq 0.01$.

The plasma ChE inhibition observed in the 3 volunteers was calculated relative to their individual mean pretest activities. A comparison with pretest plasma ChE inhibition seems reasonable since group numbers were low ($n=3$) and the interassay variability for controls relative to their respective mean pretest activities was only 3%. Therefore the significant change observed for the combined mean ChE plasma inhibition relative to the combined mean pretest values for the treated volunteers indicates a clear treatment-attributable effect. By contrast, mean erythrocyte ChE activity was not significantly inhibited (2%) at any time during treatment at 0.025 mg/kg bw/day or for the 22 days of recovery. However, unexpectedly after 22 days of recovery, the combined mean erythrocyte ChE activity declined significantly ($p < 0.05$) by 13%. Reasons for this change are obscure and the investigators did not comment; though the observation is inconsistent with a treatment-related effect.

Recovery of plasma ChE activity was evident after cessation of treatment so that by day 22 the combined mean inhibition was 10%, 6% by day 57 and full activity returned by approximately day 61. Treatment at 0.020 mg/kg bw/day resulted in a combined non-significant mean plasma ChE inhibition of 8% and recovery appeared complete after 16 days. Similarly, erythrocyte ChE activity was also not significantly affected by treatment at 0.020 mg/kg bw/day.

Based on the significant inhibition of plasma ChE activity at 0.025 mg/kg bw/day, the NOEL for this study was established at 0.020 mg/kg bw/day.

11.2 Percutaneous Absorption

Wester RC, Sedik L, Melendres J, Logan F, Maibach HI & Russell I (1993) Percutaneous absorption of diazinon in humans. Department of Dermatology, University of California,

California, USA and CSIRO-Division of Wool Technology, Belmont, Victoria, Australia. Food Chem Toxicol 31: 569-572

A 50 µL solution of acetone containing 20 µg of [2'-¹⁴C]diazinon (specific activity 66 µCi/mg) was applied to a 10 sq cm area of the ventral forearm or abdomen to groups of 6 volunteers each. A third group of 6 volunteers had 14.7 µg of the radioactive diazinon in 50 µL lanolin (wool grease) applied to a similar sized area of the abdomen and the application area of all treatment groups was left uncovered for 24 h. Any remaining diazinon was washed off after 24 h and the surface of the application site stripped repeatedly (10 times) with adhesive cellophane tape 7 days later. For each volunteer the radioactivity, in the surface wash, attached to the cellophane tape, and present in 24 h pooled urine collected daily over 7 days, was measured using liquid scintillation spectrophotometry.

After adjusting for the residual radioactivity at the application site (24 h wash, 0.5%, 1.40%, 0.35%; tape strip, 0.01%, 0.01%, 0.04%), the cumulative 7-day urinary excretion of radioactivity was calculated to be 2.2%, 1.8% and 1.6% for the forearm, abdomen 'acetone' and abdomen 'lanolin' groups respectively. Since a concurrently performed Rhesus monkey study (see under monkey, section 2.1) had shown that approximately 56% of an intravenously administered dose was excreted in the urine over 7 days, it was reasoned, based on an assumption that the pharmacodynamics in monkeys were similar to humans, that the human percutaneous adsorption rate for the 3 groups, after correcting for incomplete or other route excretion, was 3.8%, 3.2% and 2.9% respectively. The failure to reflect any statistical difference between the absorption rates of diazinon when applied in the presence of acetone or lanolin was attributed to the small numbers of subjects used in each test group. Perhaps though, more importantly, the possibility that the loss of about 95% of the applied dose being due to evaporation or by smearing on clothing as proposed by the investigators, reduces the value of the study. Clearly, if the amount of diazinon remaining in contact with the skin for 24 h is uncertain, then the reliability of the calculated absorption rates is in doubt.

In Vitro Study

Abdominal skin (500 µm thick) cut from 2 male human cadavers (23 and 56 years old) with a dermatome and stored in Eagle's minimum essential medium at 4 °C for no more than 5 days was used to estimate the percutaneous absorption rate of diazinon in an *in vitro* flow-through cell. [2'-¹⁴C]diazinon (specific activity 66 µCi/mg) in acetone at a concentration of 0.25 µg/cm² was applied (volume not reported) onto the 1 sq cm skin samples in 6 separate cells for each donor. After 24 h without occlusion and a buffered saline flow rate of 1.25 mL/h (1 reservoir volume), the skin samples were removed and washed to quantify any residual surface radioactivity. Radioactivity remaining in the skin was determined by tissue solubilisation and counting using liquid scintillation spectrophotometry.

The surface wash accounted for 48.3% and 34.6% respectively of the total radioactivity for the 23- and 56-year old cadaver skin specimens, whereas the skin digest had 5.6% and 4.8% respectively. Radioactivity in the receptor fluid was 8.5% and 19.7% respectively, so the total radioactivity recovered was 62.4% and 59.1% respectively. It was speculated that the balance of the applied radioactivity (~ 40%) had evaporated over the duration of the study.

11.3 Skin Sensitisation

Lisi P, Caraffini S & Assalve D (1987) Irritation and sensitization potential of pesticides. Istituto di Clinica Dermatologica e Venereologica, University of Perugia, Perugia, Italy. Contact Dermatitis 17: 212-218

Pesticides were patch tested in 652 subjects to establish the optimal test concentration, and the frequency of irritant and allergic reactions. Allergic reactions to fungicides were found in 46 subjects, with captan folpet and difolatan the most common. Irritancy and allergic reactions to other pesticides (insecticides and herbicides) were rare. Diazinon did not produce either irritant or allergic reactions. It was noted that pesticide sensitivity was more common in individuals who worked, or who had worked in agriculture.

11.4 Occupational Exposure

Soliman SA, Sovocool GW, Curley A, Ahmed NS, Sorya E-F & Abdel-Khalek E-S (1982) Two acute human poisoning cases resulting from exposure to diazinon transformation products in Egypt. US EPA Health Effects Laboratory, Triangle Park, North Carolina, USA & Alexandria University, Alexandria, Egypt. Arch Env Health 27: 207-212

Two Egyptian workers experienced acute toxicity after using a 60% diazinon EC formulation, which had been packaged in tin-plated sheet steel (previously packaged in aluminium containers). Both workers were experienced sprayers, having used diazinon for more than 18 months, and were accustomed to applying 1200 to 1500L diluted diazinon (0.1%) once weekly by backpack spray without using either mask or gloves. They noted when preparing this batch of spray that crystals had formed in the storage container.

