AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY

CHEMICAL REVIEW PROGRAM

Consolidated Human Health Risk Assessment

FOR

DIAZINON

Part 2 – Toxicological Hazard Assessment

prepared by

Office of Chemical Safety
Department of Health and Ageing
Canberra

Contents

2. PART 2 - TOXICOLOGICAL HAZARD ASSESSMENT

2.1. OVERVIEW

Diazinon[1] is 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 since the 1950s. As of 23 September 2011, there were seven diazinon active constituents approved and 45 registered products containing diazinon, which together have a multitude of approved uses in Australia. Diazinon is presently in Schedule 6 (in Schedule 5 for dust preparations containing 2% or less of diazinon) of the SUSDP. Following the removal of Appendix L from the SUSDP in 1994, which specified a maximum impurity limit of 2 g/kg for dithiono-tetraethyl pyrophosphate (S,S-TEPP) only, the APVMA 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 monothiono-tetraethyl pyrophosphate (O,S-TEPP). The current Australian acceptable daily intake (ADI) is 0.001 mg/kg bw/day and the acute reference dose (ARfD) is 0.01 mg/kg bw.

This component report brings together toxicological hazard assessments, for the human health assessment of diazinon, prepared by the Office of Chemical Safety (OCS) since a review of diazinon was initiated by the APVMA in December 1996. Prior to the publication of the consolidated report, a number of parts contained within the report have been published previously, but may have been updated or superseded. An attempt to amend errors and omissions in previous reports has been made, and to update the content to reflect the existing regulatory environment. However, due to the size of the report minor inconsistencies may persist. These do not alter the final position of the regulator, as detailed in the findings component of the review report.

2.1.2. Toxicokinetics and metabolism

2.1.2.1 Intravenous administration

Diazinon (10 mg/kg bw) administered intravenously (IV) to sixteen male Wistar rats, followed by plasma sampling at various times up to eight hours after dosing, was rapidly cleared (clearance, CL = 4.69 L/h/kg) resulting in a half-life of 4.7 hours in plasma. The volume of distribution at steady state was calculated to be 20.01 L/kg and the area under the concentration-time curve (AUC) 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 IV injection into the left jugular vein, was 54.8% and 47.7% with doses of 5 mg/kg bw or 10 mg/kg bw respectively. The unbound fraction in plasma, determined by ultrafiltration over the range of 0.4-30 μg/mL, was 10.9% (Wu et al., 1996).

2.1.2.2. Oral administration

Diazinon administered to sixteen male Wistar rats by stomach tube at a dose of 80 mg/kg bw was detected in blood by gas chromatography (GC; fitted with an electron capture detector) after 30 minutes (first sampling time), with the mean maximum concentration (Cmax 1.22 μg/mL) being

[1] IUPAC nomenclature: *O*,*O*-diethyl-*O*-(2-isopropyl-6-methylpyrimidin-4-yl)-phosphorothioate

observed at two hours (Tmax). The plasma AUC 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 corresponded to a half-life in plasma of 2.86 hours. 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 (Wu et al., 1996).

2.1.2.3. 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 hours. 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 two hours, suggesting probable ingestion of some of the dermally applied dose (Williams & Marco, 1984). Given the rapid rate absorption of diazinon from the gastrointestinal (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 occupational health and safety purposes was not possible (Wu et al., 1996).

Diazinon (20% w/v), applied to a dorsal neck skin region of dogs at 20 mg/kg bw was detected in the blood within one hour. The maximal mean concentration of diazinon (Cmax=19 ng/mL) was achieved after 8-12 hours 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 hours and 84 hours were calculated (Ferrandes, 1990).

During daily dermal application of ring-labelled [^{14}C]-diazinon (~40 mg/kg bw) in acetone to sheep for three 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).

2.1.2.4. Tissue distribution

In male Yok:ddY outbred strain mice, kidney, liver and brain concentrations of diazinon after intraperitoneal (IP) administration of 20 or 100 mg/kg bw were higher than in the blood (no other tissues examined). The calculated half-life in plasma for unchanged diazinon was approximately 2.5 hours and the highest concentration was observed in the kidneys. In tissues other than kidneys, the concentration had declined to ≤ 8% of the peak concentration (Tmax ~1 hour) eight hours after dosing. Maximal tissue concentrations in kidney, liver and brain were 45-, 20- and 2.5-fold respectively greater than that in blood, i.e. 750 and 100 ng/mL for 20 and 100 mg/kg bw respectively at Tmax (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 one to two hours and the half-life was approximately three hours. However, the diazinon concentration in the liver eight hours 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) two hours after IV administration to male Wistar rats (Wu et al., 1996).

Unlabelled diazinon (dose not specified, although was said to be one third of an oral LD_{50}) 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 four 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 [^{32}P]-diazinon by capsule ten hours 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 diethyl phosphate 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 diethyl phosphate (694 μg eq/mL, combined radioactivity) five hours after dosing (Millar, 1963).

Oral administration of ring-labelled [^{14}C]-diazinon (150 mg/day) in gelatin capsules for four consecutive days resulted in substantial levels of radioactivity being present in the kidney (2 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 hours 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 hours 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 seven-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) which gradually declined so that 14 days after cessation, the levels were undetectable (<0.05 μg/g).

2.1.2.5. Excretion

Although about 70% of the total radioactivity found in the urine of rhesus monkeys was excreted during the first 24 hours after IV dosing, faecal excretion of radioactivity continued at a steady rate over a seven-day monitoring period suggesting an enterohepatic recirculation (Wester et al., 1993).

Biliary excretion is also an important elimination pathway in rats; individual [2'-^{14}C]-labelled metabolites, G27550, GS31144 & metabolite (i) (see Figure 2.1 for metabolite structures), injected intravenously (to ascertain the formation sequence) were excreted in faeces (7 to 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 hours. An earlier study by Mücke et al. (1970) reported slightly lesser quantities in urine after a 4 mg/kg bw dose, i.e. 75%. Most of the remaining dose in each study was excreted in faeces, i.e. 2.5-2.8% and 20% for the Capps (1989) and Mücke, et al. (1970) 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 four days) with the mean concentration ranging between 0.33 and 0.46 μg eq/mL. This concentration was similar to that found in whole blood namely, 0.36 to 0.43 μg 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 hours. During the first 24 hours after dosing, approximately 25 to 33% of the detected 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 conversion processes for diazinon have been identified (see Figure 2.1 for pathway and chemical structures). These involve: transformation of the P-S moiety to the P-O derivative (i.e. formation of O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphate; i.e. 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; i.e. metabolite G27550),
- oxidation of the methyl substituent leading to the corresponding alcohol (metabolite (vii)),
- oxidation of the isopropyl substituent leading to the corresponding tertiary alcohol (O,O-diethyl O-[2-(α-hydroxyisopropyl)-4-methyl-6-pyrimidinyl] phosphorothioate; i.e. metabolite CGA14128), and
- glutathione mediated cleavage of the ester bond leading to the glutathione conjugate (glucuronide conjugate of G27550; i.e. metabolite (vi)).

Significant amounts of the pyrimidinol derivative, G27550 are oxidised at the isopropyl moiety thereby giving rise to the hydroxy derivatives, GS31144 & metabolite (i). This was demonstrated by injecting these individual [2'-^{14}C]-labelled metabolites intravenously in rats. Apart from some conversion to several uncharacterised aqueous polar metabolites for GS31144 (approximately 17% of recovered radioactivity in urine), GS31144 & metabolite (i) were excreted unchanged whereas G27550 was excreted in combination with approximately equal amounts of GS31144 & metabolite (i). These results have been substantially confirmed in a study by Capps (1989). The absence of any exhaled radioactive carbon dioxide after oral administration of radiolabelled [2'-^{14}C]-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 diethyl phosphate, 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 [^{32}P]-diazinon. Approximately 70% of the radioactivity excreted in urine was found to be in the form of diethylphosphorothionate and diethyl phosphate (Robbins et al., 1957). The same two urinary metabolites were also observed in a dog given [^{32}P]-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 three major esterified phosphorothionate metabolites in urine after 72 hours, as judged by co-1migration with control standards on thin layer chromatography (TLC) and their reactivity with esterases. Spectroscopic techniques, i.e. nuclear magnetic resonance, mass and infrared, confirmed their identity as hydroxydiazinon (CGA14128), isohydroxydiazinon (metabolite vii) and dehydrodiazinon (metabolite 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 six hours 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), i.e. in rate order, isohydroxydiazinon (metabolite vii) > hydroxydiazinon (CGA14128) >>> dehydrodiazinon metabolite (v) > G24576 (diazoxon) > metabolite (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-^{14}C]-diazinon suggested that diazoxon (G24576) was rapidly produced and subsequently hydrolysed to diethyl phosphate 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-^{14}C]-diazinon gave rise to both diethyl phosphate and diethylphosphorothioate, whereas with [ethoxy-^{14}C]-diazoxon, only diethyl phosphate was formed. No direct evidence of any desethylation was found.

Radioactivity in goat 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).

2.1.3. Acute toxicity

The acute toxicity of technical diazinon in mammals is moderate (LD_{50}50-500 mg/kg bw). The oral LD_{50} of stabilised diazinon in rats ranged from 300 to 1350 mg/kg bw in a variety of vehicles (Boyd & Carsky, 1969; Bathe, 1972b, 1980; Gains, 1969; Nissimov & Nyska, 1984a; Piccirillo, 1978; Schoch, 1985a, b; Yoshida, 1978). Non-stabilised diazinon, i.e. diazinon produced and used in pre-1969 studies, also had moderate acute oral toxicity (LD_{50} ranging between 76 - 466 mg/kg bw) (Bruce et al., 1955; Boyd & Carsky, 1969; Gains, 1960).

The acute dermal toxicity of stabilised diazinon was moderate in rats (LD_{50} 876 - >2150 mg/kg bw) and rabbits (LD_{50} 960 - 3500 mg/kg bw) (Bathe, 1972c; Yoshida et al., 1978).

The acute inhalation toxicity of stabilised diazinon was low in rats. Whole-body inhalation toxicity (LC_{50}) ranged between 3100 and 4370 mg/m^3 (Hardy & Jackson, 1984; Holbert, 1994; Jackson et al., 1987; Sachsse, 1972e), whereas for nose-only exposure the lethal concentration was in excess of 5437 mg/m^3 (Cummins, 1985; Holbert, 1994). In mice, death from whole-body inhalational exposure to stabilised diazinon occurred at a markedly lower concentration relative to rats, i.e. the LC_{50} was determined to be 1600 mg/m^3(Sachsse 1972a).

Signs of acute toxicity (oral, dermal, inhalation, IP) were those typically seen in organophosphate intoxication and included muscarinic effects (diarrhoea, hypersalivation, pupil constriction), nicotinic effects (muscle fasciculations and fatigue) and central nervous system effects (ataxia and convulsions) (Aardema et al., 2008)).

Technical diazinon was a slight eye and skin irritant (Sachsse, 1972h, g; Nissimov, 1984a, b; Hayashi & Yoshida, 1979a, b; Kuhn, 1989c, d). Similarly, there was evidence of skin sensitisation (Cummins, 1987; Kuhn, 1989e).

The acute oral toxicity of formulated products varied, with LD_{50}s ranging from 293 to >5050 mg/kg bw. Dermal toxicities were generally low (>1000 mg/kg bw) (Edson & Noakes, 1960; Hartmann & Schneider, 1987b; Lheritier, 1989a, b; Mercier, 1995a, b; Syntex, 1985) Microencapsulated formulations also have very low acute oral (LD_{50} >5000 mg/kg bw) and low dermal toxicity (LD_{50} >2000 mg/kg bw) (Mallory, 1993a, b; Kuhn, 1993a, b). Generally, the products were slight eye and skin irritants and did not sensitise skin (Armondi, 1993; Kuhn, 1993c, d, e; Mallory, 1993c, d; Mercier, 1989a, b; Mercier, 1995c, d, e; Schneider & Hartmann, 1987a, b; Schneider & Gfeller, 1987).

2.1.3.1. Effect of ageing of diazinon 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 technical grade active constituent 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 diethyl phosphate (Margot & Geysin, 1957). However, these intermediates are able, under catalytic influence of poorly characterised factors, to combine to form highly toxic tetraethyl pyrophosphate (O,O-TEPP), O,S-TEPP or S,S-TEPP. Median lethal dose studies, performed in 1989 but not reported until 1995, have confirmed the markedly increased toxicity of these three compounds in female rats, i.e. 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 (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 technical grade active constituent (Spindler, 1969; Sterling, 1972). However, a 1993 survey sponsored by the NRA (now the APVMA) 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 active ingredient (ai) (for S,S-TEPP). All seven (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 ai established for O,S-TEPP. The third

potential impurity in diazinon, namely O,O-TEPP, was not quantified due to the absence of an appropriate reference standard. Five of the seven batches in which O,S-TEPP was detected also contained S,S-TEPP in excess of the benchmark limit. Hence, 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. Thirty-five batches tested (or 22%) were found to exceed the label-stated diazinon concentration by ±10% (McDonald, 1993, 1994; Allender & Britt, 1994).

The acute oral LD_{50} in rats (strain and source not specified) of high performance liquid chromatography (HPLC)-purified diazinon, 90% technical diazinon or 90% technical diazinon stored for one year was 470, 170 and 30 mg/kg bw respectively. The composition of 'new' and 'aged' 90% diazinon used in this study highlights increases in S,S-TEPP (4x), O,O-TEPP (7x) and isodiazinon (19x) concentration with ageing. The acute oral LD_{50} of pure isodiazinon was 65 mg/kg bw and together with S,S-TEPP and TEPP may have been responsible for the increased toxicity of 'aged' diazinon (Nichol et al., 1982).

S,S-TEPP in three commercial and three 'military' diazinon formulations, i.e. as a dust, emulsifiable concentrate (EC) and in an oil solution, was detected by gas chromatography/ mass spectroscopy (GC/MS). Although the percentage of S,S-TEPP 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 S,S-TEPP, it was reasoned that the formulation age was not a useful predictor for the estimating S,S-TEPP content in formulations. The authors concluded that S,S-TEPP was most likely to be formed during the synthesis of diazinon with diethyl thiophosphorylchloride (Meir et al., 1979).

Several different diazinon formulations (EC; liquid concentrate, LC; and dust) at various strengths 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).

In the presence of a small quantity of water, in the order of 0.2 to 2.0%, diazinon decomposes to give the highly toxic degradation products. It is important to exclude water by addition of additives that absorb water and hence prevent hydrolysis of diazinon. The stability of hydrocarbon-based EC formulations depends on several factors including composition of the formulation, water content of the formulation (traces of moisture may be present in solvents and other excipients used), storage conditions (temperature, moisture uptake, container type, ultraviolet light etc.), and amount of stabiliser added. Products containing diazinon that are based on hydrocarbon solvents formulated without adequate stabiliser are considered a risk to public health and animal safety.

2.1.3.2. Antidote studies

The effects of atropine-oxime therapy on cholinesterase (ChE) activity after acute diazinon poisoning, was investigated in rats and rabbits. Rats treated with atropine and 2-PAM demonstrated significant reactivation of diaphragm ChE levels, i.e. 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 two hours 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).

2.1.3.3. Enzyme activity effects

Technical diazinon administered by IP injection to eight female Wistar rats at a dose of 40 mg/kg bw resulted in tremors and convulsions. Treated rats euthanased after these effects became maximal, i.e. after two hours, 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 because of the stimulatory effects of diazinon (Matin & Husain, 1987).

Very little regional variation (i.e. 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 red blood cells (RBCs) where significant inhibition became apparent at the next higher dose of 150 mg/kg bw. Irrespective of its location (brain, spinal cord, plasma or RBCs), ChE activity became inactivated (significantly) after three hours but the maximum inactivity was observed nine hours after dosing. Little or no recovery of ChE activity was evident 24 hours 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).

2.1.3.4. 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 two-fold rise in mean intraductal pressure, an increased secretory rate (four-fold), acinar cell vacuolisation, hyperamylasemia (seven-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 butyryl ChE in the pancreatic acinar cells and ampullary-sphincteric smooth muscle. In guinea pigs, another species with abundant tissue-fixed butyryl ChE, pancreatic lesions similar to those observed in dogs were found. It was concluded that inhibition of pancreatic butyryl ChE 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 butyryl ChE present in periampullary nerves and pancreatic smooth (sphincter) muscle whereas acetyl ChE activity in the periampullary 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 organophosphate, OP) or acetylcholine had a similar number of acinar cell cytoplasmic vacuoles. 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).

2.1.4. Short-term repeat-dose toxicity

Wistar rats fed diazinon in the diet at 2 ppm for seven days or at 25 ppm for thirty days had no clinical signs although significant ($p \leq 0.05$) inhibition of ChE in plasma and RBCs 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 seven-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 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(i.e. 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/m^3 for 6 h/day, 5 days/week for 21 days were found to have clinical signs of poisoning (exophthalmos and diarrhoea) and a significant ($p \leq 0.01$) reduction in brain and plasma ChE activity at concentrations $\geq$ 245 mg/m^3. 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/m^3, this cannot be considered biologically plausible due to the absence of a corresponding significant inhibition of blood ChE activity (i.e. in plasma and/or RBCs). 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/m^3 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/m^3 after one day to a maximum of 1.21 µg/m^3 after 30 days (no further measurements taken), however, no corresponding inhibition of ChE activity was detected in either RBCs 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/m^3 in whole-body chambers for 6 h/day, 5 days/week for 28 days had clinical signs of cholinergic intoxication (tremors, abnormal respiratory movements, hypersalivation and gasping) at 710 mg/m^3 and significant dose-related reductions in RBC and plasma ChE activities at all tested concentrations

(i.e. 15, 97 and 710 mg/m^3). Relative to males, females tended to have more marked clinical signs together with a correspondingly increased degree of ChE inhibition in plasma, RBCs and brain. Other changes, possibly related to treatment at the highest concentration of 710 mg/m^3, were significantly ($p<0.05$) reduced total protein (males 6%, females 11%), globulin (males 15%, females 11%), creatinine (males 17%), together with an elevated A/G (males 20%, females had reduced albumin - 11%) and alkaline phosphatase (AP) (males 25%, females 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/m^3 for 6 h/day, 5 days/week for 21 days had no clinical signs of toxicity but had significant dose-related reductions in brain, RBC and plasma ChE activities in females at concentrations $\geq$ 0.46 mg/m^3. 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/m^3 and some RBC parameters, i.e. reduced RBC and haemoglobin (Hb), and increased mean corpuscular volume (MCV) in females at 11.6 mg/m^3 (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 three weeks caused the death of 4/5 males at 100 mg/kg bw within six days. The dose was reduced to 50 mg/kg bw/day on day seven resulting in a reduction in the number and severity of clinical signs among survivors. Whereas anorexia, hypoactivity, ataxia, fasciculations, muscular hypotonia, tremors, hypersalivation 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 two) 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 albumin/globulin (A/G) ratio at 100/50 mg/kg bw/day together with reduced ChE activity in plasma at all doses and in RBCs at 100/50 and 5 mg/kg bw/day was observed among females. Irritation of the stomach and reduced ChE activities in plasma and RBCs were the major findings for the sole surviving male at 50 to 100 mg/kg bw/day (Tai & Katz, 1984).

2.1.5. Subchronic toxicity

A series of three 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). This may be explained by the reduction in both brain excitatory neurotransmitters (Asp and Glu) and inhibitory neurotransmitters (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 approximately 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 RBC ChE activity was reduced by more than 20% relative to controls from week two at 5 mg/kg bw/day and from

week twenty at 0.5 mg/kg bw/day. The RBC 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 electromyography 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 RBC ChE activities were observed at the lowest tested dose of 0.5 mg/kg bw/day, a no observed effect level (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, RBCs 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 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. Cholinesterase inhibition in the brain did not achieve significance ($p \leq 0.05$) at any dose, whereas significance in RBC ChE inhibition was achieved after 42 days at concentrations in excess of 1 ppm, and in plasma ChE inhibition 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 (approximately 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 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 mid-period bodyweight, enabled the dosage level in each treatment group to be calculated. For males, it 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 (Singh et al., 1988).

Ophthalmoscopic examination did not reveal any changes attributable to treatment. The haematological assessment in females revealed dose-dependent changes in erythrocytic parameters (i.e. erythrocyte count, 1.3, 1.8, 2.8 and 9.5% respectively; Hb, 1.5, 2, 3.6 and 4.1%; haematocrit

(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 ($p\leq0.01$) inhibition of ChE activity in the RBCs of females and the plasma of males and females at 5 ppm, whereas ChE activity in the brain was significantly inhibited only once, when 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 (males 2.2%, females 1.6%; $p<0.01$ for both) at 2500 ppm, that was associated with non-significant reductions in urine volume (males 20%; females 17%) and water consumption (males 17%; females 12%), urinalysis was similar among treatment groups (Singh et al., 1988).

Macroscopic inspection of organs at autopsy 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 RBC ChE inhibition in females at 5 ppm (Singh et al., 1988).

A published report indicated that diazinon fed to groups of ten 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 ai /kg bw/day) for sixteen weeks resulted in no treatment-related deaths or clinical signs. At the conclusion of the study RBC ChE activity was depressed to 90% and 81% of controls at 1 and 5 ppm respectively. At 25 ppm and 125 ppm, RBC ChE activity decreased to 54% and 20% respectively of the controls after four 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 RBC 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 dosed at 0.04 mg/kg bw/day uneventfully delivered six pups on day 26 of the study (the author does not state 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 units 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 sixteen days at 0.02 mg/kg bw/day, to 38% after eight days at 0.04 mg/kg bw/day and 62% at day 33 at 0.08 mg/kg bw/day. In RBCs, ChE activity was in excess of the pre-test value at all doses and all sampling times except for 25% inhibition at 0.08 mg/kg bw/day on day eight (Weir, 1957b). 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.

In a published study designed to monitor plasma and RBC ChE inhibition, diazinon at 0, 0.25, 0.75, or 75 ppm (as ai) 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 twelve weeks. However, treatment-related effects other than ChE inhibition were not measured or reported. Although the results for plasma and RBC 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 RBCs, the ChE activity was only significantly inhibited (by 55%) at 75 ppm. Recovery in plasma was complete after about four weeks whereas the ChE activity in RBCs 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, Beagle dogs (4/sex/group) were fed diazinon 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 (by 34%) resulting from treatment was observed in females at 150 ppm and in both sexes at 300 ppm (males, 33%; females, 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 (Barnes et.al., 1988).

Ophthalmoscopic examination did not reveal any observable 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 (i.e. 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 RBCs, plasma and brain with significant inhibition ($p\leq0.01$) being achieved among all dogs at 150 and 300 ppm. Males at 0.5 ppm also had significant ($p\leq0.05$) 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 (Barnes et al., 1988).

Macroscopic examination of the tissues removed at the euthanasia 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 *Pancreatitis*). 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 eight 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, one or both dogs at each of the tested doses died. Autopsy, performed on dogs surviving more than a day (i.e. 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 eight dogs examined. 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 (Earl et al., 1971).

At the highest dose of 20 mg/kg bw/day, all three males and a female died or were euthanased *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 were observed among the dogs prior to death or euthanasia. 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 three 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 (Earl et al., 1971).

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 euthanased. 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 five 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 two male dogs. Markedly increased serum amylase were also observed in these two dogs that died, as well as in the three surviving dogs at 10 mg/kg bw/day and another at 5 mg/kg bw/day (extent and dog gender not reported) (Earl et al., 1971).

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 five of six dogs, marked oedematous thickening of the jejunum or duodenum occurred with one having a ruptured duodenum resulting in peritonitis while 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; and that 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 (Earl et al., 1971).

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 eight months. Dose selection was based on a dose-ranging study in which survival, necroscopy and histopathology of four 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 euthanased *in extremis*, except for one in the 500 mg/kg bw/day group that survived treatment. Autopsy on pigs treated at 5, 10, 25, or 50 mg/kg bw/day, revealed similar lesions as for dogs (see above), i.e. 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 6/8 pigs from which sections were prepared and examined (Earl et al., 1971).

In the main study, at the highest dose of 10 mg/kg bw/day, only one female survived treatment. The other five 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, i.e. after four months of treatment. At 5 mg/kg bw/day there were no deaths and only one pig had signs that persisted for the first six months of treatment, whereas at 2.5 mg/kg bw/day the only pig that developed clinical signs after 19 weeks was euthanased *in extremis* during week 20. In four of the five pigs that died at 10 mg/kg bw/day, oedematous thickening of the jejunum wall was observed, and one pig had localised mucosal erosion that penetrated into the muscular layers resulting in a marked mucosal seepage throughout the intestines. Three of the five 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 euthanased 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 one pig were associated with this euthanased pig (no individual animal data) (Earl et al., 1971).

Haematological parameters were normal except for a slight elevation in the myeloid:erythroid ratio (two-fold) and a reduced reticulocyte count (four-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 (Earl et al., 1971).

2.1.6. 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, which indicated no change in survival or bodyweight at concentrations up to 200 ppm in mice and 800 ppm in rats (Wheeler et al., 1979).

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, (i.e. 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), there was no treatment-related increase in the total number of tumours in treated mice relative to controls. A NOEL for clinical signs could not be established (Wheeler et al., 1979).

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), i.e. 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 (Wheeler et al., 1979).

Thus, under the conditions of this study, 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 seven and 157 days, however, a stability analysis showed that by day seven, 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 eight months on the study outcome could not be determined (Kung et al., 1980).

During the first eleven 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 tumours, was initiated. After month fourteen, the frequency of palpation and clinical signs monitoring was reduced to monthly intervals (Kung et al., 1980).

Apart from a marked increase in the number of deaths following a three-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 was comparable in all groups, i.e. 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, i.e. alopecia, skin lesions, and skin irritation and piloerection (Kung et al., 1980).

Significant ($p<0.05$ - 0.01) loss of bodyweight was observed among all treated males during the first two 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 (i.e. 13%, 11% and 12% at 4, 20 and 100 ppm respectively at month eighteen) throughout the study except for months 6, 13and 19. Macroscopic examination of tissues taken from decedents and those at the scheduled euthanasia 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 uterii 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 were no evidence of any inflammatory, degenerative, proliferative or neoplastic lesions amongst treated males and females (Kung et al., 1980).

In view of the unknown doses actually administered, this study can only be considered as supplementary data to assess carcinogenicity potential. However, for rats fed diazinon in their diet at a concentration that caused significant bodyweight loss, no increase in tumour incidence was observed (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 eleven 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 seven of an unspecified number of occasions in females. However, although the overall food consumption in males was approximately 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 (Goldsmith & Craig, 1983).

Few haematological changes appeared to be related to treatment except for a reduced number of segmented neutrophils in females after 24 months. This reduction, i.e. 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, i.e. liver (by 12%) and kidney (by 11%) in females at 400 ppm after 24 months are also possibly treatment related (Goldsmith & Craig, 1983).

No consistent treatment-related increase in incidence of palpable masses occurred among mice, the number of palpable masses per mouse or percent of mice with palpable masses over the duration of the study. A statistical analysis of the tumour 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 tumours 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 euthanasia was a reduced Hct among males at 1000 ppm. Reductions in ChE activity in brain, RBCs and plasma were dose related, with the greatest reduction being observed in plasma followed in order by that in RBCs and the brain (statistical significance levels not available). However, the reporting of the gross and histopathological observations was brief that independent evaluation of the study was not possible. 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 (approximately 20%) at the highest dose of 22.5 mg/kg bw/day could be a treatment-related effect, although not statistically significant or dose related. 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, i.e. ulceration (males, twelve; females, nine relative to four and three respectively in controls), acanthosis (fifteen, twelve relative to six and nine), hyperkeratosis (fifteen, fourteen relative to six and nine), granulated submucosal tissue (sixteen, eleven relative to six and six) and hyperplasia of epithelium (seven, six relative to zero and one), 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 euthanased *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 ($p<0.05$) (Ashby & Danks, 1987).

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 seventeen and sixteen weeks respectively whereas significance for the incidence of perianal staining was delayed until week 43. The incidence of perianal staining also achieved significance for males, however all treatment groups were affected unlike females. Somewhat surprisingly, the incidence among males in the control group was initially significantly higher than those in the treatment groups, though after ten weeks this reversed and remained significantly less than observed at 0.1 and 1.5 mg/kg bw/day for the next fourteen 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, i.e. slightly delayed relative to females at 22.5 mg/kg bw/day. No signs were reported for the second study (Ashby & Danks, 1987).

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, i.e. for weeks one to 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, i.e. 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 twelve in females ($p<0.001$) and week one 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 (Ashby & Danks, 1987).

Probable treatment-related changes in haematology were observed for the erythrocytic parameters and lymphocytes in males after twelve weeks of treatment. The RBC, Hb and Hct were reduced in an approximate relationship with dose, i.e. 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%** (where * $p<0.05$; ¶$p<0.01$; ** $p<0.001$). The lymphocyte counts were similarly reduced for the three respective treatment groups, i.e. 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 (males 11%, $p<0.05$; females twenty 2%, $p<0.01$) after twelve weeks and maintained until week 51. Another change in clinical chemistry that was possibly related to treatment was a significant increase ($p<0.001$) in the concentration of uric acid at 25 weeks, i.e. two-fold in males and 1.7-fold in females. However, there were clear treatment-related changes in both acetyl ChE and butyryl ChE activity in plasma at all dose levels with females being appreciably more sensitive to the enzyme inhibition. In contrast, acetyl ChE inhibition in RBCs only achieved significance ($p\leq0.001$) 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, i.e. 22.5 mg/kg bw/day (Ashby & Danks, 1987).

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 euthanasia. 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 (eight relative to zero in controls), acanthosis (nine relative to zero in controls) and hyperkeratosis (eight relative to zero in controls) of the keratinised cells in the stomach that achieved significance ($p<0.05$) among females (Ashby & Danks, 1987).

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 acetyl ChE and butyryl 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 (i.e. with 26.5 ppm of epoxidised soybean oil (ESO); the stabiliser present in the technical formulation), 0.1, 1.5, 125, or 250 ppm ai (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 (Kirchner et al., 1991; Mann, 1993).

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-response 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 (i.e. 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, as 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 (Kirchner et al., 1991; Mann, 1993).

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 RBC 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 four weeks after 52 weeks of treatment had reduced inhibition of plasma (to 6% in males and 10% in females), RBC ChE (1% in males and 7%in females) and brain ChE (5% in males and 9% in females). Despite the increased activity, significance ($p \leq 0.01$) was still achieved for impaired brain and RBC ChE activities in females. Controls fed the diet supplemented with ESO had no inhibition of ChE activity (Kirchner et al., 1991; Mann, 1993).

No treatment-related changes in the incidence of neoplasms, histopathological lesions or tissue weight were 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 two years later by a 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 autopsy, 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 euthanasia, none were increased relative to the control incidence (Kirchner et al., 1991; Mann, 1993).