In the first case study, a 33-year-old male developed nausea and vomiting, followed by progressive weakness and muscle twitching in his arms and legs. He presented at hospital where he was treated with 1 mg atropine sulphate IM; a dose repeated 2 h later. He was discharged the next day. Relative to a control group of unexposed males his plasma ChE activity was inhibited >20% on day 8 after poisoning, with recovery at day 15. Erythrocyte ChE activity was inhibited >20% on day 18 and had returned to control levels by day 28.

In the second case study, a 50-year-old male developed nausea and vomiting after a full day's spraying. This progressed to burning eyes, blurred vision and difficulty breathing, as well as a severe headache persisting for 3 days. He did not seek any medical advice, but recovered by 3 days after exposure. Plasma ChE activity was inhibited >20% on day 10 after the incident, with recovery on day 17. Erythrocyte ChE activity was inhibited >20% on day 17 after the incident with recovery on day 20.

A sample of the crystallised material found in the batch was examined using GC-MS. The major component was 2-isopropyl-4-methyl-6-hydroxypyrimidine, in 2 tautomeric forms. Small amounts of other products were also found, including 4-ethoxy-6-methyl-2-(1-methyl-ethyl)-pyrimidine, 4-thioethoxy-6-methyl-2-(1-methyl-ethyl)-pyrimidine, 4,4'-thiobis[6-methyl-2-(1-methyl-ethyl)-pyrimidine], 4,4'-dithiobis[6-methyl-2-(1-methyl-ethyl)-pyrimidine], O,O,O'-triethylphosphorothioate; and O,O,S-triethylphosphorothioate. Sulfotepp (O,O,O',O'-tetraethyldithiopyrophosphate) and monothiono-TEPP (monothiono-tetraethylpyrophosphate) were also found in small quantities. TEPP (tetraethylpyrophosphate) was not found. It is likely that the presence of these toxic metabolites resulted in the clinical signs observed.

Loosli R (1983) Final Report G 24'480/Basudin 10G. Simulated field exposure study in human volunteers. Report no. 830206. Lab: Ciba-Geigy Ltd, Basle, Switzerland. Study date: Jun 27, 1983. Report date: Aug 24, 1983.

Five male volunteers, aged 44 to 55 years and weighing from 60 to 85 kg were exposed to diazinon over a 30-min period. Volunteers stirred a dry granular formulation of diazinon (10% diazinon; no other formulation details provided) in a plastic bucket with either their right or left bare hands. During the 30 min period of stirring, the volunteers stood with bare feet in water containing diazinon at 1.7 ppm. These conditions were designed to imitate the conditions of use of diazinon in rice paddies. At the end of the exposure period, each volunteer carefully washed their hands and feet with soap and water.

Plasma and erythrocyte ChE activity was determined twice pre-test, 2 to 4 h and 4 days after the test. No volunteers reported any effects following exposure. There were no bodyweight losses observed. Plasma ChE activity was inhibited from 17% to 27% within 4 h of test completion, in comparison to pre-exposure values. Activity 4 days later was returning to normal, with inhibitions of 9% to 14% of pre-exposure values. There was no inhibition of erythrocyte ChE activity after the test.

Wecker L, Mrak RE & Dettbarn W-D (1985) Evidence of necrosis in human intercostal muscle following inhalation of an organophosphate insecticide. Dept of Pharmacology, Louisiana State University Medical Center, New Orleans, Louisiana, USA JEPTO 6: 171-176

A 51-year-old male herded 3 cows into a closed shed and sprayed them with a commercial mixture containing malathion and diazinon. He was found unconscious several hours later and treated with atropine prior to admission to hospital. He was unresponsive to all stimuli except pain (which produced withdrawal). Neurological examination revealed increased muscle tone and neuromuscular excitability. Pupils were small and unreactive, and corneal reflexes and Doll's eye movements were absent. There was a sinus tachycardia, and chest x-ray showed a mild increase in heart size with increased pulmonary vasculature. Plasma ChE activity was inhibited by 75% relative to the normal values. On the day of admission, the patient suffered a cardiorespiratory arrest and was resuscitated. On the second day he was areflexic and unresponsive to all stimuli. On the 4th day he suffered a second cardiac arrest and died.

A postmortem examination was conducted 4 h after death. In addition to standard gross and microscopic examination, samples of intercostal muscles were taken for histological examination and a total neuromuscular ChE activity determination. Comparative samples of intercostal muscle from autopsies of patients dying of cardiac arrest with no previous chronic illness or medication were also taken.

On postmortem examination, there was diffuse subarachnoid intraventricular and cerebral cortical haemorrhage with autolysis at the base of the brain. Microscopic examination revealed haemorrhagic necrosis without inflammatory or glial response. There was moderate left ventricular hypertrophy, with no dilation or hypertrophy of the other cardiac chambers. Microscopic examination of intercostal muscle revealed mild pathologic changes, with subsarcolemmal granular basophilic inclusions, and scattered necrotic fibres. Lesions were randomly scattered throughout the muscle tissue; these types of lesion were not seen in control samples. The neuromuscular ChE activity in the intercostal samples obtained from the patient

with pesticide poisoning were approximately half those seen in control patients. Necrosis had been seen previously in muscle tissue from patients dying of acute OP poisoning

Coye MJ, Barnett PG, Midtling JE, Velasco AR, Romero P, Clements CL & Rose TG (1987) Clinical confirmation of organophosphate poisoning by serial cholinesterase analyses. Department of Internal Medicine, University of California, San Francisco, USA. Arch Intern Med 147: 438-442

Eighteen workers at a mushroom farm were exposed to diazinon when the doorway into the room they were working in was sprayed. Within 15 minutes, 17 workers developed cholinergic signs, including headache, blurred vision, dizziness, fatigue, nausea and vomiting. Four workers went to hospital, where they were treated with atropine and admitted. At this time their plasma and erythrocyte ChE activity was at the low end of the normal range. Two of these workers developed nausea and vomiting after returning to work 2 days later. Eight other workers sought advice within 48 h of exposure, and ChE activity was determined. All of these workers had a follow-up ChE test 15 days later. In all cases, plasma and erythrocyte ChE activity increased between these tests. If the levels found at 15 days after exposure were taken to be the normal levels for these workers, the mean plasma ChE inhibition seen was 29% and mean erythrocyte ChE inhibition was 27%. This may be an underestimate of the degree of inhibition, as in many cases ChE activity has not returned to normal within 15 days of exposure, depending on the degree of inhibition initially seen. Following this work, it was recommended that where there are cholinergic signs, a history of exposure, no baseline values for the individual and plasma ChE levels are at the low end of normal values the worker should be kept from work and retested by the same laboratory 3 to 5 days later. If there is an increase in ChE activity between the tests, a further test should be done 3 to 5 days later. A rise in activity between these tests would be confirmatory that pesticide exposure and ChE inhibition had occurred.