In conclusion, there was no evidence of carcinogenicity in rats at concentrations that caused a significant reduction of ChE activities in brain, RBCs 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 ai (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, (i.e. 10% and 24% that for controls in males and females respectively) that indicated a dose in excess of the maximum tolerated dose (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 among males at 150 ppm from day fourteen to day seventy. 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 (Rudzki et al., 1991: Mann, 1993).

Apart from significant plasma ChE inhibition among females dosed at 0.5 ppm, all dogs had significant ChE inhibition in plasma and RBCs 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, i.e. 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 two to three fold increase in activity was the 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 (Rudzki et al., 1991; Mann, 1993).

Following literature reports of possible retinal degeneration following administration of organophosphates, the eyes and optic nerve samples preserved from this study were re-assessed two 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 euthanasia (Rudzki et al., 1991; Mann, 1993).

In conclusion, diazinon administered to dogs at 150 ppm (equal to 4.7 mg/kg bw/day in males) in their food caused significant bodyweight loss in males, and inappetence in both sexes. 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 oral (PO) by stomach tube to Rhesus monkeys at doses of 0, 0.05, 0.5, or 5 mg ai/kg bw/day for 6 days/week resulted in clinical signs and reduced bodyweight. The clinical signs appeared treatment-related and predominantly observed at 0.5 and 5 mg/kg bw/day . 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 one 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 (Cockrell et al., 1966).

The median plasma ChE activity was substantially depressed at 0.5 mg/kg bw/day (average 55%), whereas the median ChE activity in RBCs became substantially inhibited (average 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 one monkey at the highest dose and in two monkeys that died during the study following exposure at 0.05 and 0.5 mg/kg bw/day respectively (Cockrell et al., 1966).

The reporting of many important parameters in this study was so limited that independent assessment of treatment-related changes other that for ChE inhibition was not possible. However, since plasma ChE inhibition is normally the most sensitive maker 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).

2.1.7. Reproductive toxicity

Female mice were exposed to diazinon throughout gestation at doses of 0, 0.18 or 9 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 F_2 hybrid mice given diazinon at doses of 0 (control), 0.18 or 9 mg/kg bw/day until parturition or day 22. There were two concurrent control groups. Within six hours 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 euthanased 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 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 treatment-related. This study was not adequate for regulatory purposes (Spyker Cranmer et al., 1978).

In a two-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 euthanased on day four. 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, or 10 mg/kg bw/day. No parental animals died or were euthanased 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 one to four period. The relationship between these findings and treatment was not clear. Gross and histopathological examinations did not reveal any effects that were considered 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 a diazinon wettable powder formulation 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 this dose and no higher dose was tested in this study, 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 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 male fertility, even after removal of the males from the test diets for 60 days (Abd El-Aziz et al., 1994).

2.1.8. Developmental toxicity

Diazinon technical was given to groups of pregnant Sprague-Dawley rats by oral gavage at doses on 0, 15, 50, or 100 mg/kg bw/day on days six to fifteen 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 mg/kg bw/day on days seven to seventeen 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 sternebrae in foetuses at 4 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 Sprague-Dawley rats were given technical diazinon by oral gavage at doses of 0, 10, 20, or 100 mg/kg bw/day from days six through fifteen 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 to 10%; days ten, fourteen, and twenty), 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 foetuses, 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 foetotoxicity (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 foetal 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 six through eighteen at doses of 0 (carboxymethyl cellulose, 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 foetal 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 six through eighteen 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, hypersalivation, 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 foetal 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 a 19.9% diazinon 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 total ChE activity was inhibited at all doses on day seven and at 60 and 100 mg/kg bw on day two after treatment. This effect was reversible. No adverse effects were observed on other biochemical or haematological parameters, or on reproductive parameters (Cameron, 1995). No NOEL could be set for this study because of the significant plasma total ChE inhibition at the lowest tested dose (Carmen, 1995).

2.1.9. Genotoxicity

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 (i.e. 22)

of the 26 studies cited were negative indicating by weight of evidence that diazinon is not genotoxic. Of four studies reporting genotoxicity, the two 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, i.e. human lymphocytes (CCL 156) or PHA-stimulated human lymphocytes (or V79 cells in the presence of a slightly reduced diazinon concentration, i.e. 80 µg/mL 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 (Murli, 1990a; Strasser & Arni, 1986).

Jones and Wilson (1988) reported an increased number of histidine revertants in *Salmonella typhimurium,* strain TA 1535, in the absence of metabolic activation. However, three 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.

2.1.10. 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, i.e. 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, i.e. 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, i.e. 1.4 and five 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 to 12%) after dosing. Cholinesterase inhibition in plasma was almost complete at all doses after 24 and 48 hours, 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 RBCs was observed at any dose after either 24 or 48 hours. However, as anticipated there was a substantial reduction in the neurotoxic esterase activity in the brain and spinal cord at 24 and 48 hours 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 twelve-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 nine to twelve hours 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 one, eight and fifteen revealed significant effects only on day one, when the acute OP poisoning effects (clinical signs) were maximal. No histopathology related to treatment was observed, however, significant

plasma ChE inhibition was observed nine to eleven hours after dosing in both sexes at 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 thirteen weeks lost bodyweight and had characteristic clinical signs of OP poisoning at the highest concentration tested. Functional observational battery (FOB) studies revealed reduced fore and hind-limb grip strength throughout treatment among all rats at 3000 ppm with additional findings, i.e. reduced hind-limb 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 RBC ChE inhibition at 30 ppm in both sexes (Pettersen & Morrissey, 1994)

2.1.11. Porphyrin biosynthesis studies

Prompted by the observation that chlorinated hydrocarbons cause experimental porphyria similar to the human disease, *porphyria cutanea tarda*, and a published report implicating diazinon exposure as a possible cause, a research group investigated and published four 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 (approximately 114 or 228 mg/kg bw/day) to female Dark Agouti rats for twelve 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 eight weeks ($p<0.025$) and 4.9-fold after twelve 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 eight weeks but was after twelve weeks (3.8 fold, $p<0.005$). No significant elevation in porphyrin excretion in urine was evident after four, eight, or twelve weeks of treatment, irrespective of dose or administration route. Electrophoresis of the pooled faecal porphyrins from treated rats revealed a number of decarboxylated intermediates in the conversion of uroporphyrinogen to protoproporphyrinogen, 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 S,S-TEPP were found to increase the porphyrin concentration in these cultures, i.e. 900, 1700 and 540 pmoles/mg/24 hours respectively in the presence of insulin or 730, 1600 and 220 pmoles/mg/24 hours in the absence of insulin. Solvent controls had porphyrin concentrations of 18 and 15 pmoles/mg/24 hours 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 (approximately 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 approximately 15 mg/kg bw/day, a 1:9 mixture of isodiazinon and purified diazinon, or with xylene (vehicle control). A (provided) graph for the 1:9 isodiazinon:diazinon mixture (no other data were shown) revealed that the excretion of porphyrin in faeces was elevated after about sixty days and continued to rise for the duration of the treatment. 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*, i.e. 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 *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 (Collins et al., 1982). This hypothesis was tested in the following published report by Nichol et al., 1983.

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. Excretion of porphyrin in faeces was increased above control group levels by about day seventy in the isodiazinon and isodiazinon:diazinon 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 ferrochetalase 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, their ratio to one another remained unchanged; a result inconsistent with the reduced ferrochetalase and coproporphyrinogen oxidase activity (Nichol et al., 1983).

Diazinon and isodiazinon appear to be acting synergistically to produce porphyria. Alone, neither is particularly effective in inducing porphyria (Collins et al., 1982; Nichol et al., 1983).

2.1.12. Immunotoxicity

In order to detect humoral changes in the adult immune system because 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 (i.e. 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 autopsy confirmed pulmonary congestion and mucosal infiltration consistent with acute bronchitis. However, longevity for post-weaning mice was not significantly different at day 800 (i.e. 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 (Barnett et al., 1980).

Prenatal exposure to diazinon had no effect on levels of the serum immunoglobulins IgG2b, IgM or IgA. 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 three time intervals, there were no data to indicate that any impairment of immunocompetence had occurred. Longevity and incidence of disease among groups were comparable, so the biological significance of these immunoglobulin changes is speculative (Barnett et al., 1980).

2.1.13. Human studies

2.1.13.1 Acute toxicity

Diazinon in epoxidised soybean oil was administered PO to clinically normal, healthy, adult male subjects between the ages of eighteen and 48 as a single dose at 0, 0.03, 0.12, 0.20, 0.21 or 0.30 mg/kg bw (to 11, 7, 7, 7, 8 & 1 subjects, respectively) via a gelatine capsule. The subjects showed dose-related, toxicologically and statistically significant plasma ChE inhibition commencing at about four hours after dosing (32-78% inhibition), with maximal inhibition compared to baseline values at about six hours after dosing (42-93% inhibition). At 0.3 mg/kg bw, time-related plasma ChE inhibition was observed relative to placebo controls (2-93%), with maximal inhibition occurring at about six hours after dosing. Enzyme activity showed recovery with time, commencing at approximately eight hours post treatment. However, seven individuals had still not recovered to below 20% plasma ChE inhibition by study completion (i.e. at fifteen days after treatment). With respect to RBC ChE inhibition, the effects at 0.2 mg/kg bw were not statistically significant (1 to 8% inhibition) compared to placebo controls. At 0.21 mg/kg bw, the inhibition was slightly greater than that seen at 0.2 mg/kg bw and statistically significant (4 to 11% inhibition) compared to placebo controls. Inhibition at 0.21 mg/kg bw also persisted from day one until the study completion on day fifteen after dosing. Therefore, the dose level of 0.21 mg/kg bw may be considered as a LOEL for RBC ChE inhibition in this study. At 0.3 mg/kg bw, the level of RBC ChE inhibition ranged from 4 to 13% relative to placebo controls. The NOEL for plasma ChE inhibition was 0.03 mg/kg bw, and the NOEL for RBC ChE inhibition was 0.2 mg/kg bw (Boyeson, 2000).

2.1.13.2. Metabolism and toxicokinetics

Urine and plasma samples from the same subjects as the acute toxicity study by Boyeson (2000) were analysed for diazinon and one possible metabolite (G-25770). Diazinon in plasma was detected in only some subjects at 0.12, 0.20 and 0.21 mg/kg bw. The plasma levels ranged from 1.3-3 ppb at one, two and four hours after dosing. The single subject given diazinon at 0.3 mg/kg bw had a plasma level of 5.9 ppb at about four hours after treatment, which then decreased to 1.4 ppb at six hours post dosing. Plasma levels appeared to have reached maximal concentrations at about four hours after dosing. The average amounts of the metabolite, G-25770 [6-methyl-2-(1-methylethyl)-4-(1H)-pyrimidinone] excreted in the urine during the first 48 hours of dosing represented about 8 to 25% of the administered diazinon dose, and generally, the amount excreted related to the diazinon dose administered. The majority of the metabolite found in urine was excreted within the first 48 hours after dosing, with the rate of G-27550 excretion being faster during the first 24 hours (Wong & Anderson, 2000).

Urine samples from the same subjects were also analysed for the presence of another major metabolite, diethylthiophosphate (DETP). Raw data were reported but no analysis was presented in this report and hence these data have not been evaluated (Hughes & Vaughn, 2000).

2.1.13.3. Short-term repeat dose studies

Diazinon technical in gelatine capsules was administered to four healthy male subjects at 0.03 mg/kg bw/d, once daily for 28 to 31 days. Treatment resulted in 22-48% inhibition in plasma ChE activity. The enzyme activity showed some signs of recovery at 31 days after cessation of treatment. Some fluctuations in RBC ChE activity were noted during the study, but these were in agreement with literature values for normal individual variations. All other tested study parameters were unaffected by treatment. However, the regulatory value of the study findings is limited as only four males were tested. The NOEL for plasma ChE activity was <0.03 mg/kg bw/d. The single dose tested, 0.03 mg/kg bw, can be considered a NOEL for RBC ChE activity (Beilstein, 1998).

A study, intended to monitor the effects of progressively increasing the oral dosage of diazinon in three volunteers from 0.05 to 5 mg/kg bw/day over thirteen weeks, was terminated after only five 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 five days was repeated and the extent of the significant plasma ChE inhibition observed in the first exposure confirmed (i.e. 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 (i.e. Hb, RBC count, total and differential leucocyte count and prothrombin time (PT)), clinical chemical (i.e. BUN, AP and AST) or urinary parameters (i.e. albumin, protein and microscopic elements), other than for plasma ChE activity. Significant plasma ChE inhibition ($p<0.05$) persisted for six days after treatment in the first cycle, and substantial though non-significant inhibition (i.e. 22%) was observed for six days after treatment in the second. No changes in RBC ChE activity were observed during treatment. Therefore, based on significant inhibition of plasma ChE activity after a five-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 four volunteers. However, although ChE activity in RBCs was apparently unaffected by the 34- or 36-day treatment, ChE activity in plasma in two 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 five-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).

Diazinon administered to groups of three 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 (i.e. Hb, RBC count, total and differential leucocyte count and PT), urinalysis (i.e. pH and microscopic elements) or clinical chemistry (i.e. BUN; AP; and alanine aminotransferase, 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 (i.e. 8%) and RBCs. However, at 0.025 mg/kg bw/day a significant change in the mean plasma ChE inhibition relative to the combined mean pre-test values for the treated volunteers indicated a clear treatment-attributable effect. By contrast, mean RBC 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).

2.1.13.4. Percutaneous absorption

In vivo

A study designed to measure the percutaneous absorption rate of [2'-^{14}C]-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 un-occluded for 24 hours. 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 seven 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 seven days. Assuming that the pharmacodynamics in humans are 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 two 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 hours from the open flow-through cells was 62.4% and 59.1% respectively. It was speculated that the 40% (approximately) of the radioactivity not recovered was lost into the atmosphere by evaporation (Wester et al., 1993).

2.1.13.5. 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).

2.1.13.6. Occupational exposure

Two workers in Egypt 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 eighteen months, and were applying between 1200 to 1500 L of diluted diazinon (0.1%) once weekly by backpack spray. The study noted that neither mask nor gloves were used during application. They noted when preparing this batch of spray that crystals had formed in the storage container (Soliman et al., 1982).

In the first case, a 33-year-old male developed nausea and vomiting after half a day of spraying, 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. His plasma ChE activity was inhibited >20% on day eight after poisoning when compared to unexposed males, with recovery at day

fifteen. RBC ChE activity was inhibited >20% on day eighteen but had returned to unexposed male levels by day 28 (Soliman et al., 1982).

In the second case, a 50-year-old male developed nausea and vomiting after a full days spraying. This progressed to burning eyes, blurred vision and difficulty breathing, as well as a severe headache persisting for three days. He did not seek any medical advice, but had recovered three days after exposure. When compared with unexposed males, his plasma ChE activity was inhibited >20% on day ten after the incident, with recovery on day seventeen. RBC ChE activity was inhibited >20% on day seventeen after the incident, with recovery on day twenty (Soliman et al., 1982).

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 diazinon degradation compounds were also found, including 4-ethyoxy-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. S,S-TEPP and O,S-TEPP were also found in small quantities. O,O-TEPP, however, was not found. It is therefore likely that the presence of these toxic metabolites as well as diazinon resulted in the clinical signs observed (Soliman et al., 1982).

In an attempt to simulate diazinon use in rice paddies, five 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 thirty minutes. No clinical effects or ChE inhibition in RBC were observed, however, relative to pre-exposure activities plasma ChE was inhibited from between 17 to 27%, four hours after exposure. Recovery was gradual so that after four days the degree of plasma ChE inhibition ranged between 9 and 14% of pre-exposure values (Loosli, 1983).

A 51-year-old male sprayed three 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 un-reactive, 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 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 fourth day he suffered a second cardiac arrest and died (Wecker et al., 1985).

On post-mortem 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 also been seen previously in muscle tissue from patients who died 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 (accidently) sprayed. Within fifteen minutes, seventeen 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 RBC ChE activity was at the low end of the normal range. Two of these workers developed nausea and vomiting after returning to work two days later. Eight other workers sought advice within 48 hours of exposure, and their ChE activity was determined. All of these workers had follow-up ChE activity tests fifteen days later. In all cases, plasma and RBC ChE activity increased between these tests. If the levels found at fifteen days after exposure were taken to be the normal levels for these workers, the mean plasma ChE inhibition seen was 29% and mean RBC 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 fifteen 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 three to five days later. If there is an increase in ChE activity between the tests, a further test should be done three to five days later. A rise in activity between these tests would be confirmatory that plasma 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 (Maizlish et al., 1987).

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 compared 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 nineteen 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 (Maizlish et al., 1987).

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 eighteen symptoms possibly related to diazinon exposure was not elevated among applicators. Thus, 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-hour 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 sheep farmer in NSW used diazinon to treat sheep without wearing protective clothing. He presented at hospital the next day with peri-umbilical 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 (Ciba-Geigy, Australia Ltd, 1993).

On post-mortem examination, there were a number of incidental findings, including left ventricular dilatation, extensive moderate atherosclerosis in the aorta, with an early infra-renal abdominal aortic aneurysm. The lungs showed moderate emphysema and pulmonary oedema, and the pleura contained 200 mL of serous fluid. The gall bladder 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 (Ciba-Geigy, Australia Ltd, 1993).

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 centrivenous 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 toxicity (Ciba-Geigy, Australia Ltd, 1993).

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). It is possible that toxic breakdown products of diazinon contributed to this death.

2.1.13.7. Poisoning incidents

Twenty-five cases of diazinon poisoning, admitted to the Sassoon Hospital in Poona, India in 1961-2, were reviewed. There were fourteen males and eleven females, with ages ranging from eighteen months to sixty years, though the majority of cases were aged from fifteen to 24 years. Each had ingested between approximately 14 and 57 grams of diazinon (presumably concentrate formulation); the exact quantities and formulation type ingested were not generally known. Two patients died following hospitalisation; the remaining 23 recovered (Mutalik et al., 1962).

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 (Mutalik et al., 1962).

Treatment generally included gastric lavage, atropine, oxygen and antibiotics as appropriate. No details of dosing were provided. Post-mortem examinations of the two deceased patients 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 mention of any changes in the pancreas in this report (Mutalik et al., 1962).

Twenty-five cases of diazinon poisoning in Ahmedabad, India occurring between October 1964 and October 1965 were reviewed. There were seventeen males and eight females admitted to the hospital, with 21 of the 25 poisoned patients being aged between ten and thirty years old. Most of the cases reportedly ingested less than 21 mL of diazinon; eleven ingested between 21 and 28 mL, although formulation type and concentration were not stated in the report. The most common clinical sign was vomiting (21/25), followed by giddiness, excessive sweating and unconsciousness observed in 7/25 cases. Other common clinical signs were tachycardia (15/25), constricted pupils (14/25) and pulmonary oedema (12/25). Treatment typically involved gastric lavage, atropine treatment and airway maintenance. Three of these 25 patients died despite treatment. However, no post-mortem findings were reported in this review (Kabrawala et al., 1965).

A nineteen-year-old male who ingested approximately 4 ounces (113 g) of a 20% formulation of diazinon (formulation not otherwise specified) was admitted to hospital approximately one to two hours 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 (Banerjee, 1967).

On the second day of hospitalisation, the patient reported retrosternal pain and 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; investigation indicated that left lower lung lobe consolidation had developed (Banerjee, 1967).

RBC ChE activity was significantly decreased (based on time taken for a reaction to occur) for two weeks after the onset of 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 four cases and haematuria in ten cases. Treatment with atropine was successful in most cases although five patients died despite 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 eight hours after ingestion. Where patients presented within three hours of ingestion, atropine therapy was successful. Post-mortem examination of the patients that died revealed congestion of viscera with or without pulmonary oedema and sub-endocardial 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. Post-mortem 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 thirty minutes 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 three children in this family also presented with acute signs of OP poisoning after eating an oatmeal breakfast. Plasma and RBC ChE activity measured seventeen 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 both plasma and RBC ChE levels 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 poisoning 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 thirty minutes; the male who ingested 60 mL did not present at the hospital until five hours after ingestion. In general, clinical signs included vomiting, profuse sweating, pinpoint pupils, hyper-reflexia 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 four to ten days after treatment; the fifth patient developed chemical pneumonitis and required extensive hospital treatment before recovery occurred (Klemmer et al., 1978).

A 54-year-old female was found dead at home. On the kitchen sink, there were two 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 post-mortem 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 (Poklis et al., 1980).

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). This conclusion is debatable given that other chemicals were found at the scene and the fact that the diazinon may have degraded to TEPPs during storage.

A sixteen-year-old female was admitted to hospital one hour after consuming 10 mL of a diazinon formulation. 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 four 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 (i.e. pupil dilation). She was also treated with 2-PAM. After two hours, 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, the effects of diazinon poisoning caused pancreatic interstitial oedema and acinar cell vacuolation, with resulting hyperamylasemia. 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 four new additional cases of acute pancreatitis following diazinon poisoning (Dagli et al., 1981).

A 58-year-old female was admitted to a hospital one hour after ingesting 30 mL of an unknown poison, later identified as a diazinon formulation. 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 grams of pralidoxine and 3 mg atropine IV. Plasma ChE activity remained around 100 mIU/mL until day eleven, with atropine therapy required until day seven. Clinical improvement was noted on days eight to nine, which was associated with a slight rise in plasma ChE activity. Plasma ChE activity increased relatively rapidly from day thirteen, returning to normal by about day twenty (Hassan et al., 1981).

A thirty-year-old female was admitted one hour after consuming a 20 % diazinon formulation. 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 hours of treatment (41 mg atropine administered in total). On day three 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 hours of treatment. Recovery was complete in one week, and the patient was discharged (Bichile et al., 1983).

A twenty six-year-old male was admitted to hospital two hours after consuming 50 mL of a 20% diazinon formulation. 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 five minutes until the pupils were fully dilated (40 mg atropine within thirty minutes). 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 twenty six-year-old male presented approximately one hour 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 the patient was producing dark, cloudy urine and with reduced frequency. On the second day of treatment, amorphous crystals appeared in the urine. IV fluids were increased, and treatment with pralidoxime commenced. Amorphous crystalluria persisted until day nine 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 twenty-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 three hours 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 hyper-reflexia 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. 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 hypersalivation and shallow respiration. His clothing smelt of a pesticide. 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 fifteen minutes. When vital signs stabilised and secretions decreased, the patient was extubated. He recovered without incident and was discharged six 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 storeroom spilled onto the back of one of the workers. As she changed her clothes, her companion mopped up the spilt pesticide with rags. Approximately three hours 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, tachypnoiec 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 creatine phosphokinase (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 ten-fold over normal levels. She was treated for acute pancreatitis and recovered, with enzyme levels returning to normal over the next three days (Lee et al., 1989).

The second worker developed nausea, vomiting, diarrhoea, abdominal pain and dizziness approximately seven hours after exposure. She was admitted to hospital four hours 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 four days to be within 25% of average (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 (Lee et al., 1989).

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). In this case it is also possible that toxic breakdown products of diazinon were also present in the open bottle at the time of exposure.

A twenty-year-old male ingested an unknown volume of diazinon, and was admitted into hospital six hours 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 twelve hours. 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 sixth and seventh cranial nerves with some effects on ninth and tenth 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 twenty hours after the first signs of weakness were noted. The patient died of respiratory failure approximately five hours 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 female child 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 (Richter et al., 1992).

Plasma ChE activity measured before clean-up of the house but after the family had been in residence for four 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 cases, 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 (Richter et al., 1992).

The wall surfaces were substantially contaminated prior to the clean-up; diazinon concentration ranged between 0.13 and 1.1 mg/m^2. The air levels were also high (2.5 μg/m^3). After the cleaning procedure, surface levels of diazinon decreased to 0.01-0.08 mg/m^2, 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 four 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 possible OP or carbamate poisoning. The ingested pesticide was identified in twelve of the patients (i.e. parathion - four, malathion - three, diazinon - two, and unspecified carbamates - three). One of the diazinon-poisoned children was diagnosed with pancreatitis, while overall 5/17 children 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 (Weizman & Sofer, 1992).

In the five 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 five patients. Four patients who did not have pancreatitis had raised serum amylase levels with no abdominal pain or increase in serum immunoreactive trypsin levels. All patients recovered without incident (Weizman & Sofer, 1992).

The study suggests that acute pancreatitis is not rare in children who have ingested OP or carbamate pesticides, since pancreatitis occurred in 5/17 poisoning cases. It also indicates that a raised serum amylase level can occur without pancreatic involvement; this may occur secondary to a range of disorders. Serum immunoreactive trypsin would appear to be a more specific and sensitive test for the diagnosis of pancreatitis (Weizman & Sofer, 1992).

A twelve-week-old infant girl developed persistent hypertonicity of the extremities, and, at the age of eight months, it was discovered that her home had been treated with an excessive and inappropriate application of diazinon three weeks prior to the onset of symptoms. At this time (six months after application), the remaining diazinon residue on the floor was 230 ng/cm^2 in comparison to the 38 ng/cm^2, which is expected immediately after a normal application. Vacuum cleaner dust contained 1700 mg/kg diazinon, while air contained 2.8 ng/m^3. Measurement of metabolites in urine revealed 60 ppb diethyl phosphate and 20 ppm diethylthiophosphate, enabled the diazinon dose to be estimated at 0.02 mg/kg bw/day. Although there was no appreciable reduction of ChE activity in the infant, 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 six weeks and normal development proceeded without any apparent sequelae. At twenty months, the last reported observation, motor abilities and speech were developing normally (Wagner & Orwick, 1994).

A forty two-year-old male farmer was admitted to hospital after ingestion of 200 mL of a pesticide containing diazinon at an unknown concentration. 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 seventh day. It returned to normal within two weeks. There was an increased output of urine of between two and five 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 increase in serum creatinine could not be attributed to any nephrotoxic substances or haemodynamic effects, i.e. the pathogenesis was not defined. No pathological examinations were completed. Previous OP poisoning experiments in rats have indicated that the predominant effect on kidneys is an increased urinary flow with low osmolality, suggesting there may be a direct effect on tubular function that is unrelated to the degree of ChE inhibition (Abend et al., 1994).

A twenty-five-year-old woman was admitted to hospital following a suicide attempt, in which she ingested acetaminophen and unknown amounts of a laboratory agent (containing 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 electroconvulsive therapy, which she had previously received without incident. For the therapy, she was anaesthetised with methohexital, *d*-tubocurare, atropine and succinylcholine. Following electroconvulsive therapy, she was slow to resume spontaneous ventilation, requiring assisted ventilation for twelve minutes. She remained weak for the next 25 minutes, 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).

2.2. 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. The acute toxicity profile is characterised by rapid absorption and distribution, extensive metabolism and fast excretion in the urine. Stabilised diazinon (e.g. diazinon in epoxidised soybean oil) is of moderate acute oral toxicity, with relatively large species differences in sensitivity. Female animals (rats, dogs) tend to be more sensitive to diazinon-induced ChE inhibition, but this finding is not consistent. 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 central nervous system effects (ataxia, convulsions). Technical diazinon is a slight eye and skin irritant and skin sensitiser.

Following oral administration to rats, diazinon is almost completely absorbed from the GI tract and has a plasma half-life of 2.9 hours, consistent with rapid elimination from the circulation. Excretion studies in rats indicate that most of the absorbed diazinon (96-97%) is present in urine as metabolites within 24 hours. The major metabolic pathway includes the oxidase/hydrolase-mediated cleavage of the ester bond leading to the low toxicity derivatives G-25770, and diethylthiophosphate.

Unstabilised or inadequately stabilised diazinon in hydrocarbon formulations can undergo an appreciable increase in toxicity if the diazinon undergoes degradation to form the more toxic TEPPs. Nichol et al. (1982) reported that at receipt a 90% pure preparation of technical diazinon resulted in a median lethal dose (LD_{50}) of 170 mg/kg bw in rats whereas after twelve months the same preparation stored under nitrogen at room temperature gave an LD_{50} 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 (e.g. 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 can promote the formation of diethylphosphorothionate and diethyl phosphate (a metabolite often monitored in human urine to assess exposure of several different OPs). 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 O,O-TEPP, O,S-TEPP and S,S-TEPP (see Figure 2.1). Median lethal dose studies, performed in 1989 but not reported until 1995, have confirmed the markedly increased toxicity of each of these three compounds in female rats, i.e. 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 diethyl phosphate is the predominant product (Kuhn, 1995a, b, c).

An alternative pathway for the formation of these toxic impurities was suggested by Meier et al., (1979), whereby traces of water present during diazinon synthesis, could react with diethyl thiophosphorylchloride (see 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 technical grade active constituents 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 chemical (Meier et al., 1979).

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 if the product is a hydrocarbon formulation and has inadequate stabilizer to prevent formation of these toxic breakdown products. Soliman et al., (1982) reported that the toxicity of some diazinon formulations increased rapidly when it was stored in tinned-steel containers instead of in inert-lined aluminium ones, suggesting it is possible that a catalyst may promote the reaction. In an attempt to minimise the formation of O,O-TEPP, O,S-TEPP and S,S-TEPP, product manufacturers have introduced a stabiliser (epoxidised soybean oil) during formulation (Spindler, 1969; Sterling, 1972).

Given that diazinon was readily available in products for use in the home (e.g. flea and tick treatments in dogs) and the farm, (e.g. blowfly dressings), steps have been taken to minimise the potential risk to the end user. This was especially important, given that it would normally be counter-intuitive to users that any product might become more toxic (and probably more efficacious) on storage. A small scale nation-wide survey conducted in 1993, indicated that of 157 unopened liquid diazinon formulations that had been randomly purchased from retail outlets throughout Australia 35 (or 22%) failed to meet the ±10% label claim for diazinon, 26 (or 16.5%) contained some S,S-TEPP and 13 (or 8.3%) of these 26 exceeded the 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 impurity limit of 0.2 g/kg ai for O,S-TEPP. Five of the 7 batches with O,S-TEPP also contained excess S,S-TEPP Hence, a total of 15 samples (or 9.5%) exceeded either one or both impurity limits. Any further degradation, which may occur after purchase, e.g. due to exposure to air and water vapour during repeated opening of the container or by washing the cap, has not been investigated (McDonald, 1993, 1994; Allender & Britt, 1994). However, regulatory action was taken in 2003 to cancel the registration of all emulsifiable concentrate products containing diazinon that did not contain adequate stabilizer (APVMA, 2003).

Cholinesterase inhibition

As ChE inhibition is the primary indicator of diazinon toxicity, a summary of the NOEL findings for ChE inhibition in a range of repeat-dose studies is shown in Table 2.1. NOELs are presented for plasma, RBC and brain ChE activity.

Table 2.1: Summary of NOELs (mg/kg bw/day) for cholinesterase inhibition following diazinon administration

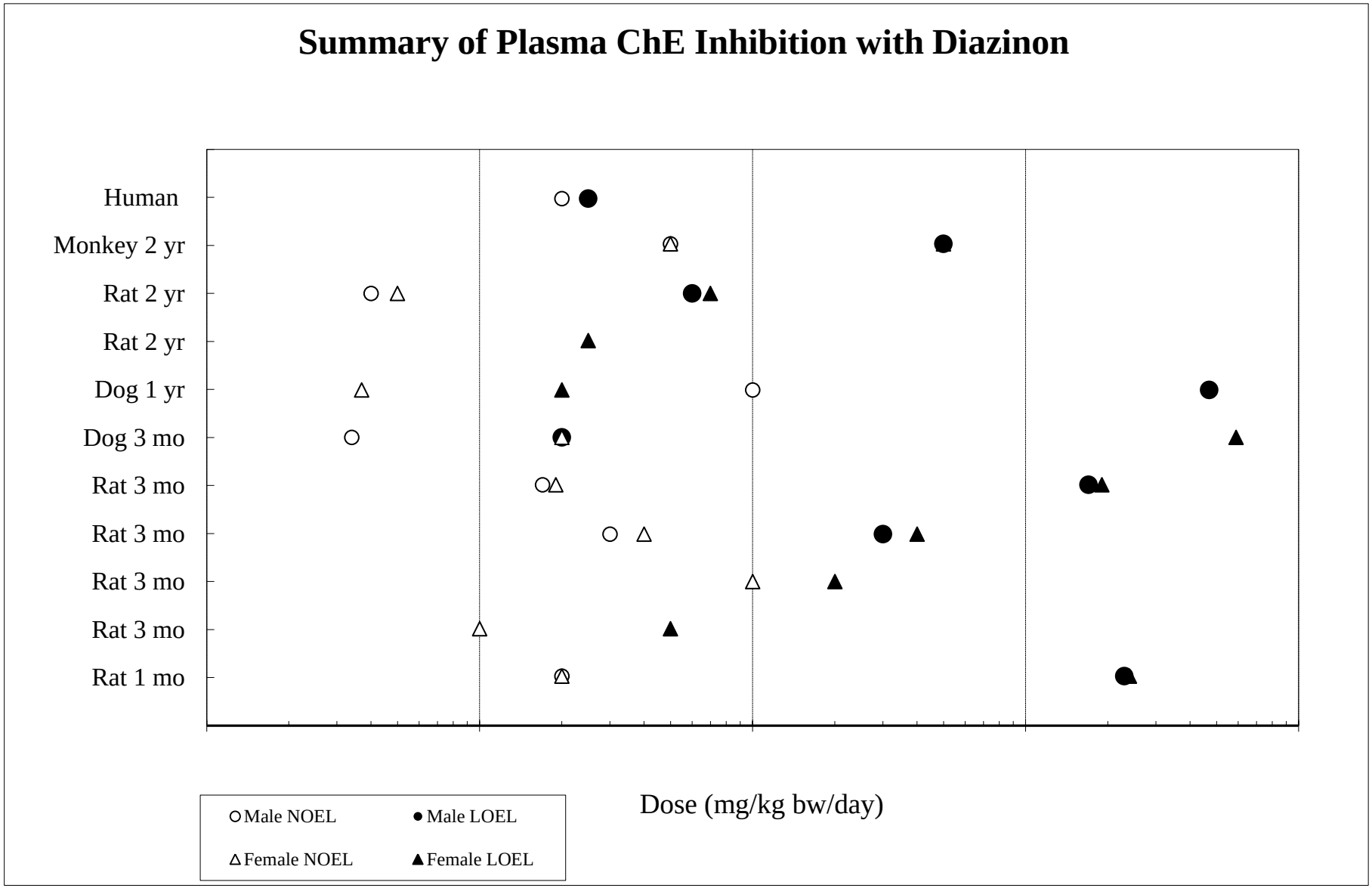
Species	Duration	Route	Plasma	RBC	Brain	E:P ratio
Rat	3 months	Diet	0.01	0.1	1.5	10
	3 months		0.1	>0.4	>0.4	-
	3 months		0.03	0.04	0.06	1.3
	3 months		0.017	0.017	1.9	1
	2 years		NE (<0.025)	0.1	1.5	-
	2 years		0.004	0.06	0.06	15

Dog	3 months		0.0034	0.02	0.02	5.9
	1 year		0.0037	0.02	0.02	5.4
Monkey	2 years	Gavage	0.05	5	ND	100

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

Inspection of the summary Table 2.1 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 RBCs 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. Note that no LOEL for any of the animal studies is much less than 0.02 mg/kg bw/day, the NOEL for humans.

Figure 2.1



Toxicokinetics and metabolism

Following oral administration to rats, diazinon was almost completely absorbed from the GI tract with only about three percent 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 hours 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 hours. 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 (Capps, 1986; Mucke et al., 1970; Wu et al., 1996).

Percutaneous absorption

Two studies, which reported percutaneous absorption of diazinon (without occlusion) essentially support a contention that a significant proportion of the 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 approximately 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 seven 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 square centimetres) was present in the atmosphere as 'volatiles' after 144 hours (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 (i.e. vapour pressure at 25^0 is 2100 mPa) dichlorvos is the most commonly used OP in pest strips, however there are also pest strips which contain diazinon. Collectively the studies detailed above 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 hours following oral administration accounted for >95% of the administered pyrimidinering labelled diazinon dose (Capps, 1990, with amendment by Carlin, 1994; Wester et al., 1993; Williams & Marco, 1984).

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 or were ingested/inhaled (noting that these are largely dipping cases). 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 (LD_{50}) 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 (i.e. from 250 mg/kg bw to 108 mg/kg bw) the increase in dermal toxicity was 4.5-fold, (i.e. 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 (Dressel et al., 1979, 1980; Frick et al., 1987). Mechanistic studies suggested that whilst diazinon was the OP under investigation, other organophosphorus pesticides could also be anticipated to elicit a similar response (Goodale et al., 1993). 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 three years by Lee et al., (1998) suggested that acute oral organophosphate poisonings in humans mimics findings in dogs. The mechanistic studies in dogs showed an increased intraductal pressure, secretory rate and induction of histopathological sequelae in the pancreas resulting from the inhibition of tissue-fixed butyryl ChE activity (Dressel et al., 1979; 1980). Moreover, there appeared to be a good correlation between the observed hyperamylasemia in human cases and the severity of the poisoning (Lee et al., 1998).

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 (Lee et al., 1998; Dressel et al., 1979, 1980; Goodale et al., 1993). Thus, the death of an Australian sheep farmer in 1994 as a result of severe acute haemorrhagic pancreatitis following exposure to diazinon, assumed to be 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 because of repeated or chronic exposure to diazinon (Ciba-Geigy Australia Ltd 1995). 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 (Ciba-Geigy Australia Ltd 1995). Support for this contention comes from the following information:

In 1993 the NRA conducted a nationwide small-scale survey (157 samples) (APVMA, 2002). The results revealed that 9.5% of off-the-shelf, unopened diazinon emulsifiable concentrate products contained O,S- and S,S-TEPP in excess of benchmark limits. The toxicity of these impurities is very high, with the oral LD_{50} in female rats being 0.46 and 3.5 mg/kg bw for O,S- and S,S-TEPP respectively, compared with 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 *Percutaneous Absorption*).

Whilst there are circumstantial data to implicate the toxic diazinon impurities as the cause of the pancreatitis and subsequent death of the sheep farmer, supportive evidence might have come from measuring the concentrations of these impurities in the diazinon product implicated as the source of the poisoning; however, there is no indication that this was done. 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 sheep farmer, 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 ai) and S,S-TEPP (1.24 g/kg ai); the benchmark limit for O,S-TEPP was set at 0.2 g/kg ai, while the presence of any O,O-TEPP in the products tested could not be determined owing to the absence of a suitable reference standard (APVMA, 2002).

Regulatory action was taken in 2003 to cancel the registration of all emulsifiable concentrate products containing diazinon that did not contain adequate stabilizer (APVMA, 2003)

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* (Marshall et al., 1976; Shirasu et al., 1976; Matsuoka, 1979; Sobti et al., 1982; Beilstein et al., 1986; Strasser & Arni, 1988; Geleick & Arni, 1990; Murli, 1990a). Similarly, there was no evidence of carcinogenicity from studies in rats and mice (Wheeler et al., 1979; Kirchner et al., 1991; Mann, 1993).

Reproduction and development

No teratogenic effects were observed in rat reproduction studies or in any of the developmental studies with rats, rabbits or pigs (Johnston, 1965; Earl et al., 1973; Tauchi, 1979; Harris, 1981; Weatherholz, 1982; Infurna, 1985; Ginkis, 1989). Although there was a published study (Abd El-Aziz et al., 1994)which reported that male rats treated with a diazinon at 1.5 or 3 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 two-generation study using technical diazinon doses of up to 10 mg/kg bw/day (Weatherholz, 1982).

Porphyrin biosynthesis

In 1992, the NHMRC Standing Committee on Toxicity 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 Brazil). The following is a summary of their findings:

Scrutiny of the supplied bibliography indicated that *porphyria cutanea tarda* occurred because of a congenital deficiency of uroporphyrinogen decarboxylase, a haem synthesis cycle enzyme. In the rats treated cutaneously each day with 20 mg of technical grade diazinon (85%) for ten to twelve 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 a hundred 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 is particularly effective in inducing porphyria. On their own, neither diazinon nor

isodiazinon are particularly effective in inducing porphyria (Nichol et al., 1983; Collins et al., 1992).

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 associated with acute cholinergic crisis following accidental or deliberate ingestion, one report suggested that diazinon may induce an additional paralytic condition called “Intermediate Syndrome”, consisting of a sequence of neurological signs that develop some 24-96 hours after poisoning (Samal & Sahu, 1990). This condition appeared to develop before the onset of delayed neuropathy (so-called “organophosphate-induced delayed neurotoxicity” or OPIDN). Clinical signs of the “Intermediate Syndrome” can be distinguished from the characteristic muscarinic, nicotinic and central nervous system effects observed very soon after exposure, as a delayed onset of muscular weakness affecting neck, proximal limb and respiratory muscles. However, there have been no reported cases of OPIDN in humans following accidental or deliberate diazinon poisoning, a result consistent with 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 Table 2.2.

Table 2.2: Summary of NOELs for Diazinon.

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 RBC ChE inhibition was 1.5 mg/kg bw/day and for brain, 22.5 mg/kg bw/day (Ashby & Danks, 1987).
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 RBC and brain ChE inhibition was 5 mg/kg bw/day (Kirchner et al., 1991; Mann, 1993).
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 RBC and brain ChE inhibition was 4.5 mg/kg bw/day (Rudzki et al., 1991; Mann, 1993).
Sprague-Dawley rat: 2-gen reproduction	5 [100 ppm]	Reduced maternal food consumption, body weight gain and body weight at next higher dose of 500 ppm (25 mg/kg bw/day) (Giknis, 1989).
	5 [100 ppm]	Reduced pup weight, viability at the next highest dose of 500 ppm (25 mg/kg bw/day) (Giknis, 1989).

Study Type	NOEL (mg/kg bw/day)	LOEL and Toxic Effect
Sprague-Dawley rat: Gavage teratology	20	Maternal body weight loss and reduced food consumption at 100 mg/kg bw/day (Infurna, 1985).
	20	Skeletal variants observed at the highest dose tested (100 mg/kg bw/day) (Infurna, 1985).
NZW rabbit: Gavage teratology	25	Maternal survival, body weight loss and cholinomimetic signs at 100 mg/kg bw/day (Harris, 1981).
	25	Delayed foetal ossification observed at 100 mg/kg bw/day (Harris, 1981).
NZW rabbit: Gavage teratology	10	Maternal body weight loss, reduced food consumption and cholinomimetic signs at 40 mg/kg bw/day (Edwards, 1987).
	40	No embryo/foetotoxicity effects observed at the highest dose tested (40 mg/kg bw/day) (Edwards, 1987).
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 RBCs observed at 0.025 mg/kg bw/day (Lazanas et al., 1966).

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 0.02 mg/kg bw/day, based on plasma ChE inhibition observed in a 37-43 day human study and using a safety factor of twenty, due to the closeness of NOEL and LOEL and the limited nature of the studies (Lazanas et al., 1966).

Acute Reference Dose

To establish a NOEL for the acute reference dose for diazinon, a summary of the NOELs determined in those studies deemed adequate for regulatory purposes are shown in Table 2.3.

Table 2.3 Summary of NOEL suitable for the ARfD

Study Type	NOEL mg/kg bw (acute exposure)	LOEL and Toxic Effect
Human single-dose po	0.2	LOEL for RBC ChE inhibition observed at 0.21 mg/kg bw (Boyeson, 2000)

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, usually during a meal or in one day, without an appreciable health risk. At the time the ARfD was set there were three human studies in the toxicology database for consideration (Sze & Calandra, 1965; Lazanas et al., 1966; Payot, 1966). The Australian ARfD of 0.005 mg/kg bw was originally based on the NOEL of 0.05 mg/kg bw/d for RBC ChE inhibition in the five-day human study of Sze and Calandra (1965). A safety factor of ten was used to derive the ARfD value.

A more recent human oral toxicity study using a single dose (Boyeson, 2000) reported a NOEL of 0.2 mg/kg bw for red blood cell ChE inhibition based on significant inhibition at the next highest dose of 0.21 mg/kg bw.

Boyeson (2000) was considered the most suitable study available for derivation of the ARfD because it is an acute oral dose study. Therefore, the ARfD has been amended to 0.01 mg/kg bw, based on the NOEL of 0.2 mg/kg bw and using a twenty-fold safety factor. The additional two-fold

safety factor over the default of ten was applied because of the limited nature of the study and the closeness of the NOEL and LOEL.

Safety Directions

The current safety directions (July 2007) are listed in Table 2.4 and should be included on all product labels:

Table 2.4: Safety directions for diazinon.

Safety Direction	Code
BL 95 g/L or less with dibutyl phthalate 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 excessive splashing or contamination is likely wear protective waterproof clothing and [or cotton overalls buttoned to the neck and wrist (or equivalent clothing), a washable hat, and PVC or rubber apron] elbow-length PVC gloves and water resistant footwear.	290 291 [or 292a 293] 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 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

Safety Direction	Code
Repeated minor exposure may have a cumulative poisoning effect	190
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	290, 291, [or 292a, 293],

Safety Direction	Code
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	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 and water resistant footwear	295, 298b
When using the prepared spray wear cotton overalls buttoned to the neck and wrist (or equivalent clothing), a washable hat and elbow-length (nominate other specific material) gloves.	279, 282, 290, 292a, 295
If excessive splashing or contamination is likely when using the prepared spray 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], elbow-length (nominate other specific material) gloves and water resistant footwear.	if excessive splashing or contamination is likely 279, 282, 290, 291 [or 292a 293] 295 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
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

Safety Direction	Code
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 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
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 excessive splashing or contamination is likely when using the prepared spray wear protective waterproof clothing [or cotton overalls buttoned to the neck and wrist (or equivalent clothing)	if excessive splashing or contamination is likely 279, 282, 290, 291 [or

Safety Direction	Code
and a washable hat and PVC or rubber apron], and elbow-length PVC gloves and water resistant footwear	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 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 excessive splashing or contamination is likely when using the prepared spray 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	if excessive splashing or contamination is likely 279, 282, 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
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

Safety Direction	Code
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

Table 2.5 lists amendments to existing FAISD entries that were recommended in the hazard assessment. These amendments will be incorporated by the OCS in an updated version of FAISD Handbook. Safety Directions listed in these Tables should appear on all associated product labels.

Table 2.5: Amended FAISD entries for diazinon

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"
Amend entry "ME 240 g/L or less" to "ME **300** g/L or less"
Amend entry "EC ULV 200 - 800 g/L" by inserting 161, 162 and 164 viz. "will irritate the eyes and skin"
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 (FAISD Handbook, 31 Dec 2008), these instructions are:

- diazinon (m);
- diazinon in dusts (a);
- diazinon in plastic resin strips (a);
- diazinon when microencapsulated in preparations containing 25 per cent or less of diazinon (a).

No changes to the current first aid directions are recommended.

Conclusion

No changes are recommended to the existing Australian ADI value of 0.001 mg/kg bw/day. However, the basis for this ADI has changed, and is derived by applying a safety factor of 20 to the NOEL of 0.02 mg/kg bw/day in a 37-43-day human study by Lazanas et al. (1966). The previously used NOEL of 0.1 mg/kg bw/d was based on plasma ChE inhibition in a three-month rat study (Weir, 1957a). The additional two-fold safety factor was applied because of the closeness of the NOEL and LOEL in this study, uncertainty surrounding the impurity profile of the administered diazinon (i.e. TEPP content) and the limited nature of the study (two doses and three subjects/group).

In 1999 an acute reference dose (ARfD) of 0.005 mg/kg bw was established for diazinon, based on a NOEL of 0.05 mg/kg bw for RBC ChE inhibition in a five-day human study (Sze & Calandra, 1965) and applying a ten-fold safety factor. The OCS evaluated additional information provided during the diazinon review, including a single-dose human oral toxicity study (Boyeson, 2000), which reported a NOEL of 0.2 mg/kg bw for RBC ChE inhibition, based on significant inhibition at the next highest dose of 0.21 mg/kg bw. As this is an acute oral dose study, it is the most suitable study available for derivation of the ARfD. Therefore an ARfD of 0.01 mg/kg bw is appropriate, based on the NOEL of 0.2 mg/kg bw and using a twenty-fold safety factor. The additional two-fold safety factor was applied because of the limited nature of the study (dose selection) and because of the closeness of the NOEL and the LOEL.

2.3. MAIN TOXICOLOGY REPORT

2.3.1 INTRODUCTION

2.3.1.1. 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. The use of diazinon is now mainly confined to the treatment of mammalian ectoparasites (i.e. in cattle, sheep, goats, pigs, horses, dogs and cats), and for insect control in bananas, 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 58 registered products containing diazinon listed on APVMA's PUBCRIS website (2008).

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 Ageing. Based on an NOEL of 0.02 mg/kg bw/day reported in a subchronic dog study, the ADI was originally set at 0.0002 mg/kg bw/day in 1965 (Weir, 1957b). 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 (Lazanas et al., 1966). To reflect intraspecies variability, a safety factor of ten was used to calculate an ADI. In 1987 the ADI was set by NHMRC at 0.001 mg/kg bw/day, derived from a NOEL of 0.1 mg/kg bw/day, based on plasma ChE inhibition observed in a 3-month rat study (Weir, 1957a).

(a) Acceptable Daily Intake

A toxicological evaluation of diazinon undertaken in July, 1999 did not recommend a change to the then existing Australian acceptable daily intake (ADI) of 0.001 mg/kg bw/day, but changed the critical study on which it was set. The ADI was originally derived from a NOEL of 0.1 mg/kg bw/day for plasma ChE inhibition in a three-month rat study (Weir, 1957a). In 1999 the ACPH recommended that the ADI be based on the lower NOEL of 0.02 mg/kg bw/day, reported in a 37-43 day human study (Lazanas et al., 1966); the existing value for the ADI for diazinon of 0.001 mg/kg bw/day was retained, but was now based on plasma ChE depression in humans at 0.02 mg/kg bw/d and the application of a twenty-fold safety factor. The additional two-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.

(b) Acute Reference Dose

In 1999 an acute reference dose (ARfD) of 0.005 mg/kg bw was established for diazinon, based on a NOEL of 0.05 mg/kg bw for RBC ChE inhibition in a 5-day human study (Sze & Calandra, 1965) and applying a ten-fold safety factor. In 2003, the ARfD was reset to 0.01 mg/kg bw, based on the NOEL of 0.2 mg/kg bw and using a twenty-fold safety factor (Boyeson, 2000). The additional two-fold safety factor over the default ten was applied because of the limited nature of the study and the closeness of the NOEL and LOEL.

2.3.1.2. International toxicology assessments

Joint FAO/WHO Meeting of Pesticide Residues

Diazinon has been reviewed by the Joint FAO/WHO Meeting of Pesticide Residues (JMPR) in 1963, 1965, 1966, 1967, 1968, 1970, 1993 and 2001. 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 1993 assessment incorporates the change in JMPR policy to use inhibition of brain ChE (or RBC ChE inhibition as a surrogate) as the toxicologically-relevant endpoint has the same ADI based on the no observed adverse effect level (NOAEL) 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 RBC 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)

In 2001, the Meeting focused solely on acute reference dose and established an ARfD of 0.03 mg/kg bw. This was based on the NOAEL of 2.5 mg/kg bw in the studies of acute neurotoxicity (brain ChE inhibition) in rats and a 100-fold safety factor. The study in male volunteers at that time was considered of limited value in establishing the ARfD, as the studies in rats provided evidence of a considerable sex difference in sensitivity to the inhibition of brain acetyl ChE activity by diazinon.

United States Environmental Protection Agency

The United States Environmental Protection Agency (US EPA), which like Australia supports the use of plasma ChE inhibition as an appropriate endpoint, has set a slightly lower reference dose (or ADI) at 0.0007 mg/kg bw/day (as at Jan, 1998) based on the NOEL (0.02 mg/kg bw/day) of a two-dose human study (Lazanas et al., 1966). An additional 10-fold safety factor in accord with FQPA requirements was not applied because there was no evidence of enhanced pre- or post-natal toxicity for pups in the reproduction studies. However, an additional 3-fold safety factor was applied because of the closeness of the NOEL and LOEL, and the use of only one gender (males) in the human study.

International Agency for Research on Cancer

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

2.3.1.3. Identification

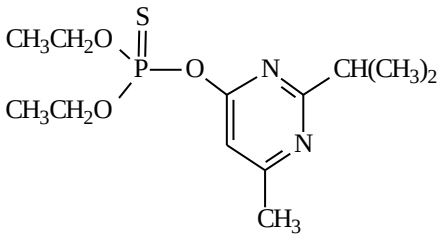
Nomenclature: *O,O*-diethyl-*O*-(2-isopropyl-6-methylpyrimidin-4-yl)-phosphorothioate (IUPAC);
O,O-diethyl-*O*-(6-methyl-2-(1-methylethyl)-4-pyrimidinyl)-phosphorothioate. (CA)

Empirical Formula: $C_{12}H_{21}N_2O_3PS$

Common Name: Diazinon

CAS no.: 333-41-5

Chemical Structure:



Molecular Weight: 304.35

2.3.1.4. Physicochemical properties

Colour: colourless

Odour: slight

Physical State: (at 25°C) liquid

Boiling Point: 84°C

Octanol/Water: partition coefficient. 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.

Diazinon technical chemistry details are found in Appendices II-IV.

2.3.1.5. 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.

2.3.2 TOXICOKINETICS AND METABOLISM

2.3.2.1. Absorption, distribution, metabolism and excretion

Excretion and metabolite profiles for rats, dogs, goats, sheep and cows are compared and presented as Tables. A generalised metabolic pathway for diazinon in mammals, generated using data described in the following studies, is shown in Figure 2.1.

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 Yok:ddY outbred strain 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 one hour. 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 Cmax and declined at a rate corresponding to that in blood.

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

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 minutes and from the remainder after 2, 4, 6 and eight hours. 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, i.e. Volume of distribution

at steady state (Vd[ss]), Clearance (Cl), Area under the curve (c), time to maximal concentration in plasma (Tmax) and half-life (T1/2); these are shown in Table 2.6.

Table 2.6: Toxicokinetic parameters for diazinon in the rat.

Dosing route	Dose (mg/kg bw)	$Vd_{[ss]}$ (L/kg)	Cl (L/h/kg)	AUC (mg/h/L)	Tmax (h)	T1/2 (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 minutes after an IV 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 µg/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 µg/g), liver (0.47 µg/g) and brain (0.45 µg/g) than in plasma (0.17 µg/mL) at two hours after IV dosing. The concentration in these tissues then declined over the next six hours at a rate similar to that in blood.

Cholinesterase activity in RBCs declined to a greater extent than that observed in plasma. Ten minutes after IV administration, RBC ChE activity was significantly reduced by 44% (estimated from graph) and maximally, 75%, after two hours. In plasma, activity was reduced by 12% after 10 minutes and by 45% after two hours. Oral dosing resulted in maximal inhibition of about 56% for RBC ChE and 20% for plasma ChE after two hours. Little ChE recovery was evident after either oral or IV dosing during the remaining six hours 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'-^{14}C]-diazinon (>98.7% pure, final specific activity 30.3 µCi/mg (low dose, lot no. RAF-V-74) or 9.7 µCi/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 euthanased 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, and in Group 4 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 hours after exposure although the high-dose females showed slower elimination. At 24 hours, 60% of the radioactivity had been excreted and after 48 hours 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, i.e. 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 ^{14}C-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), [^{14}C]-labelled in the pyrimidine ring (2' position, specific activity 4 μCi/mg; 4' position, 2.6 μCi/mg) or in an ethoxy group (3.2 μCi/mg) was investigated following gavage (or IV to ascertain the formation sequence of metabolites) administration to Wistar WU rats (source not given). Following daily oral gavage administration of [2'-^{14}C], [4'-^{14}C] or [ethoxy-^{14}C]-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 males & 2 females for [2'-^{14}C]; 1 males for [ethoxy-^{14}C]; not reported for [4'-^{14}C]) housed in metabolic cages. Metabolites in excreta were isolated using TLC and silica gel column chromatography then characterised by infrared, ultraviolet, nuclear magnetic resonance and mass spectrometry. In a second series of experiments to determine tissue distribution, [2'-^{14}C]-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 euthanased at 0.25, l, 2, 5, and 8 days after cessation of treatment. Total radioactivity in excreta, CO_2 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 hours was estimated to be twelve hours irrespective of gender after administration of the 2'- label and seven hours 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 (males 80.0%, females 68.9%) and faeces (males 16.0%; females 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 six hours after dosing was only 2.92%. The slowest rate of decline was observed in fat where the 0.23% present after six hours was reduced to 0.18% 1 day later. The most rapid decline was observed in oesophagus/stomach where 0.25% was detected after six hours and only 0.02% after 1 day.

Thin Layer Chromatography resolved four radioactive fractions in the excreta with three 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 (designated as metabolite M3)). The median lethal doses of G27550 and GS31144 were shown to be substantially less than diazinon (250 mg/kg bw), i.e. 2700 & >5000 mg/kg bw respectively. The relationship of these metabolites (i.e. the degradation sequence) was determined by IV 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 (mixture of polar compounds). 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 one to two hours. 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. Eight hours after dosing 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 three hours.

Eight hours after dosing at 100 mg/kg bw, the diazinon residue in the kidney was 500 times greater than in the liver and 11 times greater than in the brain. Diazinon is thought to be rapidly metabolized in the liver. In all tissues, there was a significant decrease (approximately 5-fold) in residue level between 8 and 24 hours.

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

Garcia-Repetto R, Martinez D & Repetto M (1995) Coefficient of distribution of some organophosphorus 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 concentrations 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 LD_{50} (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-^{14}C]-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 five minutes and seven hours. These two dogs together with another two 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 hours 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 six hours (363 minutes) after IV dosing. Cholinesterase activity in plasma was significantly inhibited by approximately 39% relative to predose levels after only 10 minutes. Activity continued to decline thereafter, albeit at a much slower rate, so that the maximal ChE activity inhibition of about 50% was detected 2.5 hours after dosing.

Urine collected over 24 hours from [ethoxy-^{14}C]-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 diethyl phosphate (16%). By contrast, the radioactivity in the urine of ring-labelled diazinon treated dogs accounted for 86% of the administered dose after 24 hours. Silica gel column chromatography also resolved two 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 ^{32}P labelled diazinon in dog tissues after oral administration. Wallaceville Animal Research Station, Department of Agriculture, Wellington, New Zealand. NZ Vet J 11: 141-144

[^{32}P]-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 caused excessive salivation immediately and vomiting after 30 minutes, followed by a further bout of vomiting and the onset of uncoordinated movement two hours later. The dog was euthanised ten hours after dosing. Of the 14 tissues removed, 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 death had 694 µg eq/mL of radiolabelled 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 diethyl phosphate.

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'-^{14}C]-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-^{14}C-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 ^{14}C-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 ^{14}C-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 ^{14}C-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 appear to be the same as that described in report no. ABR-88118.]

[2'-^{14}C]-diazinon (>98.8% pure, specific activity 9.7 µCi/mg (lot no. CL-XIV-23) was administered in capsules to 2 lactating goats (one of the treated goats was of unknown breeding and the other was an Alpine cross, whereas the control goat was a Nubian-Alpine cross; all were procured from the Ciba-Giegy Research Center). Each goat received one capsule (containing 150 mg) per day for four days, i.e. approximately 4.3 mg/kg bw. Goats were housed in metabolism cages for the duration of dosing, after which they were euthanased (23.27 hours and 23.5 hours respectively after the last dose); a gross necroscopy 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 two and day four; predose samples were collected for four 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 although the reports noted that bodyweight declined by 2% and 6% respectively and milk production declined 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 four 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 (i.e. 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 two, the average radioactivity present in blood was 0.43 μg eq/mL (0.17% of the dose), whereas at the time of euthanasia it was 0.36 μg eq/mL (0.15%). A similar amount of radioactivity was detected in milk where the range over four days was 0.33-0.46 μg eq/mL (or 0.07-08%) so that at the end of the experiment 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 four 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 two 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 (e.g. hydroxydiazinon) so that more than three quarters of the radioactivity found in these tissues was in this form. In milk there was proportionally more free GS31144 and G27550 relative to urine because there were fewer diazinon conjugates.

Gross pathology examination did not reveal any changes attributable to treatment in the cardiovascular, respiratory, urogenital, musculoskeletal, lymphatic or GI systems, or in 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 two sheep (gender, breed and source not given) produced "moderate symptoms" of poisoning (though these were not detailed). Unchanged diazinon and hydroxydiazinon (CGA14128) were quantified in the blood collected at 3, 6, 24 and 48 hours from one of the sheep. Metabolites were extracted and characterised by silica gel column

chromatography, Florisil-alumina columns, TLC, GLC, infrared, nuclear magnetic resonance and mass spectroscopy. The maximal diazinon concentration in blood (5.4 μg/mL) was achieved 24 hours after administration whereas the hydroxydiazinon concentration was greatest (1.9 μg/mL) at 48 hours.

The distribution of diazinon monitored after 48 hours (in one sheep) showed that the concentration of unchanged diazinon in the tissues (taken) was; liver (18 μg/g), brain (15 μg/g), muscle (14 μg/g), kidney (12 μg/g), fat (624 μg/g), and blood (4.9 μg/g). 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 accumulated in fat and hydroxydiazinon accumulated in the liver.