Maizlish N, Schenker M, Weisskopf C, Seiber J & Samuels S (1987) A behavioural evaluation of pest control workers with short-term, low-level exposure to the organophosphate diazinon. Occupational and Environmental Health Unit, University of California, California, USA. Am J Industrial Med 12: 153-172

Neurobehavioural effects of diazinon exposure in workers in California were investigated. All subjects were paid volunteers, either employed in the application of diazinon through residential areas or non-applicators employed as supervisors, pest-inspection or other non-pesticide related work. Initially 99 volunteers were included in the study. Diazinon granules (14%) were applied at 40 lbs/acre to the soil surface with a variety of application equipment, followed by watering in. All workers involved in the application were under direct supervision at all times. Applicators were required to wear disposable overalls, rubber boots and rubber gloves while in the treatment area. While loading machinery, workers were also required to wear face shields and full-face air purifying (cartridge type) respirators.

Diazinon dose was assessed by measuring the excretion of the diazinon metabolites diethylthiophosphate (DETP) and dimethylthiophosphate (DMTP) in the urine both pre- and post-shift. A random sample of workers was also selected for the measurement of diazinon exposure using dermal badges, hand rinses and breathing zone air samplers.

All volunteers were required to undergo a physical examination to identify any pre-existing disease, trauma, medication or other condition unrelated to pesticide exposure which may have affected behavioural test performance. The examination included an evaluation of the cranial

nerves, pupil reaction, extraocular movements, nystagmus, visual fields and acuity, tremor, reflexes, coordination and gait. Each subject also complete a questionnaire which covered demographic and physical characteristics; medical history; history of exposure to neurotoxic substances; consumption of drugs, alcohol, caffeinated beverages and tobacco; and 18 symptoms possibly related to pesticide exposure.

Seven behavioural tests were completed by each volunteer both pre- and post-shift. The tests were selected on the basis of their sensitivity, reliability, ease of administration, subject acceptance and inclusion of CNS functions known to be affected by pesticide exposure. The tests are detailed in the Table below.

Behavioural Tests

Test	Psychologic function	Task required
Continuous Performance Task	Attention/vigilance	Letters flashed on screen; subject required to react to the letter 'S'
Hand-Eye Coordination Task	Visual/motor accuracy	Subject required to trace a computer generated sine wave.
Symbol-Digit Substitution	Visual/motor speed	Subject shown 9 symbols paired with digits, and is required to enter the correct digit on being presented with a symbol.
Pattern Comparison Test	Visual perception	3 patterns presented, 2 of which were identical; subject required to select odd pattern.
Pattern Memory Test	Visual memory	Subject presented with pattern for 5 sec. After 3-second delay, subject asked to select memorised pattern from presented selection.
AFQT Vocabulary	Verbal ability	Subject presented with a word and asked to choose a synonym from 4 choices.
Finger Tapping	Motor speed	Subject instructed to tap keys for 10 second with L and R index fingers, then alternate between 2 keys with preferred hand.

In the continuous performance test there were slight, non-significant decreases in accuracy and speed in the applicator group after adjusting for confounders. Results were correlated more strongly with age, increased alcohol consumption and decreased vocabulary score than with urinary DETP levels (measure of pesticide exposure).

For hand-eye coordination, there were non-significant increases in absolute mean error in applicators in comparison to controls, however this was not associated with DETP levels. In the Symbol-Digit substitution test applicators were significantly ($p=0.02$) slower in the post-shift test than non-applicators, however this was not associated with DETP levels, but rather with increasing age and decreasing vocabulary score. There were no significant differences found in pattern comparison, pattern memory or finger tapping between applicators and non-applicators.

There was no increase in reported symptoms in applicators in comparison with non-applicators. There did not appear to be an effect associated with diazinon treatment in this behavioural trial. The post-shift median DETP level was 24 ppm, and the median diazinon exposure was 2.1 mg/day.

Weisskopf CP, Seiber JN, Maizlish N & Schenker M (1988) Personnel exposure to diazinon in a supervised pest eradication program. Dept of Environmental Toxicology. University of California, California, USA. Arch Environ Contam Toxicol 17: 201-212

Workers were involved in application of 14% diazinon granules in a primarily residential area using four types of application equipment. Planters and shrub areas were treated using a coffee can filled with the formulation with holes punched in the bottom. On lawn areas, a V-shaped spreader with a single line of holes perpendicular to the line of travel was used. In areas of high grass, a spreader mounted on three wheels and releasing pesticide about 18 inches above the ground was used, while for pasture or high grass a broadcast spreader held by a strap around the neck was used (belly grinder). After application, the pesticide was watered into the top layer of the soil.

All workers involved in pesticide application wore disposable polyethylene protective overalls, rubber boots and rubber gloves. Supervisors wore boots while in the treatment areas. Untreated controls wore coveralls to aid in standardising exposure measurement. Coveralls were changed at the lunch break, while gloves were changed at the discretion of the workers when they were torn or became dirty. While filling application devices, a full-face cartridge-type respirator and face shield was required; this protective equipment was available for use by applicators, but most applicators generally did not use it.

Exposure was, in most cases, determined on the basis of testing of urine for diethylthiophosphate (DETP). Additionally, fifteen project workers and four controls were monitored for dermal and respiratory exposure using dermal patches both on top of and under coveralls and a battery powered personal sampling pump. Worker's hands were washed in ethyl alcohol prior to removal of gloves at breaks. Body surface exposure was determined from patch concentrations, with penetration of the pesticide through clothing estimated to be 10%. For simplicity, differences in dermal absorption for different body areas was not considered, and 100% of the inhaled diazinon was included in the total.

Urine samples were collected at single time intervals, rather than over 24-h periods. Urinary metabolite levels were normalised by converting from ng DETP/mL urine to µg DETP/g creatinine, as creatinine excretion varies less between individuals than does urinary output.

Total diazinon exposures ranged from 0.1 to 11 mg/day for workers, with total and respiratory levels being highest in workers using the belly grinder. Urine diethylthiophosphate (DETP) levels ranged from <0.1-360 µg/g creatinine. Afternoon urine DETP levels were correlated with morning diazinon exposures.

Dermal exposure for crew members (except those using the belly grinder) were relatively low, with slightly higher respiratory exposures. These results are consistent with those generally seen for granular formulations. DETP concentrations were a less reliable indicator of total dose than diazinon exposure measurements in this study. A correlation was found between measured morning diazinon exposure and afternoon DETP levels, however there was variable recovery of DETP.

Ciba-Geigy Australia Ltd (1995) Adverse experience report - NSW State Coroner's Office report on death of a sheep farmer related to Topclip Blue Shield Dip (diazinon 200 g/L).