Two other metabolites were characterised and one of these was quantified (isohydroxydiazinon) in urine and appeared to be excreted at an erratic rate over the 48 hours 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 [^{32}P]-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 10^8 cpm/g) was monitored in urine (0-36 hours), blood (at 0.5, 1, 2, 3, 6, 9, 12, 15, 18, 24, 36, 48, 72, 96 and 120 hours), faeces (0-36 hours) and milk (6, 12, 18, 24, 36, 48, 72, 96, 120, 144 and 168 hours). In blood, maximal radioactivity (3.3 μg eq/mL) was detected nine hours after dosing and was no longer able to be detected at 96 or 120 hours. Analysis by paper chromatography revealed that only 18.1% of the radioactivity at Cmax was associated with unchanged diazinon. Similar kinetics, albeit slightly delayed, were observed in milk where the maximal radioactivity occurred at eighteen hours (2.3 μg eq/mL) and 25–33% of the radioactivity in the 6–24 hours 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 hours. Analysis of the pooled 0-36 hours urine indicated that only 0.2% of the radioactivity was in the form of unchanged diazinon whereas diethylphosphorothioate and 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 four) were sprayed once weekly for 16 weeks with about 1 to 1.5 gallons of a 0, 0.05 or 0.1% suspension of a wettable diazinon powder formulation mixed in water. 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 2Δ-^{14}C-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'-^{14}C]-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 cm^2 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) euthanased at 2, 8, 24, 48, 72 and 144 hours, 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 hours 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 euthanased until 144 hours after exposure. Monitoring of expired carbon dioxide in the preliminary experiment indicated that the total radioactivity excreted in this form over 72 hours 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 hours after treatment. Tissues, carcass, faeces and blood were combusted in a sample oxidiser prior to liquid scintillation counting.

Recovery of radioactivity 144 hours 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 hours 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 hours and 10.2 hours for males at 1 and 10 mg/kg bw respectively, and 5.2 hours and ~ 3 hours (though incorrectly reported as 5.25 hours) 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. It is not possible to calculate the extent of the overestimate 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 hours; the loss after two and eight hours 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 hours,

i.e. exponential, then most of the cumulative loss will occur during the first two hours followed somewhat less for the next six hours 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, were calculated to be 28.1 hours and 24.1 hours for males at 1 and 10 mg/kg bw respectively, and 26.8 hours and 20.3 hours 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 two hours after dermal application at 10 mg/kg bw [i.e. %Dose: males - 1.64, 0.28, 0.14, 0.11; females - 2.22, 3.25, 2.97, 5.79, after two hours 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 two hours (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, measures taken appear to have been inadequate to prevent some oral ingestion of the dermally applied dose.

This study has been compromised by the oral ingestion of at 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, 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, applied in approximately 1 mL, or approximately 4 mg/kg bw ai) 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 hours after treatment using a GC/MS method.

Diazinon was detected in the plasma of all dogs one hour after treatment and a Cmax of 19 ng/mL (range 14.5-28 ng/mL) was achieved eight to twelve hours after dosing except for one dog in which the maximum occurred after 48 hours. The concentration in plasma declined slowly thereafter, falling below 0.5 ng/mL after seven days, and reaching undetectable levels (<0.25 ng/mL) at 14 days in five of the six dogs. Mainly because of the large time intervals between blood sampling, the kinetic profiles generally did not permit calculation of plasma diazinon half-lifes, however for two dogs in which this was possible, values of 25 and 84 hours respectively were calculated.

Sheep

Capps T (1990) Characterization and identification of major metabolites in tissues of sheep treated dermally with ^{14}C-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 ^{14}C-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'-^{14}C]-diazinon (specific activity 3.7 μCi/mg) in acetone was topically applied to two sheep daily for three 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 euthanased six hours after the last dose and pooled urine (one sheep only) and some tissues collected for residue analysis and metabolite identification. The tissues analysed for residues and metabolite characterisation were liver, kidney, heart (one sheep only), leg muscle (one sheep only) and back fat (one 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 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, i.e. 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 (breed not stated), cow (breed not stated), 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 μg/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 metabolic 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 the metabolites formed by the microsomal preparations, only the rates of metabolite production 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 avian species/breeds tested, their respective LC_{50}s differ by about two orders of magnitude between rat and avian species/breeds. It was suggested that this difference in LD_{50} might be explained by differing rates of clearance and/or detoxification of the oxon metabolite.

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 one hour and the metabolites analysed 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 minutes and essentially complete after one hour. Ion-exchange chromatography of the water-soluble metabolites resolved two major radioactive peaks, with the smaller (23%) being diethyl phosphate and the larger (69%) being diethylphosphorothioate. It was reasoned that the presence of diethyl phosphate indicated the transient formation of diazoxon because diethylphosphorothioate is not metabolised by microsomes further.

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-^{14}C]-diazinon (0.2 μmol) or [ethoxy-^{14}C]diazoxon (amount not stated) for an unspecified time interval generated a number of metabolites that were analysed by paper and Dowex 1-X8 ion exchange chromatography. The main two metabolites found were diethyl phosphate and diethylphosphorothioate whereas diazoxon was metabolised to diethyl phosphate 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 GI tract.

Excretion: The Table 2.7 is a summary of the results obtained in excretion studies following oral administration of [^{14}C]-diazinon by gavage to rats and by capsule to dogs, goats and cows. Excretion of labelled metabolites after IV administration of [^{14}C]-diazinon to Rhesus monkeys and after oral administration of [^{32}P]-diazinon to a lactating cow are also shown. Diazinon was radiolabelled with [^{14}C] at the 2' or 4' position of the pyrimidine ring or within the ring-linked ethoxy group (E).

Table 2.7: 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)	References
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: Metabolite 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 [^{14}C] 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 is also shown. Metabolites were analysed by techniques such as DEAE-Sephadex, HPLC, TLC, GC/MS or LC/MS with or without prior hydrolysis (i.e. acid, aryl sulfatase, glucuronidase). Metabolites formed after oral administration of [^{32}P]-diazinon to a lactating cow were investigated using paper chromatography. Quantitative results (expressed as percent of recovered radioactivity in sample) are summarised in Table 2.8, while the sequence of metabolite formation is shown in the pathway Figure.

Table 2.8: Metabolism of diazinon in rats, dogs, goats and sheep (expressed as % identified)

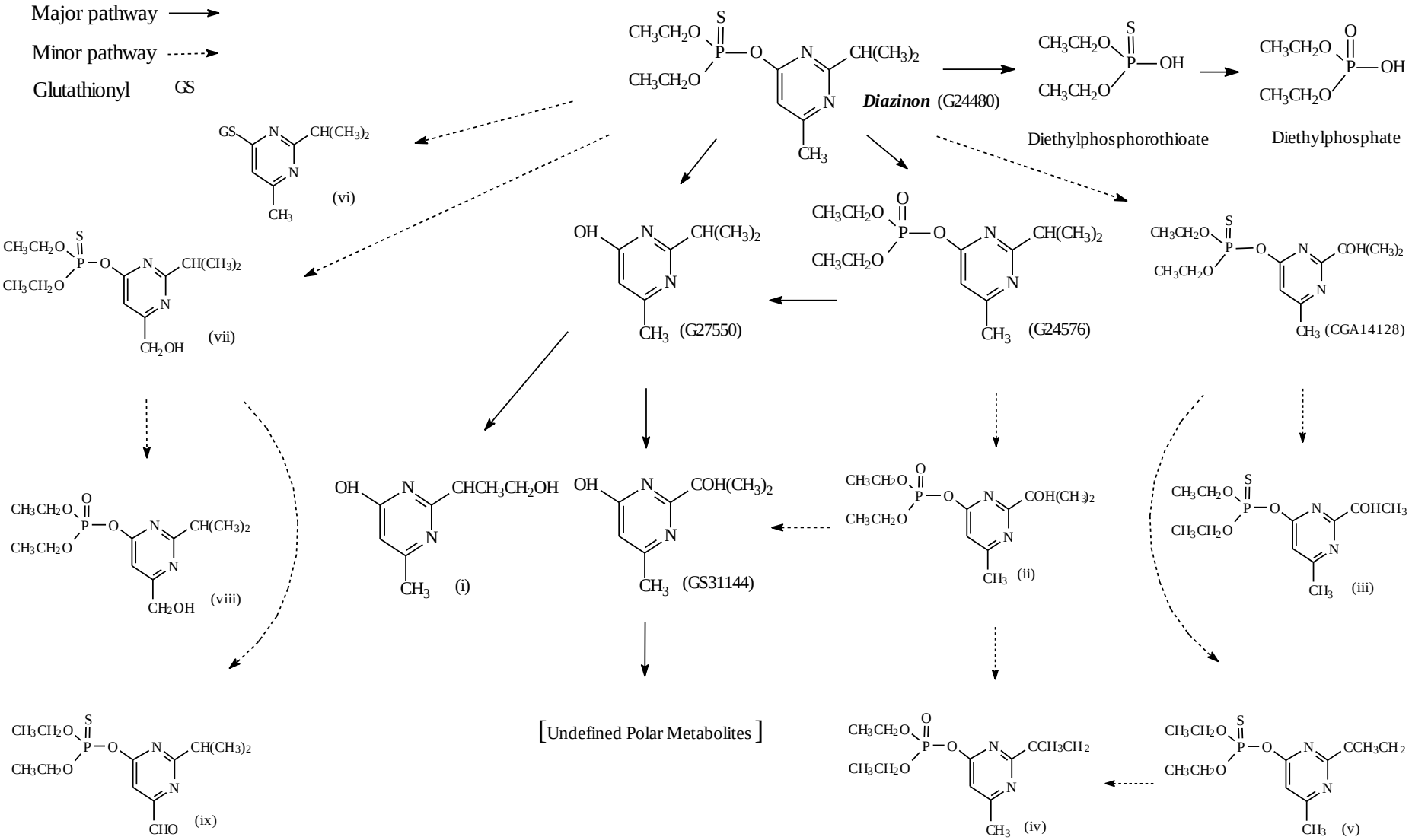
Metabolite		Rat			Dog	Goat		Sheep**
Code	Name†	Urine¤	Faeces¤	Urine§	Urine¢	Urine¶	Faeces¶	Urine‡
G24480	Diazinon	0.11	T	ND	ND	ND	T	ND
G24576	Diazoxon	0.08	T	ND	ND	ND	0.2	ND
CGA14128	O,O-diethyl O-2-(α-hydroxyisopropyl)-4-methyl-	0.14	T	ND	ND	ND	0.1	ND
G27550	2-isopropyl-6-methyl-	38.2	0.08	22.5	10	4.5	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 synthesis pathway Figure 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 six hours after dermal application; ** Capps, 1990; § Mücke et al., (1970); Capps, 1989; ¢ Iverson et al., (1975).

Figure 2.1

Proposed Metabolic Pathway of Diazinon in Mammals



2.3.3. ACUTE TOXICITY

2.3.3.1. Technical Grade Active Constituent

Median lethal dose studies

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

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, hypersalivation, ataxia, pupil constriction dyspnoea, ruffled fur, abnormal posture, tonic-clonic muscle spasms, trismus and hypoactivity. No changes in gross pathology were observed.

Table 2.9: Median Lethal Dose Studies (Oral)

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)
Rat [STD-Wistar]	M/F	10/sex	Corn oil	97.7	418, 503, 603, or 723 (M)	521 (M)	Yoshida et al. (1978)
Rat [Sprague-Dawley]	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 [Sprague-Dawley]	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 [Sprague-Dawley]	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=carboxymethyl cellulose; PEG=polyethylene glycol; * OECD guideline no. 401; ¶ US EPA guideline 81-1; † See section 3.1, Spindler, 1969 & Sterling, 1972; § Refers to stabilised product

Table 2.10: Median Lethal Dose Studies (Dermal)

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=carboxymethyl cellulose; * OECD guideline no. 402; ¶ US EPA guideline 81-2; † See section 3.1.2, Spindler, 1969 & Sterling, 1972.

Table 2.11: Median Lethal Dose Studies (Intraperitoneal)

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=carboxymethyl cellulose

Table 2.12: Median Lethal Dose Studies (Inhalation)

Species [strain]	Sex	Mode	Group Size	Vehicle	Purity (%)	Doses Tested (mg/m^3)	LC_{50} (mg/m^3)	Reference [GLP]
Inhalation {4 h}								
Mouse [Yok:ddY]	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 [Sprague-Dawley]	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]
Rat [Sprague-Dawley]	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 [Sprague-Dawley]	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.

Eye and dermal irritancy & sensitisation studies

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

Table 2.13: 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 hours occlusive	Non-irritant	Sachsse (1972g)
	Rabbit [NS]	F	6	0.1 mL/abraded and non-abraded/4 hours 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

Table 2.14: 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 seconds, 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: 0.1 mL of technical diazinon (purity 98.6%, batch number 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 minutes 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 hours, 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 one hour. This redness was still evident at 24 hours 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 males & 1 females), was 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. An assessment of ocular irritation/damage was made at 1, 24, 52 and 73 hours. No corneal or iridial changes occurred but chemosis was observed in 2 of 4 rabbits with reddened conjunctivae (grade 1) at one hour. All eyes were normal at 24 hours. 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 (approximately 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. 0.1 mL of test material was placed into the conjunctival sac of the right eye of all rabbits (6 females, 3 males) and then rinsed out of the eyes of 3 (females), 30 seconds later. The remaining 6 rabbits (3 males, 3 females) had their eyes rinsed with water after the 24 hours recording. An assessment of ocular irritation/damage was made at 1, 24, 48 and 72 hours. For rabbits whose eyes were rinsed soon after instillation, reddened conjunctivae (grade 1), chemosis (grade 1) and discharge (grade 1) were observed at one hour in 3/3, 3/3 and 2/3 rabbits respectively and in 2/3, 0/3 and 1/3 at 24 hours, 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 one hours and with somewhat reduced incidence and severity after 24 hours (i.e. grade 1 redness in 6/6, no chemosis and grade 1 discharge in 3/6) and 48 hours (i.e. grade 1 redness in 3/6 and no chemosis or discharge), and none at 72 hours. The maximum irritation score for rinsed eyes was 5.3 and 9 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 hours should be less than 2.5 and 2 respectively to permit the classification to be slight; this was observed with actual redness and oedema mean scores of 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 hours. Observation of the treated site at the time of removal (i.e. 24 hours) revealed that only 1 female rabbit had grade 1 erythema on both intact and scarified skin. At the next (last) observation (72 hours 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 four hours. Observation of the treated site at the time of removal (i.e. 4 hours) revealed that 4/6 with intact skin and 6/6 with abraded skin had grade 1 erythema. At the next observation (24 hours after application), the incidence had decreased to 1/6 for intact and 2/6 for abraded skin. At the final observation (48 hours) all rabbits appeared to have recovered.

Nissimov (1984b), OECD US GLP statement provided: Tests in a local strain of albino rabbits (2 males, 4 females; 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 hours, there were no systemic effects or skin irritation effects observed. Similarly, at 1, 24, 48, or 72 hours 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; approximately 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 cm^2 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 four hours and the residual test material removed by gentle swabbing. Application sites were examined for irritation at one hour and then 1, 2, 3, 7, 10 and 14 days following patch removal. Grade 2 erythema was observed in 6/6 rabbits at 1 hours, 4/6 at 24 hours, 3/6 at 48 hours and 2/6 at 72 hours, whereas grade 1 erythema was observed in 2/6 rabbits at 24 hours, 3/6 at 48 hours, 3/6 at 72 hours, 3/6 at 168 hours and 2/6 at 240 hours. 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 hours and in 2 at 72 hours; no oedema was observed after 72 hours. The irritation score of 2.8 from a maximum of eight at one hour 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 hours should be less than 2 to permit the irritation to be classified as 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 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 induction with 10% sodium lauryl sulfate (to maximise dermal absorption) 24 hours earlier. Skin contact was for 48 hours under occlusion. Challenge with 30 µL of diazinon occurred on day 22 with the application being covered by an occlusive dressing for 24 hours. These concentrations used in the main study were chosen based on preliminary testing (where 18/20 either died or were euthanased *in extremis* after an intradermal injection of 50% v/v diazinon).

Although 2 guinea pigs (1M + 1F) died in the main study, only 1 (male) was attributable to treatment after displaying frank signs of OP toxicity (i.e. ataxia, pallor, hypersalivation and bodyweight loss) prior to death. Autopsy was unable to determine a cause of death for the other diazinon-treated guinea pig where 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 hours caused dermal exfoliation in all diazinon-treated guinea pigs. Although dermal challenge with distilled water caused 2/10 females to have patchy, slight erythema after 24 and 48 hours, diazinon challenge caused 4/9 females to have slight, confluent erythema (grade 1) after 24 hours and after 48 hours, 2/9 had slight erythema, 4/9 had grade 1 erythema and 1/9 had moderate confluent erythema (grade 2). For males, 1/9 had slight erythema and 2/9 had grade 1 erythema after 24 hours, whereas after 48 hours, 3/9 had slight erythema. Thus, diazinon has the potential to cause a delayed skin sensitising (contact allergenic) 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 (approximately 7-10 weeks of age (weighing 285-360 g), supplied by Sasco Inc., The Woodlands, Texas; 10/group), no guinea pigs had any irritation response 48 hours 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 zero was calculated for the test group at 48 hours after challenge and therefore the diazinon formulation was classed as 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.

Effect of ageing of diazinon 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 diethyl phosphate. However, both these intermediates are able, possibly under the catalytic influence of other by-products, to combine to form the highly toxic products O,O-TEPP, O,S-TEPP or S,S-TEPP; see Figure 2.2. These products were reported to have an oral LD_{50} of 1 mg/kg bw in rats, with an attendant increased ChE inhibition. Many of the median lethal dose studies performed before 1963, when this degradation problem was not recognised, were generally much lower than found in later studies using a stabilised formulation (see 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.

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

Nichol et al., (1982): The acute oral LD_{50} 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 highlights increases in S,S-TEPP (4x), TEPP (7x) and isodiazinon (19x) concentration with ageing. The acute oral LD_{50} of pure isodiazinon was 65 mg/kg bw, and together with S,S-TEPP 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 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 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 statewide sampling programs for diazinon impurity levels, suggested the composition 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 testing 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. 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 laboratory in Sydney.

All of the registrants who responded to the request for information on the stability of their products indicated that they used technical grade active constituent sourced from either Makhteshim Agan or Ciba Geigy. The quantity of the stabiliser (epoxidised soybean oil, or ESO) added by the two manufacturers to the technical grade active constituents 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 added 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 their diazinon concentration and the presence of two of the three potential major impurities, namely O,S-TEPP or S,S-TEPP (Figure, section 3.1.3). 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 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 ai (as listed in the former Appendix L of the SUSDP) and 0.2 g/kg ai 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 Table 2.15. None of the other tested products had any detectable impurities.

Table 2.15: 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)†**
Sheep-dip 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***
Sheep-dip 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***

Product	Diazinon Label claim	Diazinon Measured	O,S-TEPP		S,S-TEPP	
	35	33.0	<0.05	-	***0.34***	***10.3***
Sheep-dip 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***
Sheep-dip 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
Sheep-dip 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
Sheep-dip 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 results which exceed the benchmark limits for impurities, i.e. 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 16.6%) contained S,S-TEPP, and thirteen (or 8.3%) of these exceeded the benchmark limit. All seven (or 4.5%) of the batches which were found to contain the highly toxic O,S-TEPP also exceeded the benchmark limit. Five of the seven batches with O,S-TEPP also contained S,S-TEPP in excess of the benchmark. Hence, 15 batches (or 9.6%) exceeded either one or both benchmark standards. Thirty-five batches (or 22%) exceeded the labelled diazinon concentration by more than 10% . 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 samples of 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–TEPP and O,S-TEPP. The results of this analysis are shown in Table 2.16.

Table 2.16: Water content in diazinon formulations containing O,S-TEPP and S,S-TEPP

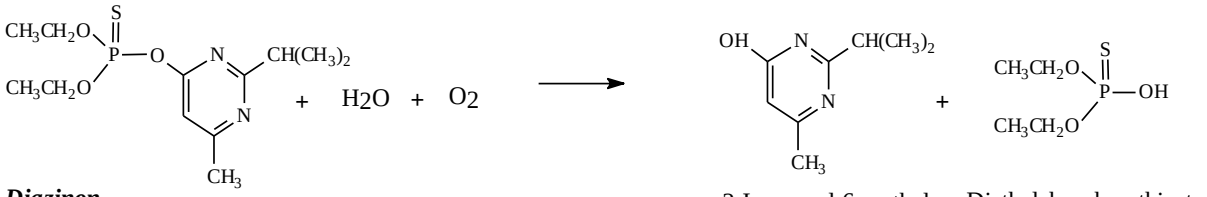
Product	Origin	Label claim Diazinon (g/L)	Water Content (mg/mL)	O,S-TEPP (g/L)	S,S-TEPP (g/L)	Diazinon (g/L)
Dog Wash	NSW	100	0.5	0.14	0.27	97.2
Dog Wash	NSW	150	0.7	1.55	0.42	94.7
Sheep-dip Blowfly Suppressant	Qld	96	2.2	4.67	<0.03	24.5
Sheep-dip Blowfly Suppressant	Qld	96	3.4	5.49	0.48	21.0
Stock Spray*	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

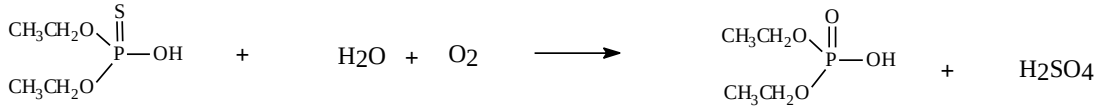
Of the 169 randomly selected samples, 26 (or 15.4%) **that also** failed to meet the ±10% label claim for diazinon were found to contain O,S-TEPP and/or S,S-TEPP. However, only eight (or 4.7%) of these samples, three from New South Wales and five from Queensland had both degradation products. Eight of these 26 products, specifically three insect killer products, two dog wash products, two sheep dip products and one stock spray 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 2.2 Proposed Formation of Diazinon Degradation Products

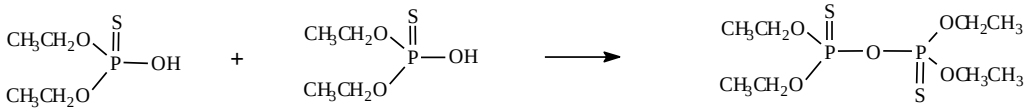
During Storage



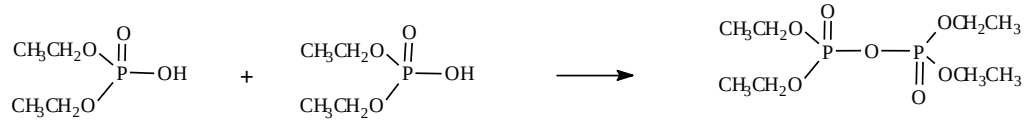
Diazinon — 2-Isopropyl-6-methyl-4(1H)-pyrimidinone — Diethylphosphorothioate



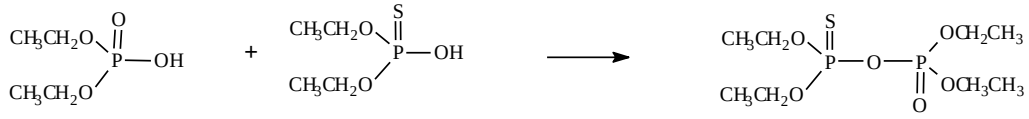
Diethylphosphorothioate — Diethylphosphate



Diethylphosphorothioate — Diethylphosphorothioate — Dithionoteraethylpyrophosphate (SulfoTEPP or S,S-TEPP)

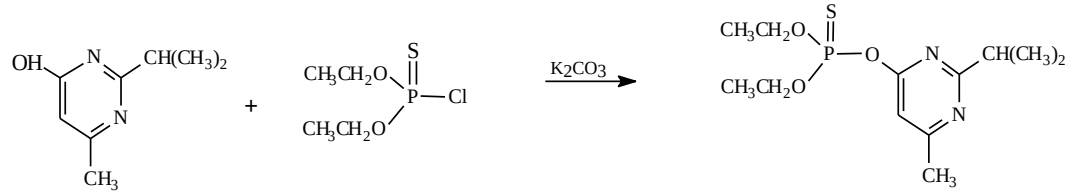


Diethylphosphate — Diethylphosphate — Tetraethylpyrophosphate (TEPP or O,O-TEPP)

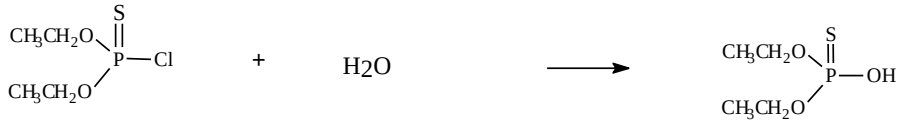


Diethylphosphate — Diethylphosphorothioate — Thionotetraethylpyrophosphate (O,S-TEPP)

As a By-Product of Manufacture



2-Isopropyl-6-methyl-4(1H)-pyrimidinone — Diethyl thiophosphorylchloride — ***Diazinon***



Diethyl thiophosphorylchloride — Diethylphosphorothioate

2.3.3.2. Isomers, metabolites, and degradation compounds

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 Table 2.17.

Table 2.17: 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 [Sprague-Dawley]	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 [Sprague-Dawley]	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)							
Rat [NS]	NS	NS	NS	NS	NS	5 (NS)	Fest & Schmidt (1973)
Rat [NS]	NS	NS	NS	NS	NS	~10 (NS)	Tomlin (1994)
Rat [Sprague-Dawley]	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.

2.3.3.3. Products

Capstar CS 500 g/L

A summary of findings of acute dose studies with a diazinon CS 500 (micro-encapsulated) formulation is shown in Table 2.18. In all studies, doses quoted refer to the product.

Note: The formulation used in the following studies was 'Capstar CS 500g/L', a dog wash product containing 470 g/L micro-encapsulated diazinon

Table 2.18: 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. #Tif:RAIf=Sprague-Dawley derived strain.

Eye and dermal irritancy & sensitisation studies

Table 2.19: Eye and Dermal Irritancy 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; NZW=New Zealand White.

Table 2.20: Sensitisation Study

Route	Species	Sex	Group size	Method	Result	Reference
Skin	Guinea pig [PW]	M/F	10/sex	Induction applications†	Non-sensitiser	Schneider & Gfeller (1987)

† 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 hours. Bodyweights recorded before and three days after treatment revealed that one rabbit had a slight and insignificant weight loss (2%) after treatment. No corneal changes occurred but grade 1 conjunctival redness was observed in 2/3 rabbits after one hour although this had resolved by 24 hours. 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 cm^2 gauze patch applied to an area of clipped unabraded skin. The patch was covered with aluminium foil and held *in situ* with surgical adhesive tape for four hours (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 one hour 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 two rabbits after one hour. At 24 hours this reaction had resolved in one but was evident in a third, previously unaffected rabbit (although absent again at 48 hours). The severity of the erythema in the other rabbit that had a reaction at one hour had increased to grade 2

at 24 hours and was accompanied by grade 1 oedema. This reaction persisted at day 3 but declined to 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 (weighing 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 fourteen days later (0.1% solution in physiological saline), and epicutaneous challenge after ten more days (1, 5, 10, 30% concentrations in Vaseline). There were no skin responses 24 or 48 hours after intra- or epicutaneous challenge. Bodyweight gains of all treated animals were normal, and there were no other clinical signs of toxicity.

Diacap 300 CS FL-93048

A summary of findings of acute dose studies with diazinon CS 300 (micro-encapsulated) formulation is shown in Table 2.20. In all studies, doses quoted refer to the product.

Note: The formulation used in the following studies was 'Diacap 300 CS FL', a 270 g/L micro-encapsulated diazinon concentrate.

Table 2.21: 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 [Sprague-Dawley]	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 [Sprague-Dawley]	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. NZW=New Zealand White.

Eye and dermal irritancy & sensitisation studies

Table 2.22: Irritancy 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 hours occlusive§	Slight	Kuhn (1993d)

† All studies conform with US GLP; ¶ US EPA guideline 81-4; § US EPA guideline 81-5; NZW=New Zealand White.

Table 2.23: Sensitisation study‡

Route	Species	Sex	Group size	Method	Result	Reference
Skin	Guinea pig [H]	M/F	10/sex	Induction applications†	Non-sensitiser	Kuhn (1993e)

‡ 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 (approximately 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 females, 3 males) and then rinsed out of the eyes of three (females), 30 seconds later. The other six rabbits (3 males, 3 females) had their eyes rinsed with water after the 24 hours recording. An assessment of ocular irritation/damage was made at 1, 24, 48 and 72 hours. For rabbits whose eyes were rinsed soon after instillation, reddened conjunctivae (grade 2) in all three rabbits and chemosis (grade 1) in one rabbit were observed at one hour but not thereafter. Similarly, for those with unrinsed eyes, reddened conjunctivae (grade 1) were seen in all six rabbits at one hour but not thereafter. Chemosis (grade 1) and discharge (grade 1) were also observed in 2/6 rabbits at one hour 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; approximately 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 cm^2 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 four hours and the residual test material removed by gentle swabbing with water. Application sites were examined for irritation at 3/4 hours and then one, two and three days following patch removal. Grade 1 erythema was observed in 5/6 and grade 2 erythema in the sixth rabbit after 3/4 hour. Only the rabbit with grade 2 erythema after 3/4 hour had grade 1 erythema at 24 hours. Oedema was observed in 4/6 rabbits at 3/4 hour but not thereafter. The irritation score of 0.5 from a maximum of 8 at one hour 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 (approximately 7-10 weeks of age (weighing 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 hours 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 zero was calculated for the test group at 48 hours after challenge and therefore the diazinon formulation was classed a non-sensitiser.

Knox-Out 2FM

A summary of findings of acute dose studies with diazinon (micro-encapsulated) formulation is shown in Table 2.24. In all studies, doses quoted refer to the product.

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

Table 2.24: Median lethal dose studies†

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference

PO	Rat [Sprague-Dawley]	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; NZW=New Zealand White.

Eye and dermal irritancy & sensitisation studies

Table 2.25: Irritancy studies†

Route	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; NZW=New Zealand White.

Table 2.26: Sensitisation study‡

Route	Species	Sex	Group size	Method	Result	Reference
Skin	Guinea pig [H]	M/F	10/sex	Induction applications†	Non-sensitiser	Armondi (1993)

‡ 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; approximately 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 hours. No ocular reaction was observed in any rabbit up to 72 hours 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; approximately 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 cm^2 gauze patch applied to an area of clipped unabraded skin. The patch was secured with a dental dam and surgical adhesive tape. The patches were removed after four hours and the residual test material removed by gentle swabbing with water. The application sites were then examined for irritation at 30-60 minutes and then 1, 2 and 3 days following patch removal. Grade 1 erythema was observed in 5/6 rabbits between 30 and 60 minutes but not thereafter. No oedema or more severe irritation was observed in any rabbit. The irritation score of 0.21 at one hour 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, two male and two female guinea pigs 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 solution was applied undiluted in the main study. In the main study the test solution was applied to shaved and occluded back skin in ten male and ten female Hartley guinea pigs (4-6 weeks old; Buckberg Lab Animals, Tomkins Cove, NY) for 6

hours, once per week, for three weeks. Negative control animals (5/sex) received distilled water. Positive control animals (three males and two females) were treated with 0.3% of 1-chloro-2,4-dinitrobenzene. The test animals were challenged at a naive skin site fourteen 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 hours 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% microencapsulated diazinon, was not a skin sensitizer, as assessed by the Buehler method, in male and female Hartley guinea pigs.

Duogard Collar Powder

Duogard Collar powder contains two active ingredients, diazinon (150 g/kg) and pyriproxyfen (2.5 g/kg). A 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.

Table 2.27: Median lethal dose studies†

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO	Rat [Sprague-Dawley]	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. NZW=New Zealand White.

Eye and dermal irritancy & sensitisation studies

Table 2.28: Irritancy studies†

Route	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; NZW=New Zealand White

Table 2.29: Sensitisation study†

Route	Species	Sex	Group size	Method	Result	Reference
Skin	Guinea pig [H]	M/F	10/sex	Induction applications	Non-sensitiser	Mercier (1995e)

† 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 three months of age (weighing 2-3 kg), had 80 mg (corresponding to a volume of about 0.1 ml) of Duogard Collar Powder introduced into the conjunctival sac of the right eye. The eyes were not rinsed and examined at 1, 24, 48 and 72 hours 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 hour, 1/6 at 24 hours and 1/6 at 48 hours. Slight conjunctival chemosis (grade 1) was

seen in 5/6 rabbits at one hour and in 2/6 at 24 hours. Slight congestion (grade 1) of the iris was present in 3/6 rabbits at one hour. Circumcorneal injection of the iris was reported in 4/6 rabbits at 1 hour, 6/6 at 24 hours, 5/6 at 48 hours, 3/6 at 72 hours, and 1/6 on day 7, but the reaction was not quantified. The mean score (of the 24, 48 and 72 hours readings) was 0.11 for conjunctival redness and chemosis. Since the severity of circumcorneal injection, which persisted for up to seven 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 three months of age (weighing 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 four hours. No signs of irritation were observed at 1, 24, 48, or 72 hours after dosing. Duogard Collar Powder 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 Collar Powder was studied in albino Hartley guinea pigs (obtained from Charles River France) of about six 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 six hours. The dosing volume was 0.5 ml. The animals were treated at the same site three times at seven day intervals. A control group of ten animals (5/sex) were similarly treated with distilled water. Fourteen days after the final induction treatment, both the control and treatment groups were challenged with 0.5 ml water and the test material as 68% paste in water under occlusive dressing for six hours. The skin reactions were examined at 24 and 48 hours after the challenge exposure. No reactions were observed after challenge exposure. The test substance was not a skin sensitiser in guinea pigs. Although this study was claimed to be conducted in compliance with the OECD guideline, no positive control studies were reported.

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.

Table 2.30: Median lethal dose studies

Route	Species	Sex	Group size	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
PO	Rat [Sprague-Dawley]	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.

Eye and dermal irritancy & sensitisation studies

Table 2.31: Irritancy studies

Route	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 hours, 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). An assessment of ocular irritation/damage was made at 1, 24, 48 and 72 hours.

At 1 hour, 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 grade 4 (more than three quarters). Iridial changes, characterised by circumcorneal injections and congestion (not quantified), were evident in all (6/6) rabbits after one hour 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 hour, 1/6 at 24 hours, 2/6 at 48 hours, and 1/6 at 72 hours. Conjunctival reactions, i.e. chemosis (oedema) and enanthema (redness), that were both grade 2 (i.e. obvious swelling of the eyelids and diffuse crimson conjunctiva) in all (6/6) rabbits after one hour showed a gradual reduction in mean intensity with time, i.e. for chemosis it reduced from 1.82 at 24 hours to 1.67 at 48 hours, and 1.50 at 72 hours whereas for enanthema the mean reduction was from 2 at 24 hours to 1.83 at 48 hours and 1.67 at 72 hours. 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 cm^2 gauze patch to an area of clipped unabraded skin. The patch was covered with a semi-occlusive dressing (perforated adhesive tape) for four hours (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 one hour and then at 24, 48 and 72 hours following patch removal. Erythema (grade 1 - primary irritation index) was observed in 4/6 rabbits after one hour. At all times thereafter no erythema was observed in any rabbit, however, slight desquamation was noted in 1/6 rabbits at 48 and 72 hours. No oedema was evident at any time in any of the treated rabbits. Therefore based on the 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).

'Preventef' (Flea collar with 15% diazinon)
'Basudin 20ES' (20% EC)
'Basudin 20WP' (20% WP)

A summary of submitted and published findings of acute median lethal dose studies for some products of diazinon, i.e. 'Preventef' (Flea collar with 15% diazinon), 'Basudin 20ES' (20% EC) and 'Basudin 20WP' (20% WP) are shown in Table 2.32.

Table 2.32: 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

2.3.3.4. Antidote studies

Harris, LW, Fleisher JH, Innerebner TA, Cliff WI & SimVM (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 LD_{50} (i.e. 235 mg/kg bw) followed 10 minutes later by an intramuscular (IM) injection of atropine (16 mg/kg bw). Twenty-four hours later, two groups of six 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 euthanased rats one hour after 2-PAM treatment.

For rabbits, diazinon was administered subcutaneously at 1600 mg/kg bw (LD_{50}=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 ChE 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 hours; from graph).

Rats treated with atropine and 2-PAM demonstrated significant reactivation of diaphragm ChE levels, i.e. 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 two hours 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.

2.3.3.5. Single dose metabolic effects

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

Although no deaths occurred, typical signs of OP poisoning (loose stools, general muscle fasciculations, diarrhoea, and/or hypersalivation) 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 three hours at 600 mg/kg bw, though the majority of these effects were observed at nine hours at doses of 300 and 600 mg/kg bw, with some reduction in observed incidence by 24 hours.

The mean percentage reductions in ChE activities are shown in Table 2.33.

Table 2.33: 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**
RBC	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**

ChE Location	Interval (h)	Male				Female			
		Dose (mg/kg bw)							
		2.5	150	300	600	2.5	150	300	600
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;
* $p \leq 0.05$; ** $p \leq 0.01$ (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 RBCs where significant inhibition became apparent at the next higher dose of 150 mg/kg bw. Irrespective of its location (brain, spinal cord, plasma or RBCs), ChE activity became inactivated (significantly) after three hours but the maximum inactivity was observed nine hours after dosing. Little or no recovery of ChE activity was evident 24 hours 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 IP injection to eight female Wistar rats (source not reported) at a dose of 40 mg/kg bw resulted in tremors and convulsions. Treated rats euthanased after these effects became maximal, i.e. after 2 hours, had significantly reduced ($p<0.05$) brain ChE (by 57%) activity but elevated fructose 1,6 diphosphatase activity (by 28%) 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.

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. 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 six 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 five dogs having their minor pancreatic duct and pylorus clamped, secretin (1 U/kg/h) stimulated mean major-duct pancreatic secretion flow rate was increased from 0.13 to 0.56 mL/min after three consecutive doses of 25 mg/kg bw diazinon injected IV 5 minutes apart; this effect was also abolished by IV atropine (0.075 mg/kg bw).

In the third experimental series 2/8[2] dogs, infused with IV secretin (2 U/kg/h) for one hour and given three bolus IV doses of diazinon (25 mg/kg bw) five minutes apart after 15 minutes, died 1 and 2 days after treatment respectively. All eight dogs developed acute hyperamylasemia (i.e. mean serum amylase increased to 11,580 SU/dL at two hours from a pre-infusion level of 1610 SU/dL; $p<0.02$) and hyperlipasemia (i.e. mean serum lipase increased to 8.75 STU/mL at two hours from a pre-infusion level of 0.41 STU/mL; $p<0.02$) during treatment. Serum lipase was still elevated 24 hours 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 one day after treatment, post-mortem 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 two hours that were not evident in those euthanased at 72 hours. Under microscopic examination acinar cell vacuolization was the predominant finding in pancreata at 2 hours, 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 hours and all controls appeared normal.

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 four pentobarbital-anaesthetised dogs one hour 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 five control dogs, butyryl ChE activity was identified in the nerves of the duodenum, the pancreatic duct, and those nerves innervating the smooth muscle of the ampullary region. Acetyl ChE 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 butyryl ChE activity was observed in either the smooth muscle or nerves whereas acetyl ChE, albeit with reduced activity, was still present. It was reasoned based on the results that the pancreatic ductal hypertension that occurs following diazinon treatment is due to selective reduction in pancreatic smooth muscle butyryl ChE 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 acetyl ChE and butyryl ChE it was reasoned that the comparative investigation of a species with an abundance of acinar cell (tissue) fixed butyryl ChE (e.g. guinea pig) with one having an absence (e.g. cat) might help explain the propensity for dogs (with

[2] It was stated that 12 healthy dogs were divided into four treatment groups, i.e. with or without diazinon and euthanased after 2 or 72 h, yet the number of dogs in the groups totalled 13.

abundant tissue-fixed butyryl ChE distribution) to develop pancreatitis after acute diazinon treatment.

In a second study in this series (Dressel et al., 1979), secretin (1 U/kg/h) was slowly administered IV by infusion together with three bolus IV doses (in different veins) of diazinon (25 mg/kg bw) five minutes apart to eight anaesthetised mongrel dogs, three cats and three guinea pigs (strain and gender not stated) and all were euthanased, after three hours for dogs and two hours for cats and guinea pigs. Another regimen, with diazinon alone, was given to three cats and twenty guinea pigs; cats were euthanased at three hours or 6 hours, whereas for guinea pigs it was three hours (eight guinea pigs), six hours or 24 hours (six 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 (Dressel et al., 1979). 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 ($p<0.001$) three hours after administration but changes in the cat and guinea pig were not significant. In contrast, serum butyryl ChE 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 three hours confirmed the results of a previous study (Dressel et al., 1979) where dogs had disseminated vasiculitis, focal perivascular exudation and basolateral vacuolisation of the acinar cells after diazinon treatment in the presence or absence of secretin, although these changes were more pronounced with the combination treatment. Atropine pre-treatment (0.2 mg/kg bw) prevented formation of these lesions. In guinea pigs, vasculitis developed after two hours or three hours in the presence or absence of secretin, and perivascular exudation and basolateral vacuolisation of the acinar cells became apparent at three hours and six hours, although some regeneration was apparent at 24 hours; atropine again prevented the development of these lesions. Degeneration of mitochondria in guinea pigs 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 two, three and six hours were 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 μM echothiophate (an OP) for one hour and then with 10 μM acetylcholine for two hours 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, i.e. approximately 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 two 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, mean corpuscular haemoglobin, MCHC, RBC count, WBC, Hct, reticulocytes, differential blood count, PT, 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, albumin, globulin and A/G ratio together with RBC and serum ChE activity. In addition to the four 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 once pre-test (for ~16 hours) 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.

Necroscopy was performed on the dogs that either died or were euthanased during the study as well as those euthanased at the euthanasia end of the study. Absolute and body-relative organ weights of the heart, liver, kidneys, testes/ovaries, adrenals, thymus, prostate, thyroids, uterus and brain were also recorded euthanasia. 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 plasma ChE activity. The mean percentage reduction in plasma ChE activity is shown in Table 2.34.

Table 2.34: 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.

2.3.4. SHORT-TERM REPEAT-DOSE TOXICITY

2.3.4.1. Rat

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 zero 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 RBC ChE activity measurements were collected from random groups of ten 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 six euthanased 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 two studies are shown in Table 2.35.

Table 2.35: Maximum ChE Inhibition (mean percentage reduction)

Dose (ppm)	Duration (days)	Plasma ChE		RBC 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; * $p \leq 0.05$, 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 (i.e. plasma and RBCs) 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 (Crl: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. females-00176), 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. Cholinesterase activity in plasma, RBCs, 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 weight change 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 one 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 three measurements are shown in Table 2.36.

Table 2.36: 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**
RBC	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;
* $p<0.05$; ** $p<0.01$ (Dunnett's t test).

Significant and dose-related inhibition of plasma and RBC ChE was evident in males and females at concentrations equal to and greater than 30 ppm (2.4 mg/kg bw/day) from week one onwards. Cholinesterase 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 one 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 RBCs was at least an order of magnitude more sensitive to diazinon treatment than that observed in regional areas of the brain.

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/m^3 for six hours per day, five days per week for 21 days. The inhalational particle diameter distribution was assessed as being predominantly less than 7 μm (83.3-90.7%) with 34.6-43.5% being less than 1 μm. Eight rats (4/sex) from the control and 559 mg/m^3 groups were kept for an additional recovery period of 25 days after the daily treatment phase, whereas all others were euthanased 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/m^3 groups were assessed using 5 rats/group and 9 rats/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 rats/group were used for all ChE activity estimations. Haematology measured Hct, Hb, RBC count, WBC, a leucocyte differential count, thrombocyte count, Heinz body count, reticulocyte count, PT and RBC 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 euthanased at the end of treatment except for the eight allowed to recover; these were euthanased on day 25. 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. In treated animals exophthalmos, hypersalivation, diarrhoea, ruffled fur and tonic-clonic muscle spasms were observed daily within two hours after exposure at the highest concentration of 559 mg/m^3; at 151 and 245 mg/m^3, exophthalmos and diarrhoea were observed with a rapid onset, i.e. immediately after exposure. No clinical signs were evident during the 25-day recovery period. Food consumption for males and females at 559 mg/m^3 was reduced during the first three days of treatment but thereafter appeared to be within the range of the other groups. Probably reflecting reduced food-energy conversion, bodyweight gain of males and females at 559 mg/m^3

(approximately 10% and 6% respectively) and males at 245 mg/m^3 (approximately 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 RBCs, plasma and brain. The mean percentage reductions in ChE activities are shown in Table 2.37.

Table 2.37: ChE Inhibition (mean percentage reduction)

Concentration (mg/m^3)	Duration (days)	Plasma ChE		RBC 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 Table 2.37) indicates that for males at the lowest dose tested of 151 mg/m^3, no significant ChE inhibition occurred. Thus, given the clinical signs (exophthalmos 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/m^3 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 RBCs)? 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 (i.e. Cholinesterase activity (in Klett units) in females; plasma: control - 30, 41, 31, 42, 38, 38, 36, 40, 35, and at 151 mg/m^3 - 35, 44, 37, 32, 33, 32, 35, 35, 40; brain: control - 244, 178, 194, 159, 194, and at 151 mg/m^3 - 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/m^3 were found to have clinical signs of poisoning (exophthalmos and diarrhoea) and a significant reduction (p≤0.01) in brain and plasma ChE activity at concentrations at or above 245 mg/m^3. 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/m^3, logically this cannot be considered to be biologically plausible due to the absence of a corresponding significant inhibition of blood ChE activity (i.e. in plasma and/or RBCs). However, another inhalational (nose-only) study by Hartmann (1990) 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/m^3 is, for some 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 m^3 room housing eighty Sprague-Dawley rats (40/sex, Control:COBS CD (Sprague-Dawley)) 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 $\mu g/m^3$ respectively. However, no corresponding ChE inhibition in plasma or RBCs was detected in rats (5/sex) using an automated 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 (Control: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 test concentrations for the respirable aerosol, namely 0 mg/m^3 (air-only), 15, 100, or 750 mg/m^3, were based on a review of the results of an acute exposure study that determined a combined sex LC_{50} of 4370 mg/m^3 (Hardy & Jackson, 1984). The actual mean diazinon concentrations measured in the chamber by GLC analysis were found to be 15, 97 and 710 mg/m^3 respectively for the treatment groups and the percentage of respirable aerosol particles (i.e. Diameter <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 one 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, RBC and total and differential WBC counts, platelet count, and cell morphology (e.g. 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 RBC ChE activity. Gross necropsies were performed on all rats following euthanasia at euthanasia 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/m^3, where abnormal respiratory movements, hypersalivation 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/m^3 and for females at 97 mg/m^3. The significantly reduced (males 27%, females 21%; p≤0.001 & p≤0.01 respectively) mean bodyweight gain of the 710 mg/m^3 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/m^3, significance for reduced water consumption was only achieved for the first day after the initial exposure. Urinalysis investigations on day 23 (males) and 24 (females) revealed that males at 710 mg/m^3 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 RBCs 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/m^3 where significant reductions (p<0.05) in total protein (males 6%, females 11%), globulin (males 15%, females 11%) and creatinine (males 17%) were associated with an elevated A/G (males 20%, females had reduced albumin - 11%) and AP (males 25%, females 43%) in plasma. The mean percentage reductions in ChE activities are shown in Table 2.38.

Table 2.38: ChE Inhibition (mean percentage reduction)

Concentration (mg/m^3)	Plasma ChE		RBC 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 euthanasia following euthanasia 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/m^3. Epithelial hyperplasia over the arytenoid projection of the larynx was also detected in three other males at 710 mg/m^3.

Thus, Wistar rats exposed to an aerosol of diazinon at concentrations up to 710 mg/m^3 in a chamber had clinical signs of OP toxicity (tremors, abnormal respiratory movements, hypersalivation and gasping) at 710 mg/m^3 and significant dose-related reductions in RBC and plasma ChE activities for all tested concentrations (i.e. 15, 97 and 710 mg/m^3). 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/m^3, were significantly (p<0.05) reduced total protein (males 6%, females 11%), globulin (males 15%, females 11%), creatinine (males 17%), and elevated A/G (males 20%, females had reduced albumin - 11%) and AP (males 25%, females 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/m^3) 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/m^3, were based on a preliminary study (2/sex/group) that found a 62% inhibition of plasma ChE in both sexes at 3 mg/m^3 and none at 0.3 mg/m^3. Two control groups, namely air-only and ethanol at 24000 mg/m^3, 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/m^3 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 euthanasia with a pre-test sample of blood being collected to establish plasma and RBC ChE activities. Haematology parameters were MCHC, MCV, Hct, Hb, RBC and total and differential WBC counts, thrombocyte count, PT, 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 RBC ChE activity. Gross necropsies were performed on all rats at euthanasia (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/m^3 that was found dead just prior to the euthanasia completion of testing, 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/m^3 males was significantly reduced (6%; $p \leq 0.05$) relative to the ethanol controls, but not air controls, for week two, this transient effect is unlikely treatment related because of the lack of any dose relationship. Similarly, reduced food consumption for males at 0.05 mg/m^3 during weeks one and two but not for the 0.46, 1.57, or 11.6 mg/m^3 groups suggests that this change is unrelated to treatment. Apart from minor (not exceeding 5% relative to either the air or ethanol controls), although in some cases significant changes ($p \leq 0.05$) in some RBC parameters, i.e. reduced RBC and Hb, and increased MCV in females at 11.6 mg/m^3, 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/m^3 (14 and 15% respectively, $p \leq 0.05$). The mean percentage reductions in ChE activities are shown in Table 2.39.

Table 2.39: ChE Inhibition (mean percentage reduction)¶

Concentration (mg/m^3)	Plasma ChE		RBC 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**,	6, 8	10*, 12**	4, [3]	20*, 20*

		35**				
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% (e.g. RBC ChE activity in males at 0.46 mg/m^3) and yet not for others with apparently a similar degree of inhibition (e.g. 13% for plasma ChE at 0.05 mg/m^3), this 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/m^3 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 (i.e. in plasma and/or RBC).

Macroscopic and microscopic examination of tissues taken at the euthanasia completion of testing revealed no lesions that could be attributed to treatment, as their incidence was similar to results observed 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/m^3 (6%, $p \leq 0.5$ and 9%, $p \leq 0.01$ respectively) but not at 11.6 mg/m^3. 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/m^3 had no clinical signs of poisoning but had statistically significant reductions in brain and plasma ChE activities at 0.46 mg/m^3. 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/m^3 and some RBC parameters, i.e. reduced RBC and Hb, and increased MCV in females at 11.6 mg/m^3.

2.3.4.2. Rabbit

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 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, six hours 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, and haematology and blood chemistry were assessed prior to and at the end of the study. The following haematology parameters were determined; Hb, RBC 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 RBC, serum and brain ChE activity.

Necroscopy was performed on the rabbits that either died or were euthanased during the study as well as those euthanased at the euthanasia end of the study. 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 (including brain stem) were also recorded euthanasia following completion of the study.

Since four of five 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, hypersalivation 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 one of the three 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 rabbits that did not die during the treatment phase (i.e. 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. There was a significant increase in 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 that survived treatment at 100/50 mg/kg bw/day is of doubtful biological significance. The mean percentage reductions in ChE activities are shown in Table 2.40.

Table 2.40: ChE Inhibition (mean percentage reduction)

Dose (mg/kg bw/day)	Plasma ChE		RBC 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; * $p \leq 0.05$; ** $p \leq 0.01$.

Apart from diffuse dark red lesions (irritation) of the stomach in the male at 50/100 mg/kg bw/day, autopsy 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 (i.e. 20%, 26% and 28% at 1, 5 and 100/50 mg/kg bw/day respectively) achieved significance ($p \leq 0.05$) 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.

2.3.5. SUBCHRONIC TOXICITY

2.3.5.1. Rat

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 which 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 twelve 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 euthanased 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. Cholinesterase activities in plasma, RBCs and brain were also determined (by colorimetric assay). Bodyweight was recorded at 3-4 week intervals.

Although there were no overt 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 (i.e. 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, i.e. Asp and Glu, and inhibitory neurotransmitters, i.e. 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 appeared to use tissue specimens from the same 12 treated male Wistar rats whose treatment was described in a previous study (Rajendra et al., 1986), livers were

taken after euthanasia and processed for light and electron microscopy to assess any changes arising from a 28-week diazinon treatment regime.

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), were euthanased 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, there were no overt clinical signs but the diazinon-treated rats were significantly lighter (10%) 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 these observations is unclear.

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. electromyography measurements were made at 2- or 4-week intervals, and the ChE activities in plasma and RBCs were determined every two weeks.

For the electromyography 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 were applied; there was little variation in the action potential produced by each of these pulses.

No overt clinical signs were observed during the feeding period, although 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 and 5 mg/kg bw/day. RBC 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 RBC 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 electromyography examinations throughout the study. This indicates that, in rats, electromyography measurement may not be a useful indicator of

exposure to diazinon. In this 26-week study, inhibition of plasma and RBC 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 (Davies & Holub, 1980a). In order to increase the accuracy for determining a NOEL based on ChE inhibition in plasma, RBCs and brain, a second and third study, each with a reduced duration and diazinon concentration, were performed. The second study involved groups of sixteen rats being fed diazinon at 0, 1, 2, 3, or 4 ppm for 42 days while groups of ten 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 four months if stored at 3-4 ^{0}C after preparation. The measured diazinon concentration in the diet indicated that actual levels were within ±5% of the targets. Food consumption and bodyweight were recorded twice weekly and clinical signs were monitored daily.

In the three studies, blood for plasma and RBC 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 two longer duration studies where satellite groups of 6 rats were euthanased at various times (5-14 day intervals). Cholinesterase activities were measured using a radiometric method with tritiated acetylcholine as the substrate.

There were no clinical signs observed at any dose in the three 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 three studies are shown in Table 2.41.

Table 2.41: Maximum ChE Inhibition (mean percentage reduction)

Dose (ppm)	Duration (days)	Plasma ChE	RBC 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 RBCs 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. 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 (ai) 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 five, to enable ChE activity in the brain and blood (plasma and RBCs) to be assessed; ChE activities were determined using an electrometric technique.

Although two rats at 0.5 ppm died, one from pneumonia and the other because of physical entrapment in a wire food spiller, neither death was 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, and a 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 two 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 Table 2.42.

Table 2.42: ChE Inhibition (mean percentage reduction)[¶]

Concentration (ppm)	Plasma ChE		RBC 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 (approximately 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 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 twenty weeks at room temperature. Food consumption

and bodyweight were measured weekly for two 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 twelve. Similarly, ophthalmoscopy and an auditory/physical examination were also performed before treatment and then again during weeks twelve and fourteen respectively. Haematology (Hct, Hb, RBC 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 PT) and clinical chemistry including ChE activity (by colorimetric assay) were determined during week thirteen. 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 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 changes associated with treatment were 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 mid-period 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 (i.e. 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 RBCs, plasma and brain that were attributable to treatment. These mean percentage reductions in ChE activities are shown in Table 2.43.

Table 2.43: ChE Inhibition (mean percentage reduction)

Concentration (ppm)	Plasma ChE		RBC 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 RBCs 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 (males 2.2%, females 1.6%; $p<0.01$ for both) at 2500 ppm, that was associated with non-significant reductions in urine volume (males 20%; females 17%) and water consumption (males 17%; females 12%), urinalysis was similar among treatment groups.

Macroscopic inspection of organs at autopsy 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 centrilolobular 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 had reduced bodyweight gain, 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 RBC 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 not given) mixed with the diet was fed to groups of ten 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 ai/kg bw/day) for sixteen weeks. Dilutions of the formulation were prepared at seven 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. Cholinesterase activity in pooled RBCs and plasma of four 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, RBCs and brain measured in all treated rats.

No treatment-related deaths or clinical signs were observed. At the conclusion of the study RBC ChE activity was depressed to 90% and 81% of controls at 1 and 5 ppm respectively. At 25 ppm and 125 ppm, RBC ChE activity decreased to 54% and 20% respectively of the controls after four 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 RBC ChE activity in male rats at 25 ppm.

2.3.5.2 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 two 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 RBC ChE activity measurements were collected hours after dosing on day 0, 1, 3, 8, 16, or 17, 31 or 33, 64, or 66 and 90 of the study. Cholinesterase activities in blood were measured using a manometric technique and compared with the pre-test 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 six 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 sixteen days at 0.02 mg/kg bw/day, to 38% after eight days at 0.04 mg/kg bw/day and 62% at day 33 at 0.08 mg/kg bw/day. In RBCs, ChE activity was in excess of the pre-test value at all doses and all sampling times except for 25% inhibition at 0.08 mg/kg bw/day on day eight.

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; and 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

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

Treatment-related effects other than ChE inhibition were not measured or reported. Although the results for plasma and RBC 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 RBCs, the activity was only significantly inhibited (by 55%) at 75 ppm. Recovery in plasma was complete after about four weeks whereas the activity in RBCs 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 thirteen 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 two 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 five, nine and fourteen. Similarly, ophthalmoscopy and an auditory/physical examination were also performed before treatment and then again during weeks fourteen and thirteen respectively. Haematology (Hct, Hb, RBC 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 PT) and clinical chemistry were determined during treatment weeks five and nine, and then prior to euthanasia (week thirteen) except for ChE activity (by colorimetric assay) in the brain that was measured after euthanasia (week fourteen). 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 was observed in females (by 34%) at 150 ppm and in both sexes at 300 ppm (males, 33%; females, 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 PT (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 non-segmented 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 (i.e. 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 RBCs, plasma and brain. These mean percentage reductions in ChE activities are shown in Table 2.44.

Table 2.44: ChE Inhibition after 5, 9 and 13 weeks of treatment (mean percentage reduction)

Concentration (ppm)	Plasma ChE		RBC 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; * $p\leq0.05$; ** $p\leq0.01$.

The three entries for ChE activity in plasma and RBCs shown in Table 2.44 are those recorded after 29, 56 and 86 days of treatment respectively. The mean values for the three 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 euthanasia revealed no changes attributable to treatment. Similarly, although a number of isolated incidences of statistically significant changes in organ weight were observed (epididymis, 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 in a published study (25 mg/kg bw; Frick et al., 1987).

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 eight 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, one or both dogs at each of the tested doses died within a day. Autopsy, performed on dogs euthanased after surviving more than a day (i.e. 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 eight examined dogs, however, the major findings were haemorrhage in the colon, and a yellow-brown pigment in the cytoplasm of hepatic parenchymal cells and on the 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 euthanased at the conclusion of the study or found moribund (and then euthanased) or found dead during the study, an autopsy 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, gall bladder, 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, RBC 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 three males and a female died or were euthanased *in extremis* during treatment. The males died on days 14, 24 and 166 and the female on day 19. Reduced food intake, along with emesis, occasional diarrhoea and body muscle fasciculations were observed among the dogs prior to euthanasia. Similar cholinergic signs were noted in a dog (1/6) at 10 mg/kg bw/day during the first 45 days but it was not euthanased. 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 three 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 euthanased. 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 five 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 the two male dogs that died. Markedly increased serum amylase were also observed in these two dogs as well as in the three surviving dogs at 10 mg/kg bw/day and another dog treated 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 five of six dogs, marked oedematous thickening of the jejunum or duodenum occurred with one having a ruptured duodenum resulting in peritonitis while another dog 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 also been completely arrested and 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.

2.3.5.3 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 miniature pig component of a comparative study in dogs and miniature pigs.]

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 eight months. Dose selection was based on a dose-ranging study in which survival, necroscopy and histopathology of four 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 euthanased *in extremis*, except for one in the 500 mg/kg bw/day group that survived treatment. Autopsy on pigs treated at 5, 10, 25, or 50 mg/kg bw/day, revealed similar lesions as for dogs (see section 5.2), i.e. 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 six of eight 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. An autopsy was performed for those animals euthanased at the conclusion of the main study or found moribund (and then euthanased) or found dead during the study. 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, gall bladder, pancreas, spleen, kidney, adrenal, urinary bladder, stomach, small intestine, colon, testes or ovary and uterus, sciatic nerve, and skeletal muscle were examined. 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, RBC 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 one female survived treatment. The other five pigs in the group died on days 12, 16, 20, 25, and 38, and had characteristic cholinergic signs prior to death, although 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 fifteen days, was remarkable by virtue of the delayed onset, i.e. after four months of treatment. At 5 mg/kg bw/day there were no deaths and only one pig had signs that persisted for the first six months of treatment, whereas at 2.5 mg/kg bw/day the only pig that developed clinical signs after nineteen weeks was euthanased *in extremis* during week twenty. In four of the five pigs that died at 10 mg/kg bw/day, oedematous thickening of the jejunum wall was observed, and one pig had localised mucosal erosion that penetrated into the muscular layers resulting in a marked mucosal seepage throughout the intestines. Three of the five 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 euthanased 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 one pig were associated with this euthanased pig (no individual animal data).

Haematological parameters were normal except for a slight elevation in the myeloid:erythroid ratio (two-fold) and a reduced reticulocyte count (four-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 presented) whether the friability was associated

with the lobular congestion observed under microscopic examination in a pig from this treatment group.

The clinical chemistry data reported erratic levels in some pigs from all treatment groups and a NOEL was not established in this study.