A 68-year-old sheepfarmer used diazinon to treat sheep without wearing protective clothing. He presented at hospital the next day with periumbilical and upper abdominal pain. He was transferred to a regional hospital the next day, and was diagnosed with acute haemorrhagic pancreatitis. He was transferred to a major hospital two days later and placed in intensive care. There was progressive deterioration of his condition, with multi-organ failure and he died two days after admission.

On post mortem examination, there were a number of incidental findings, including left ventricular dilatation, extensive moderate atherosclerosis in the aorta, with an early infrarenal abdominal aortic aneurysm. The lungs showed moderate emphysema and pulmonary oedema, and the pleura contained 200 mL of serous fluid. The gallbladder contained numerous friable stones. There was 300 mL of blood stained fluid in the peritoneal cavity. The mid-portion of the pancreas was necrotic and adherent to the stomach. There was extensive greenish-black discoloration of the peritoneal surface, and ischaemic necrosis of the small bowel and transverse colon.

On histopathological examination, the lungs showed pulmonary oedema and focal presence of saponificated lipid material in the arterial vessels. There was early tubular hyperplasia in the kidney with vascular scarring. The pancreas showed extensive necrosis, which was also seen in the retroperitoneal tissue. There was also centrilobular necrosis in the liver. The blood, urine, liver and gall bladder were sent for toxicological examination. The blood contained pethidine and lignocaine, and the urine contain paracetamol, metronidazole and metoclopramide in quantities consistent with therapeutic use. Diazinon was not detected in the organs or tissues at this time. Blood ChE activity was 26 units (normal 80 to 150 units), which is consistent with OP exposure.

The cause of death was determined to be severe acute haemorrhagic pancreatitis caused by exposure to diazinon, probably by the dermal route.

11.5 Poisoning Incidents

Mutalik GS, Wadia RS & Pai VR (1962) Poisoning by diazinon, an organophosphate insecticide. Dept of Medicine, B.J. Medical College, Poona, India. J Indian Med Assoc 38: 67-71

Twenty five cases of diazinon poisoning, admitted to the Sassoon Hospital in Poona, India in 1961-2, were reviewed. There were 14 males and 11 females, with ages ranging from 18 months to 60 years, though the majority of cases were aged from 15 to 24 years. Each had ingested between approximately 14 and 57 g of diazinon (presumable formulation); the exact quantities ingested were not generally known. Only two patients died following hospitalisation; the remaining 23 appeared to recover uneventfully.

Symptoms experienced by the patients included: vomiting (18/25); abdominal cramps (10/25); stupor (8/25); restlessness (6/25); giddiness (3/25) and sweating (3/25). Additionally there were single instances of fever, diarrhoea, hiccup and coma. Signs on clinical examination included: pulmonary oedema, detected by rales heard on auscultation (15/25); hypertension (12/25); tachypnoea (10/25); tachycardia (9/25); albuminuria (7/25); azotaemia (6/25); nystagmus (2/25); hypotonia (2/25) and cyanosis (1/25). There was no report on any clinical chemistry or haematological examination of any of the patients.

Treatment generally included gastric lavage, atropine, oxygen and antibiotics as appropriate. No details of dosing were provided. Post-mortem examination of the two patients dying following

poisoning were performed. There were petechial haemorrhages in the brain, kidney, pericardium, respiratory tract and liver. Congestion was seen in the brain, and the kidney was hyperaemic, particularly in the medullary region. There was no report of any changes in the pancreas in this examination.

Kabrawala VN, Shah RM & Oza GG (1965) Diazinon poisoning (A study of 25 cases). K.M. School of Post-Graduate Medicine and Research, Sheth V.S. Hospital, Ahmedabad, India. The Ind Pract Oct: 711-717

Twenty-five cases of diazinon poisoning occurring in the 12 months between October 1964 to Oct 1965 in Ahmedabad, India were reviewed. There were 17 males and 8 females admitted to the hospital with 21 of the poisoned patients being between 10 and 30 years old. Most of the cases ingested less than 21 mL of diazinon; 11 ingested between 21 and 28 mL. The commonest symptom seen was vomiting (21/25), with unconsciousness, giddiness and excessive sweating seen in 7/25 cases. The commonest clinical sign was tachycardia (15/25), with constricted pupils (14/25) and pulmonary oedema (12/25) also seen. Treatment included stomach washing, atropine treatment and airway maintenance. Three patients died despite treatment; no post-mortem findings for these patients were reported. .

Banerjee D (1967) Pericarditis in acute diazinon poisoning. Armed Forces Medical College, Poona, India. Armed Forces Med J (India) 23: 187-190

A 19-year-old male ingested approximately 4 ounces (113 g) of Tik-20 (diazinon; formulation not otherwise specified), and was admitted to hospital approximately 1-2 h after ingestion. He presented with vomiting and diarrhoea and was cyanotic. He was responsive to noise and pain and had tremor and spasticity of the limbs. He presented with pinpoint, unresponsive pupils. There were excessive secretions present in the trachea and respiratory passages, and moist rales in the lungs.

He was treated with gastric lavage and IV atropine sulfate. Oxygen and antibiotic therapy were also started. A tracheostomy was required to maintain an adequate airway, and digoxin and noradrenaline were administered to counter cardiovascular abnormalities. Pupils gradually returned to normal, while tendon reflexes remained sluggish.

On the second day of hospitalisation, the patient reported retrosternal pain; examination revealed evidence of pericarditis. The pericardial friction rub persisted for two days, with no other evidence of cardiac involvement. One day after the tracheostomy was closed, the patient developed respiratory distress and an increased temperature; left lower lung lobe consolidation had developed.

ChE activity determined in whole blood was significantly decreased (based on time taken for a reaction to occur) for two weeks after poisoning; levels had returned to near-normal within four weeks. The patient recovered without incident.

Gupta OP & Patel DD (1968) Diazinon poisoning: A study of sixty cases. Department of Medicine, B.J. Medical College, Ahmedabad, India. J Assoc Physic India 16: 457-463

Sixty cases of diazinon poisoning occurring between 1963 and 1965 in Ahmedabad, India, were reviewed. (This review probably includes the cases cited by Kabrawala et al, 1965; see below.) There were 42 males and 18 females involved, aged between 11 and 60 years and most (55/60)

had suicidal intentions. The common clinical signs were vomiting, giddiness, constricted pupils and signs of bronchoconstriction with pulmonary congestion. Urinalysis showed mild albuminuria in 4 cases and haematuria in 10 cases. Treatment with atropine was successful in most cases; 5 patients died despite atropine therapy. In all cases where death occurred, the patients had ingested at least 15 mL of diazinon and treatment had been delayed by more than 8 h after ingestion. Where patients presented within 3 h of ingestion, atropine therapy was successful. Post-mortem examination of the patients that died revealed congestion of viscera with or without pulmonary oedema and subendocardial haemorrhaging.