2.3.6. CHRONIC TOXICITY

2.3.6.1 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 seven 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 four years. Dose selection was based on a dose-ranging study in which survival and bodyweight at concentrations up to 3200 ppm were assessed after thirteen-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 two weeks prior to and during treatment, and clinical monitoring was performed twice daily. For mice euthanased at the conclusion of the study (103 weeks treatment + one week recovery) and for any found moribund (and then euthanased) or found dead during the study, an autopsy 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 a marginally significant increased incidence of hepatocellular carcinoma at 100 ppm, (i.e. 4/21, 20/46 (p=0.046) and 10/48 for control, 100 and 200 ppm groups respectively) this observation was not associated with a corresponding increase in hepatocellular adenoma (2/23, 0/47, 3/49 for control, 100 and 200 ppm groups respectively), and there was no treatment-related increase in the total number of tumours in treated mice relative to controls. Therefore, under conditions of this assay, there was no carcinogenicity in mice at the maximal dose tested of 200 ppm (30 mg/kg bw/day). Further, 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 nine 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 eleven 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 tumours, was initiated. After month fourteen, 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. Autopsy and tissue specimen processing of corpses and animals euthanased at the scheduled kill were performed at the IBT labs, however, the histopathological examination of the prepared sections was not performed until two 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 three-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 rates were comparable in all groups, i.e. 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, i.e. 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 two months and then (transiently) at months seven, nine and fourteen for the 20 and 100 ppm groups. In contrast, a significantly ($p<0.05$ - 0.01) reduced bodyweight was observed among all treated females (i.e. 13%, 11% and 12% at 4, 20 and 100 ppm respectively at month eighteen)

throughout the study except for months six, thirteen and nineteen. Macroscopic examination of tissues taken from animals that died and those euthanased revealed mainly isolated incidences of kidneys with discoloration or granular surface, and enlarged spleen among males and females; urine stained perineum and subcutis oedema among males, ovarian cysts and enlarged uterine horns or uterii 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 to assess carcinogenicity potential. However, it was observed that rats fed diazinon in their diet at a concentration that caused significant bodyweight loss showed no increase in tumour incidence.

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 seven days between fresh batches. Mice were monitored for death and clinical signs twice daily for the first two and last nine 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, RBC and reticulocyte counts, total and differential leucocyte counts and platelet count) was assessed at twelve and 24 months on 10 rats/sex that were randomly selected from each group. At euthanasia some organs were removed, i.e. adrenal glands, brain with medulla, heart, kidneys, liver, lungs, ovaries/testes and spleen and weighed before undergoing fixation in preparation for a histopathological investigation.

Mice bled at twelve months (for haematology) and all survivors after two years of treatment were euthanased and a gross autopsy performed. A histopathological investigation was performed on any gross lesions (including tissue masses) and on specimens of adrenal gland, brain (at least three levels from the forebrain, midbrain and hindbrain), oesophagus, eyes (and the contiguous Harderian gland), ovaries, uterus (corpus and cervix), mammary gland, bone (including marrow from sternum, vertebrae or tibiofemoral joint), heart, kidneys, large intestine, liver (at least two lobes), lungs (including 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, four were accidently euthanased and another 57 were euthanased *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 (i.e. 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 eleven 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 on seven occasions in females. However, although the overall food consumption in males was approximately 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. The reduction was 41%, 43% and 51% at 100, 200 and 400 ppm respectively, but achieved significance ($p<0.05$) only at the highest dose. Absolute heart weight was increased for all treatment mice after twelve and 24 months, however, significance ($p<0.05$) was not achieved except for females at 400 ppm (16%) after twelve 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, i.e. liver (by 12%) and kidney (by 11%) in females at 400 ppm after 24 months are also possibly treatment related.

There was no consistent treatment-related increase in the 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:

Table 2.45: Summary of Neoplasia Incidence

Observation	Sex	Treatment Group (ppm)				
		0	**100**	**200**	**300**	**400**
Number examined	M	59	60	59	56	ND
	F	57	59	60	ND	60
Number with Benign neoplasms	M	13	14	7	13	ND
	F	7	17	8	ND	12
Number with 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

ND=Not determined.

A statistical analysis of the tumour 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 tumours in B6C3F1 mice.

2.3.6.2 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 the previous study in B6C3F1 mice (Goldsmith and Craig, 1983), 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 + two weeks recovery for males or one 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 survival and bodyweight were reduced.

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 tachypnoea among rats at 800 ppm, although 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, although 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). Given that there was no dose response evident, 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), i.e. 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 the observed results 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, there is no evidence that diazinon is carcinogenic. A NOEL for non-carcinogenic results could not be established as hyperactivity was evident among all treated males.

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 euthanasia was a reduced Hct in males at 1000 ppm.

Reductions in ChE activity in brain, RBCs and plasma were apparently dose related, with the greatest reduction being observed in plasma followed in order by that in RBCs and the brain.

In the summary reporting of the gross and histopathological observations was 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 two 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 (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 fifteen weeks throughout the two studies. The feed admixtures were found to be suitable with respect to homogeneity (6% variability), stability (5%) and concentration (coefficient of variability of 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 seven 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, RBC counts, MCV, mean corpuscular haemoglobin, 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 after week 12, 25, 51, 102 and 120 (or 121 for clinical chemistry) on 10/sex/group in the first study and on 10 females/group in study two. In the first study ChE activities (i.e. using acetyl and butyrylcholine substrates) were measured (by colorimetric analysis) at the same time as other clinical chemistry parameters were measured, except that additional blood was collected after 17 and 38 weeks, and acetyl ChE activity was not measured at week 120, nor was butyryl ChE activity measured at week 17, 25, 51 and 102. 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 sampling in the first study (weeks 18, 27, 52 and 104) and from eight females at euthanasia in the second study. At the euthanasia in both studies, some organs were removed, i.e. 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 an 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, even though the results observed are not significant. 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, i.e. ulceration (males, 12; females, 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 euthanased *in extremis* before the terminal or interim sampling points. 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 seventeen and sixteen 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 ten weeks this reversed and remained significantly less than observed at 0.1 and 1.5 mg/kg bw/day for the next fourteen 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, i.e. 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, i.e. 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) 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, i.e. 0. 025, 0.1, 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 twelve in females ($p<0.001$) and week one 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 twelve weeks of treatment. The RBC, Hb and Hct were reduced in an approximate relationship with dose, i.e. 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%** (where * $p<0.05$; [¶] $p<0.01$; ** $p<0.001$). The lymphocyte counts were similarly reduced for the three respective treatment groups, i.e. 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 (males 11%, $p<0.05$; females 22%, $p<0.01$) after twelve weeks and maintained until week 51. Other changes in clinical chemistry that were possibly related to treatment were a significant increase ($p<0.001$) in the concentration of uric acid at 25 weeks, i.e. two-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 Table 2.46.

Table 2.46: ChE Inhibition after 13, 26, 39, 52, 65, 78, 91 and 104 weeks of treatment (mean percentage reduction)

Dose (mg/kg bw/day)	ChE	Plasma		RBC		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

Dose (mg/kg bw/day)	ChE	Plasma		RBC		Brain†	
		male	female	male	female	male	female
	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 Table 2.46, 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 RBCs 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, i.e. 22.5 mg/kg bw/day.

No consistent treatment-related increase in incidence of palpable masses, macroscopic changes and organ weight were observed at 0.1, 1.5, or 22.5 mg/kg bw/day at euthanasia. 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 (eight relative to zero in controls), acanthosis (nine relative to zero in controls) and hyperkeratosis (eight relative to zero in controls) of the keratinised cells in the stomach, results 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:

Table 2.47: Summary of Neoplasia Incidence

		Treatment Group (mg/kg bw/day)				
	Sex	**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 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(i.e. with 26.5 ppm of ESO (the stabiliser present in the technical formulation), 0.1, 1.5, 125, or 250 ppm ai (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 (i.e. within 15% of the target except for two 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) so that for chronic dosing the NOEL was anticipated to be 0.1 ppm, whereas the second lowest concentration of 1.5 ppm was anticipated to be the NOAEL with only serum ChE activity being affected. Cholinesterase activities in brain, RBCs and plasma were all anticipated to be affected at the highest concentration of 250 ppm (i.e. 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 thirteen weeks and monthly thereafter. Physical and auditory examinations were performed two 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 euthanased for an interim autopsy and another10 rats/sex in the control, vehicle control (i.e. with ESO) and 250 ppm groups were fed the untreated diet for a four-week recovery period. Interim recovery rats were then euthanased for autopsy during week 57. All other rats were euthanased and autopsied during weeks 98-99 of treatment.

Urine volume and water consumption were recorded two 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 RBC 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 one week before treatment; these animals were then euthanased. At the termination of the main study, bodyweights were measured and the main organs (i.e. adrenal glands, brain (including brainstem), epididymides, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes, thymus, and uterus) were removed and weighed before fixation.

Histopathological investigation was performed on any gross lesions (including 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), epididymides, 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, non-glandular), 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, i.e. 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 treatments of 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 (i.e. 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 (i.e. time patterns or dose-response relationships) evident in haematology (Hct, Hb, RBC count, WBC, differential leucocyte count, Heinz body, platelet count, reticulocyte count, PT, mean corpuscular volume and mean corpuscular Hb 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 (i.e. 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 Table 2.48.

Table 2.48: ChE Inhibition after 13, 26, 51, 79 and 98 weeks of treatment (mean percentage reduction)

Concentration (ppm)	Plasma ChE		RBC 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]
Mean	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]

Concentration (ppm)	Plasma ChE		RBC ChE		Brain ChE†	
	male	female	male	female	male	female
Mean	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**
Mean	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**
Mean	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 RBCs shown in Table 2.48 are those recorded during treatment weeks 13, 26, 51, 79, and 98 respectively. Rats at 250 ppm that were allowed to recover for four weeks after 52 weeks of treatment had reduced inhibition of plasma (to 6% in males and 10% in females), RBC (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 RBC ChE activities in females. There was no inhibition of ChE activity in controls fed the diet containing 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 ESO-fed controls (3/20) was dismissed as being unlikely to be related to treatment, on the basis of an absence of a dose-response 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:

Table 2.49: Summary of Neoplasia Incidence

Number of rats with observation		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=epoxidised 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 (i.e. not dose related) and unrelated to treatment. In males at 250 ppm, focal islet cell hyperplasia in the pancreas was 7/20 relative to 1/20 in vehicle controls, and stomach ulcers were 2/20 relative to 0/20 in controls. In females, the ESO control group at the one year interim euthanasia 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 euthanasia, 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 two years later by a 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 autopsy, 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 euthanasia, 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 the collected 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, RBCs 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.

2.3.6.3 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 ai (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 the admixture was allowed to air dry to remove the acetone. This admixture was found to be suitable with respect to homogeneity, stability and concentration (i.e. within 15% of the target, except for one batch of the 0.1 ppm feed at week two that exceeded the target by 21%) when stored at room temperature between batches. Dose selection was based on the results of a thirteen-week feeding study by Barnes et al., (1988) so that for chronic dosing the NOEL was anticipated to be 0.1 ppm whereas the second lowest concentration of 0.5 ppm was anticipated to be the NOAEL, with only serum ChE activity being affected. It was anticipated that ChE activities in brain, RBCs and plasma would be affected at the highest concentration of 300 ppm (i.e. 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 sixteen weeks and monthly thereafter. Physical and auditory examinations were performed four

weeks before testing, and then during weeks 12, 26, 39 and 51. All dogs were euthanased and autopsied during week 52 of treatment.

Clinical laboratory determinations (haematology, biochemistry (including plasma and RBC ChE activities) and urinalysis were performed four weeks before treatment and then at weeks 13, 26, (27 & 38, urinalysis only), 39 and 52. At euthanasia bodyweight was measured and the main organs (i.e. adrenal glands, brain (including brainstem), epididymides, heart, kidneys, liver, lung, ovaries, prostate, spleen, testes, thymus, and uterus) were 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), epididymides, 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, non-glandular), thymic region tissue, thyroid with parathyroid glands, tongue, trachea, and urinary bladder.

One female at 0.5 ppm died on day twelve, whereas a male *in extremis* after one day at 300 ppm was euthanased on day two. Clinical signs preceding death were recumbency, hypoactivity, pallor, diarrhoea, dysentery, vocalization, hypo- and hyper-thermia, laboured and irregular breathing and hypersalivation in the male, and dysentery in the female prior to death. Autopsy 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 these deaths were considered to be unrelated to treatment. The two dead dogs were replaced on days fifteen and four respectively, but the treatment of the replacement dogs was not extended, so that data collected and reported for these groups refers to study days and not actual days of dosing.

A marked reduction in bodyweight gain among dogs at 300 ppm, (i.e. 10% and 24% that for controls in males and females respectively) indicated a dose in excess of the MTD and therefore necessitated a reduction to 225 ppm after fourteen weeks (day 99) of treatment. There was a significant reduction ($p<0.05$ or 0.01) in bodyweight gain observed amongst males at 150 ppm from day fourteen to day seventy. 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 (i.e. time patterns or dose-response relationships) evident in haematology (Hct, Hb, RBC count, WBC, differential leucocyte count, Heinz body, platelet count, reticulocyte count, clotting time, PT and RBC morphology) or urinalysis parameters (volume, colour/clarity, pH, specific gravity, protein, glucose, ketones, urobilinogen, bilirubin, occult blood and on microscopic examination). Similarly there were no treatment-related changes observed in the measured clinical chemistry parameters (i.e. 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 Table 2.50.

Table 2.50: ChE Inhibition after 13, 26, 39 and 52 weeks of treatment (mean percentage reduction)

Concentration (ppm)	Plasma ChE		RBC 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]
Mean	[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
Mean	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*
Mean	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**
Mean	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≤0.05; ** p≤0.01.

Apart from significant plasma ChE inhibition among females at 0.5 ppm, all dogs had significant ChE inhibition in plasma and RBCs 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, i.e. a dose-response relationship, with appreciably elevated activities being observed at the equivalent diazinon concentration; this effect can be seen in Table 2.51. Amylase activity among males was elevated at all concentrations though not in an exact dose relationship, however, significance (p≤0.05) was only occasionally achieved among both males and females. A possible explanation to account for this lack of significance despite the two to three 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.

Table 2.51: 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≤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) two 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 seen in this study.

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.

2.3.6.4 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 ai/kg bw/day 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, i.e. 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 ΔpH/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 (interval not stated). 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 parameters (blood glucose, BUN, AP, ALT, AST, and ChE activities in RBCs and plasma) were determined at week 66, 78, 91 and 104. Cholinesterase activity in the brain was measured at euthanasia. Qualitative urinalysis (parameters were not detailed) was performed at week 65, 78, 93 and 104.

Although four 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. Autopsy did not establish a cause of death in three out of the four animals, although it was concluded, based on significant bodyweight loss (emaciation) prior to euthanasia, together with a more rapid sedimentation rate and an elevated WBC, that intercurrent infections were the most likely cause.

Clinical signs that appeared treatment-related, predominantly observed at 0.5 and 5 mg/kg bw/day, were tremors, soft faeces and a degree of hypersensitivity to touch and sound, which were transiently observed throughout treatment. The number and gender of monkeys having these signs was not reported except for hyperesthesia that was observed in one monkey each 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 RBCs. These ChE reductions measured at week 66, 78, 91 and 104 are shown as a median percentage in Table 2.52. No data for brain ChE activity was supplied but the summary indicated that a significant reduction was observed in one monkey at the highest dose and in two monkeys that died during the study following exposure at 0.05 and 0.5 mg/kg bw/day respectively.

Table 2.52: ChE Inhibition after 66, 78, 91 and 104 weeks of treatment (median percentage reduction for each group)

Dose mg/kg bw/day)	Plasma ChE	RBC 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 Table 2.52, plasma ChE activity was significantly depressed at 0.5 mg/kg bw/day, whereas ChE activity in RBCs only became significantly inhibited at the dose of 5 mg/kg bw/day. Gross autopsy, absolute and body-relative organ weights (i.e. liver, heart, thyroids, brain, kidneys, lung pituitary and adrenals), and histopathology (i.e. 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 was so limited that no independent assessment of treatment-related changes other that 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 in monkeys can be established at 0.05 mg/kg bw/day due to the significant reduction of ChE activity in plasma at 0.5 mg/kg bw/day.

2.3.7. REPRODUCTIVE TOXICITY

2.3.7.1 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 F_2 hybrid mice (National Center for Toxicological Research, Jefferson, Arkansas; age not stated) were mated 1:1 with F_2 males. Mated females were given diazinon (research lot MG8-FL741305; Ciba-Geigy) at doses of zero (control), 0.18 or 9 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 six hours 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 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 ten-minute period, and a qualitative score was 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 freestanding weight, then trained to run in a 130-cm long alley for food reinforcement. All animals were euthanased at 101 days of age. Brain sections were examined microscopically.

Maternal and offspring data: As shown in the summary Table 2.53 the group mean bodyweight gain during gestation was about 14% lower in groups given 0.18 and 9 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 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.

Table 2.53: Summary of maternal and offspring data (mean ± SD)

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

*$p<0.05$ (t-test)

Developmental landmarks: As shown in Table 2.54 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 treatment-related.

Table 2.54: Summary of developmental landmarks (postnatal day of appearance; mean ± SD)

	Dose (mg/kg bw/day)		
	0	**0.18**	**9.0**
Simple righting	3.2 ± 0.4	3 ± 0.1	2.7 ± 0.4
Hair coat	4.9 ± 0.2	4.9 ± 0.1	4.2 ± 0.1
Contact placing	11 ± 0.4	13.3 ± 0.4*	9.1 ± 0.3
Acceleration righting	10.9 ± 0.3	11.2 ± 0.9	11.1 ± 0.4
Eye opening	13.6 ± 0.4	13.3 ± 0.1	14.3 ± 0.3
Ear opening	13.9 ± 0.3	13.3 ± 0.2	14.7 ± 0.3
Sexual maturity	28.4 ± 0.4	30 ± 0.4*	26.8 ± 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 mg/kg bw/day (see Table 2.55). 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 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 treatment-related.

Male and female offspring from dams exposed to 9 mg/kg bw/day displayed impaired running performance in a Lashley III maze, but only in the last ten 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 ten trials is not known. Open-field data were unaffected by treatment.

Table 2.55: Summary of endurance and coordination (mean ± SD)

Dose (mg/kg bw/day)	0	0.18	9.0
Swimming activity (sec)	200 ± 23	190 ±19	243 ± 34*
Rod cling endurance (sec)	65 ± 4	78 ± 6*	78 ± 8*
Rotarod endurance (sec)	1006 ± 621	407 ± 161	103 ± 56
Inclined plane (degrees)	120 ± 7	104 ± 8**	109 ± 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 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.

In this study, female mice were exposed to diazinon throughout gestation at doses of 0 0.18 or 9 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 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 F_2 hybrid mice (National Center for Toxicological Research, Jefferson, Arkansas; age not stated) were mated with individual F_2 males. Mated females were given diazinon at doses of 0 (control), 0.18 or 9 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 six hours 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 euthanased 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 fifteen seconds 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 twenty seconds. Animals were decapitated fifteen minutes after the swim, and immediately after euthanasia, liver and adrenal glands were excised and trunk blood was collected for analysis of plasma corticosterone. The *in vivo* reductive capacity of corticosterone by intact livers was estimated, and adrenal corticosterone production in the adrenals and plasma corticosterone levels were estimated.

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 mg/kg bw/day, a significant reduction in adrenal weight was observed in females only. In the absence of any dose-response relationship, the above findings were not considered treatment-related.

This study was not adequate for regulatory purposes.

2.3.7.2 Rat

Giknis 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 of admixtures of diazinon technical in rodent feed at concentrations of 5 ppm and 2000 ppm, confirmed the stability 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 two to

three days, it was not considered that this batch would affect the outcomes of the study. However, the batch was replaced after two to three 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 zero of the pre-mating phase.

Table 2.56: Summary of experimental design

Event	Period
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 euthanased	Days 110, 117, 118, and 119 of study.
F0 (P1) females euthanased	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 eleven 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 euthanasia. Feed consumption and bodyweight data for females were recorded weekly during the pre-mating period and on day zero, seven, fourteen and twenty of gestation. Female bodyweights were also recorded on lactation day zero, four, seven, fourteen, and 21. Females that showed evidence of mating but failed to produce a litter were weighed as scheduled during presumed gestation and at euthanasia. Parental animals that died or were euthanased were autopsied. 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 euthanased and autopsied, 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 40 F2 pups following euthanasia 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.

With regard to the 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 euthanased 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 stools) were not considered 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 Table 2.57.

Table 2.57: 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 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 fourteen only, and was not considered biologically significant. On occasions, the group mean bodyweight gain in high-dose animals was significantly ($p<0.05$) reduced compared with controls. These findings were generally isolated in nature during pre-mating, and were not considered 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 zero to seven (17% reduction, $p<0.05$), seven to fourteen (11%), fourteen to twenty (5%) and zero to twenty (10%). These decreases in bodyweight gain in high-dose females during gestation, though significant only for the day zero to seven interval, were considered treatment-related. Increases in bodyweight gain in high-dose females during lactation days fourteen to 21 may have resulted from a decrease in pup survival at 500 ppm, and the resultant decrease in viable pups present at lactation day zero. However, bodyweight gains were reduced in high-dose females on lactation days zero to four, four to seven, seven to thirteen, and zero to 21, by up to 50% compared with controls.

Pathology: No treatment-related gross observations were noted at the F0 parental autopsy examination, and histopathological examination did not reveal any treatment-related findings.

Reproductive parameters: As shown in Table 2.58, there were decreases in the survival indices for F1 pups (male and female) for days zero to four (pre-sampling) and days four to 21 (post-sampling). All other reproductive parameters were similar in control and treated groups.

Table 2.58: Summary of F1 litter survival (mean %)

	Dietary concentration (ppm)

	0	10	100	500
Pups surviving days 0-4 (pre-sampling)	88.7	97.6	95.4	61.3*
Pups surviving days 4-21 (post-sampling)	99.0	98.7	93.5*	61.7*
Males surviving days 0-4 (pre-sampling)	96.1	98.6	95.1	64.3*
Males surviving days 4-21 (post-sampling)	100.0	99.1	93.5	59.5*
Females surviving days 0-4 (pre-sampling)	89.0	97.0	95.7	60.5*
Females surviving days 4-21 (post-sampling)	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 four 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 fourteen only and females on day seven only.

With regard to the 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 apart for bodyweight gain differences discussed below. There were no compound-related deaths in any group.

Test article consumption: The ranges of diazinon consumption in F1 animals are outlined in Table 2.59.

Table 2.59: 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 to 14% compared with controls) were seen in high-dose males at every pre-mating sample interval. The food consumption was also reduced (5 to 8%; $p<0.05$) at 100 ppm, but at this dose the effect was inconsistent and 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 cumulative treatment, as the high-dose F1 males were about 25% smaller than controls at the beginning of the pre-mating period (i.e. at birth).

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 zero (birth) to 12% at day 63, and the bodyweights of high-dose males were about 14% lower than controls at euthanasia. In

high-dose females, group mean bodyweights were about 16% lower than controls at day zero pre-mating ($p<0.05$), and this difference was only 6% by the end of the pre-mating phase (i.e. eleven weeks after birth). Decreases in bodyweights ranged from 6 to 10% in high-dose females during gestation and lactation. Therefore, while bodyweights in F1 males and females were reduced by about 28% at the end of the 21-day postnatal period (lactation), the reductions in bodyweights were not so great by the end of the F1 (P2) pre-mating phase (11 weeks after birth).

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 to 6% compared with controls during this period, which was consistent with the 5% lower bodyweights in this group at day zero pre-mating (i.e. after 10 weeks of age).

Pre-mating, the bodyweight gain in F1 high-dose males was occasionally reduced compared with controls ($p<0.05$), and for the 0 to 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 zero to 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 zero to seven, fourteen to twenty, and zero to twenty. The reduced bodyweight gains over the whole lactation period were 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 Table 2.60, there was an observable decrease in the number of viable F2 newborn, and observable decreases in the fertility and mating indices in F1 (P2) males and females at 500 ppm, although 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 zero to four (pre-cull) and day four to 21 (post-cull) intervals.

Table 2.60: 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*

*$p<0.05$, [§] Number of pregnant females/Number of females with positive evidence of mating, [‡] Number of females with positive evidence of mating/Number of females co-housed, [†] Number of fertile males/Number of males with positive evidence of mating, [¶] Number of males with positive evidence of mating/Number of males co-housed

Table 2.61: Summary of F2 litter survival (mean %)

	Dietary concentration (ppm)			
	0	**10**	**100**	**500**
Pups surviving days 0-4 (pre-sampling)	96.2	98.4	97.9	44.0*
Pups surviving days 4-21 (post-sampling)	100.0	96.5	98.2	76.7*

Males surviving days 0-4 (pre-sampling)	98.2	99.5	98.8	44.6*
Males surviving days 4-21 (post-sampling)	100.0	95.3	97.6	78.0*
Females surviving days 0-4 (pre-sampling)	95.9	97.4	97.1	42.9*
Females surviving days 4-21 (post-sampling)	100.0	98.7	98.8	72.2*

*Indicates statistically significant result ($p<0.05$).

F2 pup observations: There were no treatment-related clinical signs or malformations in F2 pups, apart from differences in bodyweight gains. At 500 ppm, no treatment-related findings were observed at autopsy 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.

In summary in this two-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 euthanased 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).

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 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 three 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 145-196g for males and 109-143g for females. The parental animals for the second generation (F1) were selected from the weanling

offspring (F1a) of the preceding filial generation. After mating (one male and two females in each mating box), animals were housed individually.

Table 2.62: 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 euthanased (without autopsy)	At the end of the mating period.
F0 females deliver naturally	
All F0 females euthanased (without autopsy)	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 autopsy. Remaining weanlings euthanased (without autopsy).	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 autopsy.	Day 21 of lactation
F1 females (25/group) selected for gross autopsy. Remaining females euthanased (without autopsy).	Day 21 of lactation
F2a weanlings	
5 pups/sex/group selected for gross autopsy. Remaining weanlings euthanased (without autopsy).	Day 21 of lactation

Dietary analysis: Diets were prepared weekly and analysed for stability (pre-test and week eight), homogeneity (pre-test) and concentration of test material (pre-test, weekly for four weeks and then four-weekly for the remainder of the study). The diets were homogeneous at the pre-test sample interval. At the highest concentration, the diazinon concentration for males and females was generally lower than the target level (i.e. 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 pre-test and four-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 zero and twenty of gestation, and days one, four, seven, fourteen, 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 Table 2.62. All F0 males were euthanased 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 one, four, seven, fourteen, and 21 post-parturition. On day seven, all litters were reduced to ten pups. Selection of pups was non-random, with equal numbers of males and females selected when possible. After the lactation

period, fifteen males and thirty females per group were selected at random to grow to maturity for the next generation of the study. Animals not selected were euthanased without autopsy.

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 autopsy. Those pups not selected for gross autopsy or to continue onto the next generation were euthanased without autopsy. Ten F1 males and twenty-five F1 females per group were selected at random for gross autopsy following day 21 of lactation. Those F1 animals not selected for autopsy, and all F0 animals, were euthanased without autopsy.

The following tissues (and any gross lesions) from each autopsied 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.

Mortality and clinical signs: No parental animals died or were euthanased in a moribund condition 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 treatment-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 observed to be 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 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 day one to day four. The relationship between these findings and treatment was not clear.

Table 2.63: 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

Table 2.64: 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 treatment-related.

Pathology: Gross pathological examination did not reveal any effects that were considered 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 treatment-related.

In this study 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 mg/kg bw/day. No parental animals died or were euthanased 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 day one to day four. However, the relationship between these findings

and treatment was not clear. Gross and histopathological examinations did not reveal any effects that were considered treatment-related.

As no treatment-related adverse effects were observed at any dose in parental animals or offspring in either generation, a NOEL could not be established and the adequacy of this study for regulatory purposes was limited.

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, twenty males were mated with twenty females (ten 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, although females were not weighed during gestation and lactation. The experimental protocol is summarised below.

Table 2.65: 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 euthanased and autopsied.	
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 i.e. F1 (P2). Remainder of pups euthanased and autopsied. All F0 parental animals euthanased (without autopsy)	
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 autopsy conducted as for first generation.	

Event	Period
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 euthanasia of F3a litters followed the procedures used in previous generations.	
F3b weanlings	
At weaning, all F3b pups were euthanased 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 euthanased after weaning the F3b pups. Females that failed to bear two litters were examined post mortem for uterine implantation sites.	

F0 generation: Two control animals died during the study, and one control animal was euthanased 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.

Table 2.66: 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 eight 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.

Table 2.67: 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 five females that received 8 ppm diazinon died during the study. The cause of death was not determined in any case, and autopsies 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 treatment-related.

Table 2.68: 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). 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 three generations, and as no toxicity was demonstrated at the high dose, the adequacy of this study for regulatory purposes is limited.

In this study the reproductive toxicity potential of a 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 ppm 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.

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 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 fifteen. One group served as controls and received distilled water (0.5 mg/kg bw/day), and groups two and three 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 euthanased 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, five rats from each group were isolated. The remaining males in each group were mated individually with females (8/group) for 48 hours 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.

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 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.

Table 2.69: Summary of sex organ weight (g) (mean ± SD)

Organ		**Dose (mg/kg bw/day)**		
		0	**1.5**	**3.0**
Testicles	65-day treatment	1.72 ± 0.05	1.38 ± 0.05**	1.16 ± 0.032***
	21-day recovery	1.68 ± 0.025	1.47 ± 0.04**	1.20 ± 0.033***
Seminal vesicles	65-day treatment	0.23 ± 0.014	0.16 ± 0.008**	0.12 ± 0.014***
	21-day recovery	0.24 ± 0.015	0.17 ± 0.006**	0.13 ± 0.013***
Prostate gland	65-day treatment	0.19 ± 0.014	0.12 ± 0.008**	0.09 ± 0.002***
	21-day recovery	0.19 ± 0.016	0.14 ± 0.009*	0.12 ± 0.003**

(n = 5) Statistically significant results marked as *($p<0.05$), **($p<0.01$), or ***($p<0.001$).

Table 2.70: Semen quality (mean ± SD)

	Dose (mg/kg bw/day)		
	0	**1.5**	**3.0**
Sperm cell concentration after 65-day treatment (units not given)	1.90 ± 0.18	1.30 ± 0.064*	0.92 ± 0.08**
Sperm cell concentration after 21-day recovery	1.91 ± 0.19	1.44 ± 0.068*	1.22 ± 0.09**
Live cell (%)-after 65-day treatment	97.89 ± 1.84	83.74 ± 1.42***	80.22 ± 1.04***
Live cell (%)-after 21-day	98.22 ± 1.96	86.42 ± 1.84**	86.47 ± 1.79**

recovery			
Motility (%)-after 65-day treatment	95.82 ± 0.72	17.24 ± 0.85***	7.41 ± 0.64***
Motility (%)-after 21-day recovery	96.46 ± 0.85	27.96 ± 2.14***	17.22 ± 1.98***
Total sperm abnormalities (%) after 65-day treatment	7.68 ± 0.32	22.86 ± 1.17***	42.28 ± 1.68***
Total sperm abnormalities (%) after 21-day recovery	8.24 ± 0.42	18.37 ± 0.62***	30.02 ± 0.79***

(n = 5) Statistically significant results marked as *(p<0.05), **(p<0.01), or ***(p<0.001).