Heyndrickx A, Van Hoof F, De Wolf L & Van Peteghem C (1974) Fatal diazinon poisoning in man. Department of Toxicology, State University of Ghent, Belgium. J Forens Sci Soc 14: 131-133

A 65-year-old female was found at home after attempting suicide by cutting both radial veins. She was admitted to hospital and died some hours later. Postmortem and pathological examination failed to reveal the cause of death, however the stomach was found to contain a green, oily fluid. The lungs were oedematous.

The stomach and small intestine content, as well as samples of liver and brain tissue were extracted with petroleum ether. The extracts were evaporated and analysed by thin layer chromatography, together with reference samples of parathion and diazinon. Diazinon was found in extracts from the stomach and small intestine contents. The concentration of diazinon (quantified by GC) in the brain (30 µg/100g) was higher than that in the liver (8 µg/100g), kidney (4 µg/100g) or lung (1.5 µg/100g), but all were markedly lower than that in the stomach (756 µg/100g) and small intestine contents (262 µg/100g).

Reichert EF, Yaeger Jr WL, Rashad MN, Klemmer HW & Hattis RP (1977) Diazinon poisoning in eight members of related households. Hawaii Epidemiologic Studies Program, Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii, USA. Clin Tox 11: 5-11

Five children from a family became ill 30 min after eating breakfast (oatmeal, sugar and evaporated milk). The children had profuse sweating, nausea, vomiting and abdominal cramps, with the youngest having muscle weakness, muscle contractions and cramps. They were treated with atropine, and recovered without incident. Their mother indicated that she regularly sprayed the shelves and cupboards and painted the baseboards with a 25% diazinon formulation. This had occurred without removing packaged food (including cardboard boxes of oatmeal), dishes or glasses. An unopened box of oatmeal was given to a related family; the 3 children in this family also presented with acute signs of OP poisoning after eating an oatmeal breakfast. Plasma and erythrocyte ChE activity measured 17 or 23 days after poisoning did not appear to be significantly decreased relative to levels observed 52 or 58 days after poisoning, although there was a slight increase in all family members. Urinalysis revealed diethyl phosphate in the urine at the measuring times indicated above. There was not a major change in the excretion of this metabolite between the two time intervals. This may have reflected ongoing diazinon exposure. Diethylphosphorothioate was not found at either time intervals; it is possible that the level of this metabolite was below the limit of detection (0.02 ppm).

Klemmer HW, Reichert ER, Yaeger Jr WL & Haley TJ (1978) Five cases of intentional ingestion of 25 percent diazinon with treatment and recovery. Hawaii Epidemiologic Studies

***Program, Pacific Biomedical Research Center, University of Hawaii, Honolulu, Hawaii, USA.
Clin Tox 12: 435-444***

Five cases of successfully treated cases involving intentional ingestion of 25% diazinon were reported. Patients ingested between 60 and 180 mL of the pesticide formulation (between 15 and 45 mg diazinon). Four patients were seen at a hospital within 30 min; the male who ingested 60 mL did not present at the hospital until 5 h after ingestion. In general, clinical signs included vomiting, profuse sweating, pinpoint pupils, hyperreflexia and muscle twitching. One patient had abdominal pains that persisted for several days; it was unclear as to whether serum amylase activity was determined. In general patients were treated with gastric lavage, atropine, pralidoxime chloride and oxygen supplementation. One patient required assisted ventilation; he had ingested approximately 25 mg diazinon and had not vomited prior to hospital admission. Four of the patients recovered without incident within 4 to 10 days after treatment; the fifth patient developed chemical pneumonitis and required extensive hospital treatment.

Poklis A, Kutz FW, Sperling JF & Morgan DP (1980) A fatal diazinon poisoning. Department of Pathology, St Louis University School of Medicine, St Louis, Missouri, USA. Forensic Sci Int 15: 135-140

A 54-year-old female was found dead at home. On the kitchen sink, there were 2 vials containing chlordiazepoxide and furosemide, and a half empty 600 mL bottle of a 10% diazinon solution. A resident in the house indicated that the pesticide bottle had been stored for many years and had not previously been opened. On gross postmortem examination there were atherosclerotic plaques in the left anterior descending coronary artery. The lungs were heavy and congested. There were petechial haemorrhages through the stomach and gastric mucosa and the white and grey matter of the brain. Samples of fat tissue, bile, blood, brain, stomach contents, kidney and liver were collected for toxicological analysis.

Diazinon concentration was highest in the blood at 28 mg/dL, with high levels also found in the stomach contents (22 mg/dL) and bile (10 mg/dL). Diazinon was also found in omental fat (1.5 mg/100g), and the liver (0.4 mg/100g), brain (0.2 mg/100g) and kidney (0.01 mg/100g). Plasma ChE activity was also measured; this was found to be 0 U/mL (normal 40-80 U/mL). Based on the case history and lack of other findings, the cause of death was determined to be diazinon poisoning.

Dagli AJ, Moos JS & Shaikh WA (1981) Acute pancreatitis as a complication of diazinon poisoning. JJ Group of Hospitals, India. J Ass Phys Ind 29: 794 - 795

A 16-year-old female was admitted to hospital 1 h after consuming 10 mL of Tik-20 (diazinon). On admission, signs included nausea, headache and upper abdominal pain. On clinical examination, there was an elevated heart rate, small non-reactive pupils and epigastric tenderness. Clinical chemistry examination found normal blood glucose, blood urea, creatinine and calcium levels, but serum amylase was increased to more than 4 times the normal level (680 Somogyi units). Urinalysis was normal. She was treated by gastric lavage with potassium permanganate. Atropine was administered until an obvious atropine effect had occurred. She was also treated with 2-PAM. After 2 h, she had improved clinically, with the only abnormality being mild abdominal pain. Eight hours after admission, serum amylase was within the normal range (110 Somogyi units), and she was later discharged. The author presumed from observation and other experimental information in dogs, that functional ductal obstruction, pancreatic interstitial oedema and acinar cell vacuolation with resulting hyperamylasemia were caused from the

anticholinesterase effects of diazinon. The rapid resolution of signs in these case indicate that the pancreatic damage was transient in nature, possibly related to the rapid treatment of the case. In the addendum, the authors noted that there had been 4 new additional cases of acute pancreatitis following diazinon poisoning.