Table 2. 71: Serum testosterone concentration (ng/mL, mean ± SD)

	Dose (mg/kg bw/day)		
Time of sampling (day)	**0**	**1.5**	**3.0**
0	3.73 ± 0.09	3.48 ± 0.082	3.56 ± 0.06
14	3.66 ± 0.07	3.20 ± 0.09**	3.08 ± 0.06***
28	3.58 ± 0.07	3.16 ± 0.08**	2.76 ± 0.07***
42	3.67 ± 0.05	2.48 ± 0.10***	2.22 ± 0.09***
65	3.68 ± 0.04	2.40 ± 0.09***	1.83 ± 0.09***
After 21-day recovery	3.56 ± 0.06	2.34 ± 0.07***	1.98 ± 0.08***

(n = 5) Statistically significant results marked as **(p<0.01), or ***(p<0.001).

Table 2.72: 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

In this study when male rats were given diazinon by oral gavage at doses of 1.5 and 3 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.

2.3.8. DEVELOPMENTAL TOXICITY

2.3.8.1 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 six through fifteen 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 CMC (concentration not reported). Control animals received CMC only. Autopsies

were performed on the dams at gestation day 21, and foetuses were removed by caesarean section at this time. General condition, weight gain and signs of treatment were recorded daily. Food consumption was determined on day six, eleven, sixteen and 21. The maternal organs (ovaries and uterus, including mucosa and contents) were examined and the foetuses were inspected and weighed. The situs of the body cavities (thorax, abdomen, pelvis) was assessed. One third of the foetuses were fixed (alcohol, formalin and acetic acid) and examined. The remaining two thirds of foetuses were placed in 70% alcohol, cleared in one percent potassium hydroxide, and stained with Alizarin Red S for skeletal examination.

Bodyweights were reduced in high-dose dams throughout the treatment period, with animals losing about 5 to 10% of their bodyweight between day six and eight of gestation. The rate of bodyweight gain in high-dose animals was similar to other groups from day nine to fifteen 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 day six and eleven of gestation, but was similar in all groups between days sixteen 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.

Table 2.73: Summary of Effects on Reproductive Indices and Foetal 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 foetal resorptions	0	0	0	1
Number of dead foetuses	1	0	0	0
Number of live foetuses with malformations	0	0	1	0
Mean weight of live foetuses	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.

In summary in this study 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 day six to fifteen 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. No NOEL for developmental toxicity could be established for this study because no treatment-related effects were observed on reproductive or developmental parameters at any dose.

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 five rats by oral gavage on pregnancy days seven to seventeen, at doses of 0 (control), 1.25 (four animals only), 2.5, 5.0, or 12.5 mg/kg bw/day. Ethylenethiourea (ETU) was similarly given to groups of five 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 bw (as determined on day seven), and for ETU was 4 mL/kg bodyweight. Observations for maternal health status and bodyweight measurements were conducted every day. Maternal animals were euthanased on day 21 of gestation, and foetuses were examined for abnormalities. Cholinesterase activity in the brain homogenate of maternal animals was determined by the ΔpH/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, hypersalivation, frequent urination, reduced 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 foetal development. Mean foetal bodyweights were significantly decreased at 5 mg/kg bw/day, but as there was also an increase in the number of foetuses at this dose level, the decrease in foetal 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 to 60% compared with controls at the other doses. No foetal 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 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; ten weeks old at beginning of study; Institute for Animal Reproduction, Japan) at doses of 0 (controls), 0.53, 1.45, or 4 mg/kg bw/day on day seven to seventeen of gestation. Similarly, ETU (positive control; 50 mg/kg bw/day) was given to a group of twenty 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, twenty animals from each group were selected at random for euthanasia at day 21 of gestation, at which time the foetuses were obtained for examination. The remaining ten animals in each group were maintained through, until day three *post-partum*. At that time, the litters were adjusted to eight pups each (four males and four females), and these remaining pups were not weaned until day 21 *post-partum*. After weaning, two pups (one male/one female) from each of these litters were then used for further examinations (†see below), namely autopsy at weaning, autopsy at ten 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 foetuses were used for teratological examinations only.

The health of all animals was checked daily. Maternal animals were weighed on days zero, seven, fourteen, seventeen, and 21 of gestation, and days zero, three, seven, fourteen, 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 foetuses, number of dead foetuses (residual implants, placental residue, resorbed or dead foetuses), placental weight, weight of live foetuses, sex, gross malformations, and crown-rump length.

Half of the live foetuses per litter were used for skeletal examination, and abnormalities, variations and ossification status were recorded. The remaining half of the foetuses was used for visceral examination. Histological examinations were conducted on livers, kidneys and hearts of five 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 any gross malformations. Prior to weaning, pup bodyweights were recorded on days three, seven, fourteen, and 21 *post-partum*, and the number of deaths occurring between days zero and three *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 three *post-partum*).

A number of other parameters were also measured in this study[†]. These included morphological differentiation (auricular standing on day three *post-partum*, incisor extrusion on day ten, hair growth around nasal area on day ten, eyelid opening on day sixteen), 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.

No maternal deaths or signs of intoxication were reported at any diazinon dose. Animals exposed to ETU displayed alopecia by day seventeen, 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 seventeen of gestation, but as the decrease only represented a two to three decrease compared with controls, this effect was not considered 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 day seven to fourteen 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 day seven to fourteen and day fourteen to seventeen 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 foetal abnormalities were confined to a single stunted control foetus, and a single tail-less foetus 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 foetal weight and crown-rump lengths (males and females), and 249/252 foetuses with

gross abnormalities. At day three *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 Table 2.74, skeletal examination of foetuses (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 foetuses 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 foetuses with poorly ossified sternebrae, and in the incidence of foetuses with five ossified metatarsal centres at 4 mg/kg bw/day.

Table 2.74: Summary of Foetal Skeletal Abnormalities

	Dose (mg/kg bw/day)			
	0	**0.53**	**1.45**	**4.0**
Number of litters examined	20	20	20	20
Number of foetuses examined	140	131	135	137
Variants				
Total number of foetuses with abnormalities (litters)	1 (1)	2 (2)	2 (2)	0
Total number of foetuses with variations (litters)	0	6 (5)	3 (3)	2 (2)
Sternum: Foetuses with poorly ossified sternebrae (litters)	3 (2)	4 (2)	7 (4)	21* (9)
Vertebra: Foetuses with unossified odontoid process (litters)	63 (18)	37* (13)	40* (17)	37* (15)
Hind limb: Foetuses with five ossified metatarsal centres	131 (93.6%)	125 (95.4%)	129 (95.6%)	136* (99.3%)

Statistically significant result indicated by * ($p<0.05$)

Visceral examination: As shown in Table 2.75, 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 treatment-related. The incidence of hydroureter was significantly higher in controls than in the treated groups, again with no dose response relationship, therefore this effect was not considered toxicologically significant.

Table 2.75: Summary of Foetal Visceral Abnormalities

	Dose (mg/kg bw/day)			
	0	**0.53**	**1.45**	**4.0**
Number of litters examined	20	20	20	20
Number of foetuses examined	130	124	126	125
Variants				
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%)

Statistically significant result indicated by* ($p<0.05$).

Morphological differentiation: There were no consistent dose-related effects that were considered toxicologically significant in this portion of the study. Significant ($p<0.001$) decreases in the number of animals displaying incisor eruption at ten days were seen in all treated groups, and there

was an increase in the incidence of auricular standing at three 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 three and ten weeks *post-partum* did not reveal any consistent findings that 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 treatment related.

In this study when diazinon was given by gavage to groups of female Wistar-Imamichi rats at doses of 0 (controls), 0.53, 1.45, or 4 mg/kg bw/day on day seven to seventeen of gestation, the NOEL was 1.45 mg/kg bw/day, based on a significant but transitory reduction in maternal food consumption and a significantly increased incidence of delayed ossification of sternebrae in foetuses at 4 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 Sprague-Dawley rats; 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 day six through fifteen. Control animals received 10 mL/kg bw/day of 0.20% 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 six, ten, and fourteen.

Dams were observed daily for changes in appearance and behaviour, and were weighed on days zero, six, ten, fourteen, eighteen, and twenty of gestation. Food consumption calculations were made for gestational day zero through five; daily for days six through fifteen; days sixteen through seventeen; and days eighteen through nineteen. Dams were euthanased on day twenty of gestation and the ovaries were examined and *corpora lutea* counted. The uteri (including contents) were weighed and live foetuses, dead foetuses, and intrauterine resorption sites were counted. Viable foetuses and their placenta were weighed and the foetuses were examined for gross abnormalities. Approximately one third of the foetuses 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 foetuses from each litter were cleared, stained with Alizarin Red S and examined for skeletal abnormalities. All dams were euthanased and examined for gross pathology, and all gross lesions were excised and submitted for microscopic evaluation. Cholinesterase activity was not measured.

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 autopsy 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 day six to nine 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 ten, 7% at day fourteen, 5% at day eighteen (not statistically significant) and 5% at day twenty. Mean body weights of dams without the gravid uterus on day twenty 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 zero to day twenty 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 six to ten day interval at the high dose, with animals losing weight over this period.

Table 2.76: Summary of Mean Maternal Bodyweight (g)

Gestation (day)	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 foetuses)	306	313	303	294

Statistically significant results marked with *($p<0.01$), or **($p<0.05$).

Table 2.77: 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 foetuses	58	67	60	44*

Statistically significant results marked with *($p<0.01$), or **($p<0.05$).

Reproductive parameters: As shown in Table 2.78, there were no statistically-significant, treatment-related effects on reproductive parameters 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. At 100 mg/kg bw/day, there were slight increases in the pre-and post-implantation losses and in the mean number of resorptions, and a consequent small decrease in the mean number of live foetuses; however these findings were not significantly different to controls. Foetal sex ratios in treated groups were not significantly different to controls. Slight increases in the mean foetal 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 foetuses 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 twelve and nine, respectively,

while the range for the remainder of the high dose group was zero to two. 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.

Table 2.78: Summary of Reproductive Parameters

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 foetuses	14.2	13.4	14.2	11.9
Foetal weight-male (g)	3.4	3.5	3.5	3.6*
Foetal weight-female (g)	3.2	3.4	3.3	3.4*

Statistically significant results marked as *($p<0.05$).

Malformations: A single incidence of exencephaly was observed in a foetus at 20 mg/kg bw/day, and single instances of umbilical hernia, filament tail and sublingual extraneous soft tissue were seen in separate foetuses at 100 mg/kg bw/day. Because of the isolated nature of these findings they were not considered 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 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 result was considered to be incidental to treatment.

Table 2.79: Summary of Foetal Malformations (by foetus)

Variant	Dose (mg/kg bw/day)			
External	**0**	**10**	**20**	**100**
Foetuses 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
Visceral				
Foetuses examined	105	82	108	81
Litters examined	24	20	24	21
Situs inversus	**0**	**0**	1	**0**
Skeletal				
Foetuses 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

Statistically significant result marked as *($p<0.05$), refer to text for further information.

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 treatment-related. A significant increase ($p<0.005$) in the incidence of mottled liver (by foetus 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 artefact and not related to treatment.

Significant ($p<0.007$) increases in the incidence of rudimentary T-14 ribs (by foetus 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 foetotoxicity, 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 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.

Table 2.80: Summary of Foetal Visceral and Skeletal Abnormalities

Variant	**Dose (mg/kg bw/day)**			
Visceral	**0**	**10**	**20**	**100**
Number of foetuses examined	105	84	108	81
Number of foetuses with variations	42	32	28	25
Mottled liver	**0**	**0**	**0**	6*
Skeletal				
Number of foetuses examined	235	198	247	181
Number of foetuses with variations	228	195	246	178
Number of foetuses with variations excluding metacarpals and forepaws	183	158	190	139
Rudimentary T-14 ribs	**0**	6	6	9**
Wavy/angulated ribs	1	**0**	2	3

Statistically significant results marked as *($p<0.005$), or **($p<0.007$), refer to text for further information

In this study groups of pregnant Charles River Sprague-Dawley rats were given technical diazinon by oral gavage at doses of 0, 10, 20, or 100 mg/kg bw/day from days six through fifteen of gestation. No unscheduled deaths were reported at any dose. At 100 mg/kg bw/day, decreases in mean food consumption, mean bodyweights (5 to 10%; days 10, 14and 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 foetuses, were not significantly different to controls. An increase in the incidence of rudimentary T-14 ribs at the high dose was considered 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 foetotoxicity (increased incidence of rudimentary ribs) at 100 mg/kg bw/day. No teratogenicity was observed at the highest dose tested and a NOEL for teratogenicity could not be established.

2.3.8.2 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 foetuses were examined for gross defects when delivered by caesarean section. All foetuses with sufficient developmental form for determination of structural defects were counted in the number of foetuses/litter, even though some of these foetuses were dead when delivered. Resorption sites and foetuses that died in late embryonic life were counted as dead foetuses.

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 six through eight; eight dams) or 0.25 mg/kg bw/day (on gestation day seven or eight; five dams). Controls (eight dams) were similarly treated with the vehicle alone. Dams were euthanased on gestation day fourteen. Four foetuses were chosen at random from each litter for staining and skeletal examination.

Cholinergic signs (diarrhoea, hypersalivation, incoordination) were reported in all dams, but no hamsters died after treatment with diazinon. No major malformations or increase in foetal mortality were seen at either dose. No test for foetal viability was made, as the dams were euthanased on day fourteen of gestation.

Table 2.81: 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
Number of dams that died/Number treated	0/8	0/8	0/5
Mean number of foetuses/litter	12.9	14.3	14.4
Foetal mortality (%)	5.7	5.8	2.7
Mean foetal weight (g)	1.7	1.7	1.5

NS = not stated

In this study diazinon was given to Golden Syrian hamsters (0.125 or 0.25 mg/kg bw/day) during gestation. Cholinergic toxicity signs were observed, but no signs of developmental toxicity or foetal 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.

2.3.8.3 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 foetuses were examined for gross defects when delivered by caesarean section. The viability of rabbit foetuses was determined by placing them in a chicken-hatching incubator for six hours. All foetuses with sufficient developmental form for determination of structural defects were counted in the number of foetuses/litter, even though some of these foetuses were dead when delivered. Resorption sites and foetuses that died in late embryonic life were counted as dead foetuses.

Rabbits were given diazinon by gelatin capsule orally with the aid of a balling gun on gestation days five through fifteen, at doses of 30 mg/kg bw/day (eight does) or 7 mg/kg bw/day (nine does).

Control animals (21 does) received empty gelatin capsules. The does were euthanased on gestation day 28. The internal organs of rabbit foetuses were examined and fixed, and the skeletons stained with Alizarin red and examined for bone abnormalities.

No treatment-related malformations and no foetotoxicity 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).

Table 2.82: Summary of Effects on Reproductive Indices

Reproductive Indices	Dose (mg/kg bw/day)		
	0 (CMC control)	7	30
Number of dams that died/Number treated	0/21	0/9	6/8
Mean number of foetuses/litter	7.5	9.3	9.0
Foetal mortality (%)	16.5	2.3	0
Mean foetal weight (g)	36.2	36.3	29.6

In this study 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 foetal malformations were reported at any tested dose. 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 epoxidised soybean oil was given to pregnant New Zealand White rabbits (Hills of Home Ranch, California, USA; aged four to five months, starting weight 3kg –to 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 six through eighteen. The test material was formulated in a 0.2% aqueous solution of 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 zero, six through eighteen, 25, and prior to caesarean section on day thirty. The animals were observed twice daily for clinical signs of intoxication. Any animals that died during the study were autopsied shortly after discovery. On day thirty, does were autopsied 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 foetuses, the number and type of resorption sites, individual foetal bodyweights, and foetal external anomalies.

Visceral examination of the foetuses 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 foetus, 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 foetuses were processed with Alizarin Red S for skeletal examination.

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.

Table 2.83: 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)

Foetal 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 foetuses per litter, foetal weight, and sex ratio.

Table 2.84: 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 foetuses examined	135	146	118	81
Number of live foetuses (%)	135 (95.1%)	146 (95.4%)	118 (95.9%)	81 (95.3%)
Mean number of live foetuses/litter	7.5	8.6	7.9	6.8
Mean live foetal weight	49.5	45.6	48.6	47.3
Number of resorbed foetuses	6	6	3	4
Number of dead foetuses	1	1	2	0
Sex ratio (males:females)	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 foetal 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 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 sternebrae 5, and incomplete ossification of sternebrae 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.

Table 2.85: Summary of Foetal Skeletal Abnormalities

Skeletal Abnormalities	Dose (mg/kg bw/day)			
	0	**7**	**25**	**100**
Number of foetuses examined	135	146	118	81
Malformations				
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
Variations				
Sternebrae 5 absent	0	0	6 (5.1%)	11 (13.6%)
Sternebrae 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 arteriosis* and *ductus arteriosis* arising from the left pulmonary artery, scoliosis, and an interventricular septal defect. These findings were not related to dose, and were not considered treatment-related.

Table 2.86: Summary of Foetal Visceral Abnormalities

Visceral Abnormalities	Dose (mg/kg bw/day)			
	0	**7**	**25**	**100**
Number of foetuses examined	135	146	118	81
Malformations				
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

In this study diazinon was given by oral gavage to groups of pregnant New Zealand White rabbits on gestation days six through eighteen 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 foetal development at 100 mg/kg bw/day. It was not possible to determine a NOEL for teratogenicity in this study due to the absence of any significant adverse effect at the highest dose tested.

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 (thirteen to sixteen weeks old; Cheshire Rabbit Farms and/or Ranch Rabbits and/or Buxted Rabbit Company Ltd., UK) on days six to eighteen 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 thirteen consecutive days, with treatment beginning on day one of the study. Dose volumes were adjusted for individual animals before doses three, five, eight, and eleven. Animals were euthanased eight days after the last dose, then dissected and examined for macroscopic pathological changes. Body weights were measured prior to treatment, and on days one, four, six, eight, fourteen, and 21. 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 two hours 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 six to eighteen of gestation. Animals were dosed within two hours of test material formulation, and dosage volumes were adjusted according to body weight on days eight, ten, and fourteen. 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 six of pregnancy. In the supplementary phase of mating, an extra five to twelve mated does were allocated to each of the dose groups. At 0, 2.5, 10, and 40 mg/kg bw/day, the groups numbered seventeen, seventeen, twenty, and eighteen does, respectively, in the main mating phase, and eight, five, five, and twelve 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 two hours after preparation. The chemical stability of diazinon suspended in 5% gum arabic at ambient temperature was confirmed over a 4 hour period.

All animals were handled regularly and observed daily for signs associated with treatment. All animals that died or were euthanased were weighed and subjected to post-mortem examination. All rabbits were weighed on days one, six, eight, ten, nineteen, 23, and 29 of gestation, and food consumption was calculated at these same intervals. Animals were euthanased 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/foetal deaths, the individual foetal weights, and foetal abnormalities.

Embryonic/foetal deaths were classified as:

- early: only placenta visible at euthanasia,
- late: both placenta and embryonic remnants visible at euthanasia,
- abortion: only implantation site scars visible at euthanasia.

Live young were examined externally then euthanased, weighed, dissected to examine for visceral and skeletal abnormalities, and sexed.

Pilot study: At 100 mg/kg bw/day, the animal treated showed anorexia, hypersalivation, body tremors and abnormal posture. The animal was euthanased on day three 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 hypersalivation 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 hypersalivation, piloerection, abnormal movement and posture, and was subsequently euthanased.

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, decreased bodyweight gain was evident until day four only. There was an apparent decrease in group mean food consumption at 50 mg/kg bw/day until about day eleven 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, RBC ChE activity was inhibited at all doses, by about 70 to 80% compared with controls. No treatment-related macroscopic changes were noted at autopsy.

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, hypersalivation, and piloerection. The number of animals that died or were euthanased 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 fourteen to eighteen. 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 nineteen 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 eight 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 Table 2.86, 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 foetal 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.

Table 2.87: 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 foetal weight (g)	44.7	42.4	39.7**	40.9*

Statistically significant results marked as *($p<0.05$), or **($p<0.01$).

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.

Table 2.88: Summary of Foetal Malformations and Anomalies

Malformations	Dose (mg/kg bw/day)			
	0	**2.5**	**10**	**40**
Foetuses affected	2/133 (1.5%)	5/133 (3.8%)	3/150 (2%)	1/130 (0.8%)
Visceral anomalies-foetuses affected	11/131 (8.3%)	6/128 (4.7%)	6/147 (4.1%)	16/129 (12.4%)
Skeletal anomalies-foetuses affected	37/131 (28.2%)	13/128** (10.1%)	21/147 (14.3%)	10/129** (7.8%)

Statistically significant results marked as ** ($p<0.01$).

In this study 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 six through eighteen 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, hypersalivation, 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 foetal 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 clinical signs, reduced bodyweight gains and reduced food consumption in maternal animals at 40 mg/kg bw/day.

2.3.8.4 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 autopsied. Pups were autopsied at days one, seven, fourteen, 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.

At 1 mg/kg bw/day, 6/8 females produced pups. In one litter, all seven pups died by day thirteen *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. There effects are summarised under the term terata in the table below. No unusual autopsy 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.

Table 2.89: Summary of Effects on Reproductive Indices

	Dose (mg/kg bw/day)					
Reproductive Indices	**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
Average 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

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.

2.3.8.5 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 one 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.

Swine: At 5 mg/kg bw/day all seven sows farrowed. A single piglet in each of three different litters displayed abnormalities. One piglet 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.

Table 2.90: 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
% Piglets with terata	0	6.3	0
% Piglets stillborn	1.9	0	0

In this study, pregnant swine given diazinon at up to 10 mg/kg bw/day showed 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 sows (R Beedles, UK; 2-4 years old; 160-285 kg bw on the day prior to dosing). Groups of

four 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, eight days before treatment, and at days one and fourteen following treatment. Animals were also weighed within one day of parturition and at euthanasia (ten days post-farrowing). Piglets were weighed within one day of parturition and at euthanasia. Food consumption was recorded twice daily from the time of allocation to euthanasia, and all sows 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, one sample taken on days two and seven. Blood was also sampled within 48 hours of farrowing. Haematology parameters determined were packed cell volume, Hb, RBC count, mean corpuscular haemoglobin concentration (MCHC, MCV, reticulocyte count, WBC, differential count, platelet count, PT, and activated partial thromboplastin time. Biochemistry parameters determined were glucose, total protein, albumin, globulin, urea, creatinine, AP, ALT, AST, LDH, glutamate dehydrogenase, total bilirubin, electrolytes, cholesterol, plasma ChE, RBC ChE. Venous blood samples were obtained from all piglets ten days after parturition for determination of plasma and RBC 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 hours of birth and ten days *post-partum*, morphological or functional abnormalities in piglets, and clinical observations on litters and piglet mortality.

At the study termination, sows were euthanased 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 euthanased by IV injection of sodium pentobarbitone, but post-mortem examinations were not conducted at euthanasia.

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 RBC ChE activity were observed at 60 and 100 mg/kg bw at day two 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 seven, significant ($p<0.01$), dose related decreases in plasma ChE inhibition were observed at all doses. RBC ChE activity was decreased at all doses, but the inhibition was not dose-related or statistically significant, and inhibition ranged from 13 to 21% compared with controls. At parturition, no significant, dose-related decreases in plasma or RBC 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 RBC ChE activity was not significantly affected by treatment.

Table 2.91: ChE Inhibition in sows (mean percentage reduction in comparison to controls)

Dose (mg/kg bw)	Plasma ChE			RBC 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

Statistically significant results marked as * ($p<0.05$), or ** ($p<0.01$).

Pathology examination did not reveal any effects that were considered treatment related.

In this study treating pregnant sows on gestation day 85 with a single topical application of diazinon (19.9% formulation) at doses of 0, 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 tested doses on day seven and at 60 and 100 mg/kg bw on day two after treatment. This effect was reversible. No adverse effects were observed on other biochemical or haematological parameters, or on reproductive parameters. No NOEL could be set for this study because of the significant plasma ChE inhibition at the lowest tested dose.

2.3.9. GENOTOXICITY

A summary of submitted and published findings of genotoxicity studies with diazinon is shown in the Tables below.

Table 2.92: Summary of Genotoxicity Testing with Diazinon

Assay	Bacterial strain or Cell type	Concentration (or Dose)	Metabolic activation	Results	Reference
Gene Mutation					
S. typhimurium	TA1535 TA1536 TA1537 TA1538	50-1000 µg/plate	+, - +, - +, - +, -	-, - -, - -, - -, -	Marshall et al.,1976
	TA98 TA100 TA1535 TA1537 TA1538 *E. coli* WP2*hcr*$^-$	200-5000 µg/plate	+, - +, - +, - +, - +, - +. -	-, - -, - -, - -, - -, - -, -	Shirasu et al., 1976
	TA98 TA100 TA1535 TA1537 *E. coli* 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) & *S. typhimurium* 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.

Table 2.93: Summary of Genotoxicity Testing with Diazinon (cont.)

Assay	Bacterial strain or Cell type	Concentration	Metabolic activation	Results	Reference
Chromosomal Effect Assays (*in vitro*)					

Assay	Bacterial strain or Cell type	Concentration	Metabolic activation	Results	Reference
Chromosomal Effect Assays (*in vitro*)					
Rec-	*B. subtilis* H17/M45	Up to 20 µL	+, -	-, -	Shirasu et al., 1976
E. coli	Sprague-Dawley-4	50% (v/v)		-	Hurni & Ohder, 1970
	WP67 (*uvr*A⁻, *pol*A⁻) WP871 (*uvr*A⁻, *rec*A⁻, *lex*A⁻)	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.

Table 2.94: Summary of Genotoxicity Testing with Diazinon (cont.)

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

2.3.9.1. Gene mutation assays

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 (dimethyl sulfoxide , 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 given) 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 μg/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 μg/plate), confirmed the result for TA 1535 but cultures of TA 100 were contaminated. At the maximum tested concentration (10000 μg/plate), the mean number of TA 1535 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 WP2*uvrA*, 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 Specific Pathogen Free (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 exposed to diazinon.

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 given) 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 significant, did not achieve the required two-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, i.e. 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 fourteen-fold and four-fold respectively. Thus, this study gave limited evidence of some possible indirect mutagenic activity 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 based on 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 fifteen-fold respectively. Thus, there was no evidence of direct or indirect mutagenic activity in these experiments in mammalian cells *in vitro*.

2.3.9.2. Chromosomal aberration tests

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, *uvr*A$^-$, *pol*A$^-$; CM871, *uvr*A$^-$, *rec*A$^-$, *lex*A$^-$) *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 bactericidal effects, after two or eighteen hours at the maximum concentration tested,

i.e. 10 mg/mL. Comparing survival for WP2 with either 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, showed 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 two 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 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 μg/mL was lethal for Chinese hamster lung cells (V79) in culture after three hours. However, in the presence of S9 metabolic activation the frequency of sister chromatid exchanges observed after 24 hours was significantly ($p<0.05$) increased (27%). The only concentration tested was chosen based on 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 (e.g. 2-acetylamino-fluorene, benzo(a)pyrene), most of which gave the expected increase in SCEs but there were also some which did not (e.g. 3-methylcholanthrene and 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 zero,0 (vehicle), 10, 20, or 40 μg/mL did not significantly increase the cell generation time (i.e. calculated by assessing the percentage of mitoses at M1, M2, or M3) or the frequency of sister chromatid exchange (SCE) (i.e. 5.97, 5.63 or 5.30/cell respectively in three replicate experiments) in Chinese hamster lung (V79) cells relative to negative (5.96/cell) or vehicle controls (5.8/cell) after exposure for 29 hours and two rounds (approximately) 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 μg/mL there was no significant increase in the frequency of SCE (i.e. 6.3, 6.4, 6.0, or 6.6/cell at 10, 20, 40, or 80 μg/mL respectively) in V79 lung cells relative to negative (vehicle) controls (5.7/cell). The positive control (cyclophosphamide in DMSO, 5 μg/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 μg/mL did not significantly increase the frequency of SCE (i.e. 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 hours 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 μg/mL (13.50/cell) relative to vehicle controls (6.79/cell). Positive controls (cyclophosphamide, 0.1 μg/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 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 μg/mL to 1000 μg/mL indicated that at 200 μg/mL, approximately 50% suppression of mitotic activity occurred after a three hour exposure and a 24 hour 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 μg/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 three hours (followed by 43.5 hours of recovery) at each of the two lowest concentrations tested, i.e. 12.34/cell and 12.64/cell, at 12.5 and 25 μg/mL respectively. However, at 50 and 100 μg/mL the incidence of SCE was not significant, i.e. 11.6/cell and 11.2/cell respectively. Excessive cytotoxicity at 200 μg/mL prevented any meaningful results and the positive control, mitomycin C (0.2 μg/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 μg/mL (13.68 and 12.63/cell respectively) but not at 25, 100, or 200 μg/mL (9.61, 11.08 and 11.18/cell respectively). Positive controls (cyclophosphamide, 3 μg/mL) gave the expected statistically significant increase ($p<0.01$) in SCE (48.06/cell) relative to negative controls (9.81/cell).

Although there were small but significant increases in the incidence of SCE 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, the conclusion was that diazinon, at concentrations up to 100 μg/mL, caused a statistically significant increase in SCE but did not cause a toxicologically important increase in the frequency of SCE 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 μg/mL (or 66.8 μg/mL with metabolic activation) did not increase the frequency of SCE in phytohaemagglutinin-stimulated human lymphocytes in the absence or presence of S9 metabolic activation. Concentrations tested were chosen based on cytotoxicity (including 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 (i.e. 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 hours after dosing. Dose selection was based on the results of an acute median oral lethality study in which the combined sex LD_{50} was 76 mg/kg bw. Marrow cells harvested from each group (2/sex except for the vehicle control, one female and three males) had similar SCE frequencies (i.e. 3.89, 3.70, 4.41, or 4.06/cell for the zero, 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 carboxymethyl cellulose) 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 hours 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 hours compared with 13.18 hours for vehicle controls). SCE frequencies for vehicle controls (males, 3.22/cell; females, 4.70/cell) were within the mean historical control range (males, 2.77-3.82/cell; females, 3.1- 4.73/cell) and positive controls (cyclophosphamide, 10 mg/kg bw, IP) gave the expected increase (males, 12.35/cell; females, 16.74/cell), while for treated groups none were significantly different from controls (males, 3.12-3.69/cell; females, 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 SCE in bone marrow cells isolated thirty hours after dosing. Although a similar study (HLA 12226-0-458) had been performed three years earlier (Murli, 1990b), where the maximum dose selected (100 mg/kg bw) was based on systemic toxicity (listlessness) observed in male mice, two further 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, one mouse died and the other four 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, (i.e. 17.15, 23.88 and 20.39 hours respectively relative to the positive (12.10 hours) and vehicle control (12.24 hours) 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 and 6.35/cell. Sufficient cells in metaphase were obtained from only three of five mice at 160 mg/kg bw and two of five mice 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 twelve in the control group) for five 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, Bathe, 1972a). An excessive death rate, i.e. 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 euthanased 24 hours after the last dose and three hours after an IP injection of colchicine (10 mg/kg bw). Drop preparations of testicular parenchyma from the testes of 10/group and nine in the control group were prepared and 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 in these two studies.