Hassan RM, Pesce AJ, Sheng P & Hanenson IB (1981) Correlation of serum pseudocholinesterase and clinical course in two patients poisoned with organophosphate insecticides. Department of Pathology and Laboratory Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio, USA. Clin Tox 18: 401-406

A 58-year-old female was admitted to a hospital 1 h after ingesting 30 mL of an unknown poison (later identified as diazinon). Her pupils were small (1 mm) but equal and reactive. The patient was comatose and responsive to pain but not to verbal stimuli. Plasma ChE activity was 138 mIU/mL (normal 1800 - 4800 mIU/mL). Initial treatment was gastric lavage, supportive therapy, 2 g of pralidoxine and 3 mg atropine IV. Plasma ChE activity remained around 100 mIU/mL until day 11, with atropine therapy required until day 7. Clinical improvement was noted on days 8 to 9, which was associated with a slight rise in plasma ChE activity. Plasma ChE activity increased relatively rapidly from day 13, returning to normal on approximately day 20.

Bichile LS, Kuloor PL, Hegde AV & Nanivadekar SA (1983) Acute reversible cerebellar signs after diazinon poisoning. Department of Medicine, General Hospital, Bombay, India. JAPI 31: 745-746

A 30-year-old female was admitted 1 h after consuming a bottle of diazinon (Tik-20). On admission, she was drowsy, with bradycardia and an increased respiratory rate. Blood pressure was normal. Muscle fasciculations and pinpoint pupils were observed. She was treated with gastric lavage, atropine, 2-PAM and IV fluids. The signs of OP poisoning resolved over the next 36 h of treatment (41 mg atropine administered in total). On day 3 after admission, the patient reported being unable to hold objects in her hands. On neurological examination the patient was unable to walk, although she had normal power in all extremities. There was lateral nystagmus and gross incoordination on the finger-nose test and heel-knee test. Sensory test (pinprick, touch and temperature) were all normal, and the biceps, triceps, knee and ankle jerks were brisk. A diagnosis of bilateral cerebellar signs was made, and the patient was treated with prednisolone; coordination improved after 48 h of treatment. Recovery was complete in one week, and the patient was discharged.

Hase NK, Shrinivasan J, Divekar MV & Gore AG (1984) Atropine induced ventricular fibrillation in a case of diazinon poisoning. Dept of Medicine, L.T.M.M. College and Hospital, Bombay, India. JAPI 32: 536

A 26-year-old male was admitted to hospital 2 h after consuming 50 mL of Tik-20 (diazinon). He was drowsy, with bradycardia, pinpoint pupils, sweating, cyanosed and tachypnoeic. Chest examination indicated pulmonary oedema. He was intubated and given 5 mg atropine IV every 5 min until the pupils were fully dilated (40 mg atropine within 30 min). Ten minutes after the final atropine dose, the patient had ventricular tachycardia, which degenerated to ventricular fibrillation. He was converted to a normal sinus rhythm using an electric shock, maintained on a lignocaine drip, and recovered uneventfully. It was proposed that the hypoxia, pulmonary oedema and bradycardia induced by the muscarinic effect of diazinon lowered the threshold for ventricular tachyarrhythmia. This threshold was further lowered by the rapid atropinisation.

Wedin GP, Pennente CM & Sachdev SS (1984) Renal involvement In organophosphate poisoning. Maryland Poison Center, Maryland, USA. JAMA 252: 1408

A 26-year-old male presented approximately 1 h after ingesting a mixture (unknown concentration) of diazinon in water. He was conscious, oriented and had vomiting, diarrhoea, bradycardia and hypoactive bowel sounds. Atropine treatment improved clinical signs, and the patient was admitted and treated with IV fluids and atropine. Shortly after admission, monitoring revealed dark and cloudy urine and reduced frequency. On the second day of treatment, there were amorphous crystals in the urine that were not able to be identified by the hospital laboratory. IV fluids were increased, and treatment with pralidoxime commenced. Amorphous crystalluria persisted until day 9 of hospitalisation. Serum creatinine and urea nitrogen levels remained unchanged, indicating normal renal function. Direct renal toxicity is not generally associated with OP poisoning, however, there have been a few reports of acute renal failure in which an immune complex nephropathy was postulated. It is possible that these findings are related to a nephrotoxic substance present in the formulation, rather than being directly related to the active constituent.

Hata S, Bernstein E & Davis LE (1986) Atypical ocular bobbing in acute organophosphate poisoning. Division of Emergency Medicine, University of New Mexico, Albuquerque, New Mexico, USA. Arch Neurol 43: 185-186

A 20-year-old woman was admitted to hospital after complaining of blurred vision and collapsing. She was comatose, unresponsive to commands and had pinpoint pupils. She was intubated, and initially treated with naloxone and glucose without effect. On admission, pinpoint pupil, decerebrate positioning and vertical nystagmus were noted. Later, bradycardia, hypotension and deep coma developed, which was treated with atropine. After 3 h she was conscious and responsive, displaying full conjugate voluntary gaze with episodes of atypical ocular bobbing (ocular bobbing with preserved movement on the horizontal plane). Occasional instances of decerebrate posturing were seen, with hyperreflexia but without limb weakness. A case history of diazinon ingestion (unknown quantity) was subsequently obtained. Plasma ChE activity was depressed for 4 days. After this time the neurologic examinations returned to normal and the bronchial secretions gradually decreased. It was proposed that the ocular bobbing was produced by a disturbance in the cholinergic transmission at the brain-stem level, probably in the tectal and pretectal areas.

Halle A & Sloas DD (1987) Percutaneous organophosphate poisoning. Dept of Internal Medicine, University of Tennessee Center for the Health Sciences, Memphis, Tennessee, USA. Southern Med J 80: 1179-1181

A 58-year-old man was admitted to hospital after applying approximately 5 mL of diazinon to his genital area to treat pubic lice. He had complained of thirst and profuse sweating prior to asking to be taken to hospital. He regularly took a range of medication, including phenytoin, phenobarbital, hydrochlorothiazide, spironolactone, clonidine and insulin. On arrival, the patient had a seizure, and was unconscious with salivation and shallow respiration. His clothing smelt of insecticide. There was left periorbital oedema and bilateral chemosis. The pupils were small and minimally reactive to light. The patient did not respond to deep pain, and corneal and doll's eye signs were absent. Extremities were flaccid with no muscle fasciculations and deep tendon reflexes were absent. The patient was intubated and activated charcoal and magnesium citrate were administered via nasogastric tube (no case history was available at this time). Atropine and pralidoxime were administered IV, and his clothing was removed. He was also given α -methyldopa, phenytoin and potassium chloride. He gradually recovered consciousness after

approximately 15 minutes. When vital signs stabilised and secretions decreased, the patient was extubated. He recovered without incident and was discharged 6 days after admission. The plasma ChE activity at the time of admission was 1 IU/mL (normal 7 to 19 IU/mL). The author noted the high absorption of OP pesticides from the scrotum, and indicated that this may have contributed to the severity of the symptoms.

Zwiener RJ & Ginsburg CM (1988) Organophosphate and carbamate poisoning in infants and children. Department of Pediatrics, University of Texas Health Sciences, Dallas, Texas, USA. Pediatrics 81: 121-126

A retrospective review of 37 children admitted to hospital with either organophosphorus or carbamate poisoning was done. The children were aged between 1 month and 11 years, with a median age of 22 months. Males made up 54% of the individuals. The pesticide involved could not be identified in all cases, however six children were poisoned with chlorpyrifos, five with diazinon, two with malathion and two with chlorfenvinphos. Most poisonings occurred in the home, with 76% the result of oral ingestion, mainly following improper storage of pesticides. Sixteen percent were probably the result of dermal exposure, with five of these cases occurring within 48 h of the home being sprayed or fogged.

Signs and symptoms on admission included miosis in 73%, salivation in 70%, muscle weakness in 68%, respiratory distress in 59%, lethargy in 54% and tachycardia in 49%. Tonic-clonic convulsions were seen in 22% of cases; this is not a common sign in adults (around 2% was cited), but it was felt that hypoxia may have contributed to the increased frequency of this effect, particularly in young children. The difficulty of diagnosis in the young child was highlighted, with a note that increased urination and defecation may be difficult to determine where the child is not toilet trained, and that lacrimation may be difficult to distinguish from the normal tearing associated with distress and pain.

Most cases required repeated treatment with atropine and pralidoxime, while 36% required intubation and assisted ventilation. Erythrocyte ChE was determined in 24/37 cases: in sixteen of these levels were decreased to less than 50% of the lowest normal value. The frequency of accidental poisoning of children with pesticides in the home was highlighted and its importance emphasised.

Lee HS (1989) Acute pancreatitis and organophosphate poisoning - A case report and review. Department of Industrial Health, Ministry of Labour, Singapore. Sing Med J 30: 599-601

Two female horticultural workers were exposed to diazinon when an open bottle in a store room spilled onto the back of one of the workers. As she changed her clothes, her companion mopped up the spilt pesticide with rags. Approximately 3 h later the worker who cleaned up the spill became giddy and had diarrhoea and vomiting. She was admitted into hospital and was frothy at the mouth, cyanotic, tachypnoeic and drowsy. The history of pesticide exposure was not explained, and despite a clear chest x-ray was treated for pulmonary disease with furosemide, aminophylline and morphine, intubated and maintained on a respirator. Blood tests indicated serious disruption of the clinical chemistry, with raised LDH, ALT and CPK levels, as well as hyperglycaemia, hypokalaemia and an increased leucocyte count. She improved, and was removed from the respirator. Later in the evening she vomited and had epigastric pain. Serum amylase levels were increased approximately 10 fold over normal levels. She was treated for acute pancreatitis and recovered, with enzyme levels returning to normal over the next 3 days.

The second worker developed nausea, vomiting, diarrhoea, abdominal pain and dizziness approximately 7 h after exposure. She was admitted to hospital 4 h after first showing signs, and was treated for pesticide poisoning with atropine. Plasma ChE levels were decreased by approximately 75% in comparison to normal values, increasing over the next 4 days to be within 25% of normal values. On clinical chemistry evaluation, there was hyperglycaemia, hypokalaemia and an increased leucocyte count. Serum amylase levels were not determined, and it was therefore not possible to determine if an underlying pancreatitis was present.

Given the role of acetylcholine in stimulation of the acinar cells of the pancreas, the link between OP poisoning and pancreatitis is conceivable. It would appear that other risk factors for pancreatitis may also be involved, given that not all poisoning victims develop the symptoms of acute pancreatitis.

Samal KK & Sahu CS (1990) Organophosphorus poisoning and intermediate neurotoxic syndrome. Department of Medicine, Medical College, Burla, India. JAPI 38: 181-182

A 20-year-old male ingested an unknown volume of diazinon, and was admitted into hospital 6 h later after vomiting at home, and being treated with atropine (6 mg; route not specified). On admission the patient was conscious with no muscle fasciculations or twitching. His pupils were slightly constricted but reacted to light. He was maintained on atropine as required and 2-PAM at 1 g IV every 12 h. He recovered uneventfully until the second day after admission when he developed difficulty in swallowing and was unable to move any limbs. A neurological examination revealed deficits of function in the 6th and 7th cranial nerves with some effects on 9th and 10th cranial nerves. Distal muscle groups were more severely affected than proximal groups, and deep tendon reflexes were absent. There was no sensory loss. Some muscle fasciculation became evident, but no respiratory paralysis was present until approximately 20 h after the first signs of weakness were noted. The patient died of respiratory failure approximately 5 h after the onset of respiratory paralysis.

Richter ED, Kowalski M, Levanthal A, Grauer F, Marzouk J, Brenner S, Shkolnik I, Lerman S, Zahavi H, Bashari A, Peretz A, Kaplanski H, Gruener N & Ben Ishai P (1992) Illness and excretion of organophosphate metabolites four months after household pest extermination. Hebrew University School of Public health and Community Medicine, Jerusalem, Israel. Arch Env Health 47: 135-138

A family moved into a new apartment that had been treated with a diazinon containing preparation. Soon after moving in, the mother experienced fatigue, sleep problems and irritability. The infant had vomiting and a runny nose, and a 4.5-year-old had vomiting. The cleaner also reported dizziness, headache and heaviness in the chest when she was in the house. There was a strong odour in the house, and clothes and bedding smelt of a pesticide. Blood and urine samples were obtained from household members for determination of plasma ChE activity and urinary diethyl phosphate concentration. Air samples and wall surface samples were obtained to determine diazinon levels in the house.

Plasma ChE activity measured before clean up of the house but after the family had been in residence for 4 months were reduced by between 6% and 12% in the mother and children (the levels in the father were normal, while the housekeeper was not tested). Urinary diethyl phosphate levels were increased in all family levels, with the highest being found in the father (at 1.7 mg/L), and lowest in the mother (0.45 mg/L). After the house was cleaned by an exterminator

company that included washing the wall surfaces, plasma ChE activity of family members returned to normal while diethyl phosphate was no longer detected in urine.

The wall surfaces were substantially contaminated prior to the clean up; diazinon concentration ranged between 0.13 to 1.1 mg/m². The air levels were also high (2.5 µg/m³). After the cleaning procedure surface levels of diazinon decreased to 0.01-0.08 mg/m², and air levels were below the level of detection. The spraying practices of the company were subsequently investigated, given the high residual levels still present approximately 4 months after the spraying.

Weizman Z & Sofer S (1992) Acute pancreatitis in children with anticholinesterase insecticide intoxication. Dept of Pediatrics, Ben-Gurion University, Israel. Pediatrics 90: 204-206

The occurrence of acute pancreatitis in children who had been exposed to OP or carbamate pesticide, and showed signs of such exposure by both clinical signs and decreased plasma ChE was investigated. All admitted patients with these signs were treated with atropine and obidoxime chloride. Blood samples were taken for determination of glucose, electrolytes, calcium, urea and creatinine levels, serum amylase, immunoreactive trypsin. Arterial blood gases and plasma ChE levels were also determined.

Seventeen children were involved in the study (59% males), ranging in age from one to eight years. The pesticide was identified in 12 of the patients (parathion - 4, malathion - 3, diazinon - 2, and unspecified carbamates - 3). One of the children who had been affected with diazinon was diagnosed with pancreatitis, while overall 5/17 were diagnosed with pancreatitis. Common signs of poisoning included vomiting and diarrhoea. Abdominal pain was also seen; this was severe in two children diagnosed with pancreatitis.

In the patients diagnosed with pancreatitis, there were increased levels of immunoreactive trypsin in comparison to controls; this was also associated with an increase in the serum amylase levels. Hyperglycaemia was also seen in these patients. Four patients had raised serum amylase levels with no abdominal pain or increase in serum trypsin levels. All patients recovered without incident.

The study demonstrates that acute pancreatitis is not rare in children who have ingested OP or carbamate pesticides. It also indicated that a raised serum amylase level can occur without pancreatic involvement; this may occur secondary to a range of disorders, and may be related to hypersalivation. Serum trypsin would appear to be a more specific and sensitive test for diagnosis. It was also proposed that the hyperglycaemia seen relatively frequently following OP poisoning may result from damage to the endocrine islets, and be associated with an excess of glucagon rather than a deficit of insulin.

Abend Y, Goland S, Evron E, Sthoeger M & Geltner D (1994) Acute renal failure complicating organophosphate intoxication. Dept of Internal Medicine, Kaplan Hospital, Rehovot, Israel. Renal Failure 16: 415-417

A 42-year-old male farmer was admitted to hospital after accidental ingestion of 200 mL of a pesticide containing diazinon. On admission he had mild bradycardia, and increased salivation. Plasma ChE activity was 269 IU (normal: above 4000 IU). He was treated with activated charcoal, atropine and abidoxime, along with cathartics and enemas. He was intubated for a few hours to prevent aspiration and maintain a patent airway, but did not require assisted ventilation. One day after admission, his serum creatinine began to rise, peaking on the 7th day. It returned to

normal within 2 weeks. Urine output was between 2 and 5 L/day over this period. Urine sediment was normal, and urinary protein did not exceed 500 mg/day throughout the time. Kidneys were normal on ultrasound examination. The renal failure could not be attributed to any nephrotoxic substances or haemodynamic effects, ie. the pathogenesis was not defined. Previous OP poisoning experiments in rats had indicated that the predominant effect was an increased urinary flow with low osmolality, suggesting there may be a direct effect on tubular function that was unrelated to the degree of ChE inhibition.

Wagner SL & Orwick DL (1994) Chronic organophosphate exposure associated with transient hypertonia in an infant. Dept of Agricultural Chemistry, Oregon State University, Oregon, USA. Pediatrics 94: 94-97

A 12-week-old infant girl developed persistent hypertonicity of the extremities, and, at the age of 8 months, it was discovered that her home had been treated with an excessive and inappropriate application of diazinon 3 weeks prior to the onset of symptoms. At this time (6 months after application) the remaining diazinon residue on the floor was 230 ng/cm², in comparison to the 38 ng/cm² which is expected immediately after a normal application. Vacuum cleaner dust contain 1700 mg/kg diazinon, while air contained 2.8 ng/m³. The infant's urine contained 60 ppb diethylphosphate and 20 ppm diethylthiophosphate, which calculated to a diazinon dose of approximately 0.02 mg/kg bw/day. There was no depression of ChE level in comparison to normal values for a child of this age. When the infant was removed from the home muscle tone returned to normal within 6 weeks and development proceeded normally from this point. Motor abilities and speech were developing normally at 20 months, which was the last reported observation.

Jaksa RJ & Palahniuk RJ (1995) Attempted organophosphate suicide: A unique cause of prolonged paralysis during electroconvulsive therapy. Dept of Anesthesiology, University of Minnesota Hospitals and Clinics, Minneapolis, Minnesota, USA. Anesth Analg 80: 832-833

A 25-year-old woman was admitted to hospital following a suicide attempt, in which she ingested acetaminophen and unknown amounts of a laboratory agent (found to be diazinon). Her initial condition did not reveal any signs related to ChE inhibition, and she was stabilised without incident. The patient was admitted to the psychiatric ward for treatment. Twenty days later she requested ECT, which she had previously received without incident. For the therapy, she was anaesthetised with methohexital, *d*-tubocurare, atropine and succinylcholine. Following ECT, she was slow to resume spontaneous ventilation, requiring assisted ventilation for 12 minutes. She remained weak for the next 25 min, but was able to breathe spontaneously. Blood samples revealed plasma ChE activity of 1.1 IU/mL (normal 5.9-12.2 IU/mL). It was planned to track plasma ChE activity with serial blood tests, however the patient left the hospital the next day. The plasma ChE inhibition in this case appears particularly prolonged, especially given the lack of significant signs at the time of poisoning.

12. REFERENCES

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APPENDIX I - Effect of ageing on the LD₅₀ and composition of '90% diazinon'

(From Nichol et al, 1982)

Compound	Percentage composition		LD₅₀ (mg/kg bw)
"90% diazinon" (at receipt)	Diazinon	94.0	170
	Isodiazinon	0.8	
	2-Isopropyl-4-ethylthio-6-methylpyrimidine	0.06	
	2-Isopropyl-6-methyl pyrimidine-4-thione	0.7	
	2-Isopropyl-6-methyl pyrimid-4-one	2.5	
	SulfoTEPP (S,S-TEPP)	0.6	
	O,O-TEPP	0.4	
"90% diazinon" (stored for 1 year at room temperature under nitrogen)	Diazinon	74.0	30
	Isodiazinon	15.0	
	2-Isopropyl-4-ethylthio-6-methylpyrimidine	3.2	
	2-Isopropyl-6-methyl pyrimidine-4-thione	1.0	
	2-Isopropyl-6-methyl pyrimid-4-one	1.2	
	SulfoTEPP (S,S-TEPP)	2.5	
	O,O-TEPP	2.8	
Diazinon	Pure by HPLC		470
Isodiazinon	Pure by HPLC		65