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 to the Hool & Müller, 1981b study, 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 twelve in the control group) for four non-consecutive days (treatment administered on day zero, two, three, and five). 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, (Bathe, 1972a). An excessive death rate, i.e. 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 zero, two, three, five and nine, and euthanasia was delayed until day twelve, three hours after an IP injection of colchicine (10 mg/kg bw). Only one 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 twelve in the control group were prepared and 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), and any 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 10.5 mg/kg bw group 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 carboxymethyl cellulose 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 LD_{50} was 187 mg/kg bw, although all deaths contributing to this calculation were among males, (Bathe, 1972a). Each treated male was allowed to mate with different groups of three untreated females at weekly intervals for six weeks. Females were examined daily for the presence of a vaginal plug and its presence was deemed to be day zero of gestation.

Treated males observed daily during the first week after dosing showed signs of somnolence and dyspnoea at 45 mg/kg bw. Bodyweight gain in males, measured before and a week after treatment, were similar in all groups. There were no treatment-related effects observed on the pregnancy rate, number of live and dead foetuses or the number of resorptions in pregnant females euthanased on day fourteen 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 two consecutive days. Dose selection was based on the results of an acute oral gavage lethality study in which the combined sex LD_{50} was 76 mg/kg bw. Six hamsters (3/sex) from each treatment group were euthanased for preparation of bone marrow smears 24 hours 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 euthanased 24 hours 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, i.e. 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-Giegy 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 two of four mice died at 200 mg/kg bw and none at 120 mg/kg bw. Eight mice from each treatment group were euthanased for preparation of bone marrow smears sixteen, 24, or 48 hours after dosing. A positive control group of mice (8/sex) was dosed similarly with cyclophosphamide (64 mg/kg bw) and euthanased 24 hours 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 euthanased at the same time (24 hours after dosing). In neither experiment were there any deaths, significant increases in the frequency of micronucleated polychromatic RBCs, or changes in the ratio of polychromatic to normochromatic cells.

2.3.9.3. Other genotoxic effects

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; four 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-acetylamino-fluorene at 45 µM) gave the expected results.

2.3.10. SPECIAL STUDIES

2.3.10.1. Neurotoxicity studies

Chicken

Krinke G, Ullmann L & Sachsse K (1973) Acute oral LD_{50} 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, i.e. 3 of 4 dead at 3.59 and 10 mg/kg bw (not surprising given the doses were equal or in excess of the LD_{50}), appears to have necessitated the use of an antidote (10 mg/kg bw atropine, IM, 30-60 minutes 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 (e.g. leg paralysis) were monitored daily in all treatment groups for a total of six weeks. Surviving fowls at 2.15 mg/kg bw (one male and two female) and 3.59 mg/kg bw (one female) were euthanased 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 four 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 neurotoxicity was apparent in all positive control birds (although 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 five hens in order to established 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 four hens/group, the LD_{50} 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 LD_{50} in the definitive study) was afforded when the hens were pre-treated with atropine (10 mg/kg bw, IM) one hour 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 one and five hours 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 ten 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, i.e. 28.09 mg/kg bw (determined by GC). All hens (incl. controls) received the atropine/2-PAM therapy before and after treatment and two hens in the diazinon-treated group required one or two additional antidote treatments respectively on the second day after treatment because of persistent cholinergic signs. Signs of delayed neurotoxicity (i.e. gait and locomotor changes) were monitored three times weekly starting three 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 zero) was repeated. The second treatment (with the lower dose) required no additional atropine/2-PAM therapy after the second dose (i.e. after 5 hours).

After the second three-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 two cm above the branch to the peroneal and tibial nerves) and the left tibial nerve (distal 1.5 cm) were taken at autopsy from all hens except for those that died following treatment (i.e. one vehicle control hen after the first treatment and one diazinon-treated hen after each of the first and second treatments respectively). These tissues were then fixed and processed for light microscopy.

Reduced activity that persisted for two to four 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 two weeks, hens in the positive control group had reduced activity accompanied by progressive ataxia and bodyweight loss (in association with inappentence). Body weight or food consumption for diazinon-treated hens was 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 any potential for diazinon to cause 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 hours after diazinon administration, whereas for the combination therapy it was zero, six, twelve and eighteen hours 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 (i.e. reduced activity, diarrhoea, mild dyspnoea, ataxia, abnormal posture, recumbency) were observed for four hours 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 (i.e. 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 three-day intervals.

After the three-week observation period, tissues (cerebellum, *medulla oblongata*, cervical, thoracic and lumbar segments of the spinal cord, plus sciatic and tibial nerves) were taken from *in situ*

formalin perfused hens following deep pentobarbitone-induced anaesthesia/euthanasia and examined for macroscopic changes. These tissues were then processed for light and electron microscopy. The degree of ChE inhibition in plasma, RBCs, brain and spinal cord was determined (by colorimetric assay) in satellite groups, 24 and 48 hours after dosing. Neurotoxic esterase activity in the brain and spinal cord was also measured.

Two hens (2/10) died 48-72 hours after treatment at 100 mg/kg bw, and another died unexpectedly following treatment at 30 mg/kg bw. Although these hens were replaced, none of the dead hens were necropsied because any delayed neurotoxicity generally takes ten days to develop. All hens in all groups had reduced activity for 24 hours and ataxia was only observed in the positive control group (6/6) for four 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, (i.e. 1, 1.8 and 1.1 days at 10, 30 and 100 mg/kg bw respectively) hours. However, the duration did appear to be related to dose, (i.e. 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, (i.e. 1.4 and 5 days respectively), whereas only recumbency was observed 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 eighteen 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 to 12%) after dosing. As shown in Table 2.95, ChE inhibition in plasma was almost complete at all tested doses after 24 and 48 hours 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 RBCs was observed at any dose after either 24 or 48 hours. As anticipated, there was a substantial reduction in the neurotoxic esterase activity in the brain and spinal cord at 24 and 48 hours for the tri-orthocresyl phosphate-treated group (positive control), however, none was observed among any of the diazinon-treated groups.

Table 2.95: ChE Inhibition (mean percentage reduction)

.Dose	Plasma		RBC		Brain		Spinal Cord	
(mg/kg bw)	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;

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.

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 twelve-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 three 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 two weeks. Five rats in each group, not scheduled for the 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 one (i.e. approximately nine to eleven hours after dosing) and on day fifteen after dosing.

Neurological tests, namely FOB and figure-eight maze motor activity, were performed in the presence of 'white noise' on ten rats in each group one week before treatment, at the estimated time of maximal ChE inhibition on day one (i.e. approximately nine to eleven hours after dosing), and on days eight and fifteen 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 recorded posture, tremors, convulsions, stereotypy, bizarre behaviour, faecal colour/composition, and gait.

- Manipulative measurements recorded ease of removal from cage, respiration character, position of hind-limbs when held by tail, pupillary size, lacrimation, staining, eye prominence, palpebral closure, piloerection, fur appearance, hypersalivation, vocalization, and ease of handling.

- Open field tests recorded arousal, circling, convulsions, gait, stereotypy, tremors, number of defecations, numbers of rears, number of urine pools, bizarre behaviour and head position.

- Neuromuscular tests recorded fore and hind-limb grip strength, hind-limb foot splay and hind-limb extensor strength.

- Physiological measurements recorded body tone, rectal temperature and muscle tone.

After each FOB test series, six rats in each group were individually tested in a figure-eight maze for spontaneous activity (measured by light-beam interruption). After the day fifteen FOB test, 5 rats/group were anaesthetised, then euthanased by whole-body perfusion (with glutaraldehyde) and a gross autopsy performed. The only tissues removed for histopathological examination were brain, spinal cord with ganglia (at each level, i.e. 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 two, four and five 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. Cholinesterase activities in plasma and RBCs that were six to seven standard deviations from the mean appeared to confirm the assertion.

Clinical signs that appeared 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 one was accompanied by a corresponding significant ($p \leq 0.01$) reduction in food consumption (i.e. 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 (i.e. approximately nine to eleven hours after dosing) and at doses ≥150 mg/kg bw, but not at any dose on day eight or fifteen. In addition to the incidence of FOB effects shown in Table 2.96, males and females at 600 mg/kg bw had reduced forelimb grip strength (by 24% and 27% respectively); males had reduced hind-limb foot splay at 300 and 600 mg/kg bw (by 19% and 22% respectively); and females at 600 mg/kg bw had reduced hind-limb 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 were also reduced in both sexes (by 3.4% and 11% respectively in males and 4.7% and 12% respectively in females). Maze activity among males at doses ≥300 mg/kg bw and females at doses ≥150 mg/kg bw were reduced. Rats in the positive control group had the expected increase in excitability.

Table 2.96: Incidence of FOB effects (nine hours after dosing)

	Male			**Female**		
	Dose (mg/kg bw)					
	150	**300**	**600**	**150**	**300**	**600**
No. tested	10	10	10	10	10	10
Autonomic Effects						
Impaired respiration	0	0	10	0	0	6
Altered faecal consistency	3	9	10	0	3	3
Lacrimation	0	0	9	0	0	5
Hypersalivation	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
Neuromuscular Effects						
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 hind-limb extensor reflex	0	3	10	0	0	6
Central nervous system *Excitability Effects*						
Tremors (home cage)	0	1	3	0	0	1

	Male			Female		
	Dose (mg/kg bw)					
	150	300	600	150	300	600
Tremors (open field)	0	6	10	0	5	10
Muscle fasciculations (open field)	0	5	10	0	0	3

As shown in Table 2.97, ChE activities in plasma and RBCs at the time of maximal inhibition (i.e. approximately nine to eleven hours after dosing) on day one 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.

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.

Table 2.97: ChE Inhibition‡ (mean percentage reduction)

Dose (mg/kg bw)	Plasma ChE		RBC 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**

‡ Nine to eleven hours after dosing; Statistically significant results marked as ** ($p\leq0.01$).

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 nine to eleven hours after dosing, together with reduced food consumption for a week at doses ≥300 mg/kg bw. FOB tests performed on days one, eight and fifteen revealed significant effects only on day one, when the acute OP poisoning effects (clinical signs) were maximal. No histopathology related to treatment was observed, however, significant plasma ChE inhibition was observed nine to eleven hours after dosing in both sexes at 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 MG 87% 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®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 thirteen 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 87%; Lot no. FL-880045) were prepared monthly and stored at 4^0 C. Diets not used within 35 days were discarded and eight out of thirteen prepared blends that were tested were found to be suitable with respect to homogeneity, stability and concentration (SD ± 4.2% of targets). Doses selected were based on the results of a three-month (Singh et al., 1988) and a one to two year study (Kirchner et al., 1991), 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 three-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 FOB tests, were used exclusively as the source of blood to assess the extent of ChE inhibition (by colorimetric assay) during weeks four, eight, and thirteen. Regional brain ChE activity (i.e. cerebellum, cerebral cortex plus hippocampus, and striatum) in these five rats was then measured at week thirteen.

Neurological tests, namely FOB and figure-eight maze motor activity, were performed in the presence of 'white noise' on ten rats in each group one week before, and then again on the fourth, eighth and thirteenth 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 recorded: posture, tremors, convulsions, stereotypy, bizarre behaviour, faecal colour and composition, and gait.

- Manipulative measurements recorded: ease of removal from cage, respiration character, position of hind-limbs when held by tail, pupillary size, lacrimation, staining, eye prominence, palpebral closure, piloerection, fur appearance, hypersalivation, vocalization, and ease of handling.

- Open field tests recorded: arousal, circling, convulsions, gait, stereotypy, tremors, number of defecations, numbers of rears, number of urine pools, bizarre behaviour and head position.

- Neuromuscular tests recorded: fore and hind-limb grip strength, hind-limb foot splay and hind-limb extensor strength.

- Physiological measurements recorded: body tone, rectal temperature and muscle tone.

After each FOB test series, rats in each group were individually tested in a figure-eight maze for spontaneous activity (measured by light-beam interruption). After thirteen-weeks of treatment the FOB-tested rats were anaesthetised, then euthanased by whole-body perfusion (with glutaraldehyde) and a gross autopsy performed. The only tissues removed for histopathological examination were brain, spinal cord with ganglia (at each level, i.e. 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 dosed at 0.3 and 300 ppm.

All rats survived treatment, though clinical signs consistent with OP toxicity were observed in males (i.e. hypersensitivity to touch and sound) and females (i.e. muscle fasciculations and tremors) at 3000 ppm. At this concentration bodyweight gain was significantly ($p<0.01$) reduced for the first six weeks in males and twelve weeks in females. This reduced weight gain, associated with a reduction in food consumption that achieved significance ($p<0.01$) during weeks one and two in males (average 15%) and weeks one, two and four (average 14%) in females, resulted in a generally reduced bodyweight throughout treatment but which only achieved significance during weeks one to six (average 11%) in males and weeks one to nine (average 11%) and eleven 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 hind-limb grip strength (average for both was 16%) throughout treatment (i.e. weeks four, eight and thirteen), that did not achieve significance. However, reductions in limb grip strength, which averaged 25% among females, achieved significance ($p<0.01$) for forelimb grip strength at weeks four, eight and thirteen and at week four for hind-limb grip strength ($p<0.05$). Hind-limb foot splay was also significantly reduced ($p<0.05$) by 32% at week four and by 26% and 23% (both not significant) at weeks eight and thirteen respectively. Rectal temperature among females was significantly reduced ($p<0.05$) at week thirteen and four out of ten females had signs of dehydration at week four. 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 Table 2.98, ChE activities in plasma and RBCs 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 RBCs, 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. Cholinesterase 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.

Table 2.98: ChE Inhibition after four, eight and thirteen weeks of treatment (mean percentage reduction).

Concentration (ppm)	Plasma		RBC		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 thirteen; Statistically significant results marked as * ($p\leq0.05$); or ** ($p\leq0.01$).

No treatment-related findings were observed following gross autopsy and no additional histopathological lesions apart from the mild focal degeneration of the sacral spinal cord nerve root axons in two of ten 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 thirteen weeks lost bodyweight and had characteristic clinical signs of OP poisoning at the highest concentration tested. FOB studies revealed reduced fore and hind-limb grip strength throughout treatment among all rats

at 3000 ppm with additional findings, i.e. reduced hind-limb 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 RBC ChE inhibition at 30 ppm in both sexes.

2.3.10.2. Porphyrin biosynthesis studies

Bleakley P, Nichol AW & Collins AG (1979) Diazinon and porphyria 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 porphyria similar to the human disease, *porphyria cutanea tarda*, and a published report implicating diazinon exposure as a possible cause, a research group in Australia investigated the effect of diazinon on porphyrin biosynthesis in rats. This published report is the first of a series.

Diazinon (purity 85%; source and batch number not given) administered either orally in the diet (equivalent to 47 mg/kg bw/day) or topically (20 or 40 mg/rat/day, i.e. approximately 114 or 228 mg/kg bw/day) to female Dark Agouti rats (6/group; source not given) for twelve weeks resulted in an increased concentration of porphyrin in faeces, but not in urine. Relative to an untreated control group, the porphyrin concentration in faeces was 2.4-fold greater after eight weeks ($p<0.025$) and 4.9-fold after twelve 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 eight weeks but was after twelve weeks (3.8 fold, $p<0.005$). No significant elevation in porphyrin excretion in urine was evident after four, eight, or twelve weeks of treatment, irrespective of dose or administration route. Electrophoresis of the pooled faecal porphyrins from treated rats revealed a number of decarboxylated intermediates, indicative of the conversion of uroporphyrinogen to protoproporphyrinogen; 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 number 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 S,S-TEPP were found to increase the porphyrin concentration in these cultures (i.e. 900, 1700 and 540 pmoles/mg/24 hours respectively, in the presence of insulin, or 730, 1600 and 220 pmoles/mg/24 hours, in the absence of insulin). Solvent controls had porphyrin concentrations of 18 and 15 pmoles/mg/24 hours 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 oral and topical administration in rats (Bleakley et al., 1979).

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 hypothesis 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 (approximately 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 approximately 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 sixty 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,* (i.e. the absence of a concomitant increase in urinary excretion of porphyrin and the accumulation of coproporphyrin and protoporphyrin instead of uroporphyrin), the use of isodiazinon 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 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), and 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 seventy 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 ferrochetalase 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 ferrochetalase and coproporphyrinogen oxidase activity.

2.3.10.3. 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

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 eighteen, 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 (i.e. IgG1, G2a, G2b, M and A) were measured in the blood of pups euthanased (10/sex/group) on days 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 (i.e. 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 autopsy confirmed pulmonary congestion and mucosal infiltration consistent with acute bronchitis. However, longevity for post-weaning mice was not significantly different at day 800 (i.e. 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 concentrations were apparent at the three time intervals examined, there were no data to indicate that any impairment of immunocompetence had occurred. Longevity and incidence of disease among groups were comparable, so the biological significance of these immunoglobulin changes is unclear.

2.3.11. HUMAN STUDIES

2.3.11.1. Metabolism and toxicokinetics

Wong AJ & Anderson GD (2000) A randomized, double-blind, ascending, acute, oral dose study of diazinon to determine the No Effect Level (NOEL) for plasma and RBC cholinesterase activity in normal, healthy subjects. Part C: Analysis of diazinon in blood and G-27550 in urine. Study No. NCP-8373-Part C. Lab: Development Resources/Chemical Support Department, Novartis Crop Protection Inc. Sponsor: Novartis Crop Protection Inc. Study duration: Clinical trial was conducted during 26 July-22 October, 1998. Report No. Novartis No: 615-98, Report date: July 25, 2000.

This report presented the results of analysis of diazinon in plasma and the diazinon metabolite (G-25770 [6-methyl-2-(1-methylethyl)-4-(1H)-Pyrimidinone,] in urine from the acute human study by Boyeson (2000). In that study (clinical phase), 41 clinically normal, healthy, adult male subjects between the ages of eighteen and 48 received a single oral dose of diazinon (purity: 8% w/v, in epoxidised soybean oil, batch: 781/44, source: Novartis Crop Protection) at 0.03, 0.12, 0.20, 0.21 or 0.30 mg/kg bw administered PO via a gelatine capsule with 240 mL of water after consuming a standardised breakfast. There were seven subjects/group at 0.03, 0.12, 0.20, 0.21 mg/kg bw, whilst the 0.3 mg/kg bw group consisted of only one subject. The placebo control group consisted of

eleven subjects. Blood samples for the plasma diazinon determinations were collected at check-in (two days before dosing) and just prior to dosing on day zero (0 hours), one, two, four, six, eight, twelve, 24 and 48 hours post dosing, and also five, eight and fifteen days post dosing. Plasma diazinon and urinary diazinon metabolite, G-27550 were analysed by GC/MSD methods developed by the study performing laboratory. It was reported that the method used for plasma diazinon assay was a method validated under GLP, whilst that used for urinary metabolite determination was validated during the course of the study. The Tables below provide additional details.

Table 2.99: Table of subject identification numbers and treatment

Sample	Dose (mg/kg bw)					
	Control	0.03	0.12	0.2	0.21	0.3
Plasma	2, 6, 11, 14, 20, 21, 24, 26, 31, 34, 36, 38	1, 3, 4, 5, 7, 8, 9	10, 12, 15, 16, 17, 18, 19	32, 33, 35, 37, 39, 40, 41	13, 22, 23, 25, 27, 28, 30	29
Urine	2, 6, 11, 14, 20, 21, 24, 26, 31, 34, 36, 38	1, 3, 4, 5, 7, 8, 9	10, 12, 15, 16, 17, 18, 19	32, 33, 35, 37, 39, 40, 41	13, 22, 23, 25, 27, 28, 30	29

The following are some of the protocol deviations noted:

- *Blood collection/processing:* Blood sample were collected one to 28 minutes later than originally planned; sometimes the samples were not centrifuged within thirty minutes of collection, but were between one and sixteen minutes late.

- *Urine collection/processing:* The six to twelve hour urine sample for subject twenty (control) and the twelve to 24 hour sample for subject 33 (at 0.2 mg/kg bw) were not collected, and the six to twelve hour, day four and day fourteen urine samples for subject 21 (control) were not collected.

The samples in the preliminary recovery studies were corrected for control residues. Test samples from the clinical phase were not corrected for control residues. However, they were corrected for the procedural recovery values (if recovery was <100%). Descriptive statistics were available.

The limit of quantification of the plasma diazinon assay was 1 ppb. The urinary G-27550 assay had a limit of quantification of 1 ppb during the course of the study. Average recoveries of 70-100% were considered acceptable. The magnitude and frequency of outliers from this recovery range were evaluated as needed. In this study, the overall average recovery of diazinon from fortified plasma samples (from 1.3 to 100 ppb) was 104% (range 58-134%; SD 13%), whilst that for the urinary metabolite, G-27550, was 94% (range: 50-160%; SD 19%). Preliminary studies on the stability of diazinon in plasma and G-27550 in urine showed that these compounds are stable for at least 18 and 12 months in frozen samples, respectively, representing 96% and 104% of the fortified amounts at these two time points.

Diazinon was not detected in plasma of the control subjects, nor in plasma of treated subjects given diazinon at 0.03 mg/kg bw. Some samples collected from the 0.12 (one subject), 0.20 (two subjects) and 0.21 (one subject) mg/kg bw groups contained quantifiable amounts of diazinon ranging from 1.3-3 ppb at one, two and four hours after dosing, but not thereafter (see Table 2.100). All other plasma samples collected at different sampling times from these three dose groups contained <1.3 ppb diazinon. The single subject treated at 0.3 mg/kg bw exhibited a plasma diazinon level of 5.9 ppb at about four hours after treatment, which decreased to 1.4 ppb by six hours post dosing. The

plasma diazinon levels appeared to have reached maximal concentrations at about four hours after dosing.

Table 2.100: Plasma diazinon levels (ppb) in four subjects at different sampling times

Dose (mg/kg bw)	Subject Number	Time after dosing (h)					
		0	1	2	4	6	8
0.12	15	<1.3	2.8	<1.3	<1.3	<1.3	<1.3
0.20	32		1.9	<1.3	3.0		
	37		<1.3	2.5	<1.3		
0.21	23		2.0	<1.3	<1.3		
0.30	29		<1.3	<1.3	5.9	1.4	<1.3

The percentage of the administered dose excreted as G-27550 in urine by the subjects for different sampling periods during the 48-hours period after treatment are summarised in Table 2.101. The average amounts of G-25770 excreted in the urine during the first 48 hours of dosing represented about eight to 25% of the administered dose. Generally, the amount excreted was related to the administered diazinon dose, except at 0.20 mg/kg bw. The rate of excretion appeared to be faster during the first 24 hours after dosing.

Only individual data for urinary DETP levels were provided and no statistical analyses were conducted on the data. Generally the excretion of DETP in the urine was related to the dose administered.

Table 2.101: Percentage of the dose excreted in the urine as G-27550*

Dose (mg/kg bw)	Body weight (kg)	Sampling interval (h)				Total (%)
		0-6	6-12	12-24	24-48	
0.03	82.1	2.5	2.0	2.2	1.13	7.9
0.12	76.1	2.9	3.3	3.4	1.7	11.3
0.20	78.6	2.7	2.0	2.5	1.1	8.1
0.21	75.4	2.2	8.3	1.95	0.88	13.4
0.3	71.2	1.8	17.0	3.3	3.2	25.3

*Mean values, calculated by reviewing toxicologist.

Results indicate diazinon was detectable in the plasma of some subjects after dosing at 0.12, 0.20 and 0.21 mg/kg bw. Plasma levels ranged from 1.3-3 ppb and were present up to four hours after dosing. The single subject given diazinon at 0.3 mg/kg bw had a plasma level of 5.9 ppb at about four hours after treatment, which then decreased. Plasma levels appeared to have reached maximal concentrations at about four hours after dosing. The average amounts of the urinary metabolite, G-25770 excreted in the urine during the first 48 hours of dosing represented about eight to 25% of the administered diazinon dose. The proportion of the dose excreted in the urine as G-27550 generally increased as the dose increased (over the range tested in this study). Generally, the percentage rate of G-27550 excretion was greater during the first 24 hours after dosing.

Hughes DL & Vaughn C (2000) A randomized, double-blind, ascending, acute, oral dose study of diazinon to determine the No Effect Level (NOEL) for plasma and RBC cholinesterase activity in normal, healthy subjects. Part B: Analysis of DETP in urine. Study No. NCP-8373-Part B, 6117-394. Lab: Covance Clinical Research Unit Inc. Sponsor: Novartis Crop Protection Inc. Study duration: Clinical trial was conducted during 26 July-22 October, 1998. Report No. Novartis No: 587-98, Report date: July 25, 2000.

Raw data for individual results (urinary DETP) analysed in the study by Boyeson (2000) were provided in this report. However, these results were not considered to be suitable for further

assessment, although in general, the proportions of DETP excreted in the urine were related to the administered diazinon dose.

2.3.11.2. Acute toxicity

Boyeson MG (2000) A randomized, double-blind, ascending, acute, oral dose study of diazinon to determine the No Effect Level (NOEL) for plasma and RBC cholinesterase activity in normal, healthy subjects. Study No. NCP-8373 Part A. Lab: Covance Clinical Research Unit Inc. Sponsor: Novartis Crop Protection Inc. Study duration: Clinical trial was conducted during 26 July-22 October, 1998. Report No. Novartis No: 587-98, Report date: July 25, 2000.

Note this was a quality assured study conducted in compliance with the US Code of Federal Regulations governing the protection of human subjects (21 CFR 50), Institutional Review Boards (21 CFR 56) and the Obligations of Clinical investigators (21 CFR 312), which are consistent with the Declaration of Helsinki and Common Rule.

Forty clinically normal, healthy, adult male subjects between the ages of 18 and 48 (bw range 64.3 – 99.5 kg; mean: 78.9 kg) participated in the study. These subjects received a single oral dose of diazinon (purity: 8% w/v, in epoxidised soybean oil, batch: 781/44, source: Novartis Crop Protection) at 0.03, 0.12, 0.20, 0.21 (seven subjects/group) or 0.30 (one subject) mg/kg bw administered PO via a gelatine capsule with 240 mL of water after consuming a standardised breakfast. The placebo control group consisted of eleven subjects. The study utilised a double-blind, randomised, ascending dose design. A single individual (a dose cohort leader) in a dose-block received the next highest dose, while the remainder of the group received a lower dose that had been demonstrated to be safe and tolerable by the previous dose cohort leader (see Table 2.102 for dosing regimen).

Each dose (in a gelatine capsule) was prepared within the 24 hours period prior to dosing, stored under refrigeration, and protected from light until use. For each dose block, one extra capsule each of diazinon and placebo vehicle were prepared. These were subsequently analysed for diazinon.

Subjects were instructed to swallow the capsule whole. The subjects entered the study two days prior to dosing and abstained from consuming foods or beverages that contained xanthine or caffeine for five days, and that contained alcohol for fourteen days prior to study entry, up until study completion on day fifteen. They also refrained from consuming grapefruit/juice during the study. They were provided with standardised diets (breakfast, lunch, dinner and a snack) moderate in fibre with normal fat levels. Consumption of water was *ad libitum* throughout the study. The subjects also refrained from strenuous exercise during the study and from the use of prescription or non-prescription medications within fourteen days prior to study entry, and for the study duration.

Although it was originally planned to include females in the study after completion of male cohorts, this was not done on the advice of the sponsor. The initial dose of 0.03 mg/kg bw was based on the findings of a single dose, 28-31 day repeat exposure study in human subjects (Beilstein, 1998). It was reported that in animal studies, rats have been shown to tolerate, without effect, a single acute oral dose that is approximately 10 times the NOEL established in repeat-dose studies. Based on this relationship observed in rats, it was anticipated that humans may tolerate a dose of 0.3 mg/kg bw diazinon without effect. Dose levels were implemented based on the criteria outlined below.

All subjects were pre-screened within four weeks prior to study commencement for recording of complete medical history, physical condition, electrocardiogram, blood pressure, pulse rate, clinical chemistry tests, haematology, urinalysis, serology for hepatitis B and C, and plasma and RBC ChE assays. Only those subjects who met the following criteria were included in the study: good health,

normal resting blood pressure and heart rate, body weight between 50 and 100 kg, negative HIV and hepatitis B and C results and negative urine test results for drugs of abuse at screening and two days before the commencement of the study. Factors that excluded potential subjects from the study were the following: any acute or chronic conditions, history or clinical manifestations of significant metabolic, pulmonary, cardiovascular, hepatic or renal disease or condition, alcoholism within a year of study entry, allergic conditions, donation or loss of >400 mL blood within twelve weeks prior to study entry, and participation in other clinical studies ending within ninety days of study entry.

Table 2.102: Dosing regimen

Dose block	Dose (mg/kg bw)	Number of subjects
1	0	1
	0.03	1*
2	0	2
	0.03	6
	0.12	1*
3	0	3
	0.12	6
	0.21	1*
4	0	3
	0.21	6
	0.3	1*
5	0	3
	0.20	7

*Lead subject for the dose group.

The decision to proceed to the next dose block was based on the following criteria:

- Absence of clinically significant adverse events indicative of test material intolerance. If the lead-in subject of a dose level/dose cohort group or if two or more subjects in any one dose level developed a severe, probably-related adverse experience, or if one subject developed a serious, probably-related event that was life-threatening, then that dose level was considered an intolerable dose,
- Clinically significant physical examination, electrocardiogram findings or vital signs in two or more subjects at any dose level,
- An overall pattern of clinical changes or symptoms which could have appeared minor in terms of individual adverse events but which collectively represented a concern of safety,
- Levels of RBC ChE inhibition >20% from baseline, if concomitant with plasma ChE inhibition >20% of baseline (each subject's baseline ChE level was defined as the level just prior to dosing). If a single individual at any dose level reached >20% inhibition of RBC ChE (concomitant with >20% inhibition of plasma ChE inhibition), no further increase in dose level was attempted. However, if this occurred, the next cohort group dose level could have been adjusted downward in order to help define the upper limit of NOEL and,
- Presence of a pattern of clinical laboratory changes i.e. consistent increase or decrease within a dose level or within a randomised dose group, which might have indicated an overall safety issue of concern.

Subjects reporting for check-in two days before the start of testing were subjected to medical and clinical chemistry examinations as outlined above. Blood samples were taken for assay of diazinon and urine for assay of diazinon metabolite G-277550 (see below for details).

Serial blood samples for haematology and clinical chemistry evaluations were collected (at and 24 and 48 hours post dose, and at study completion on day fifteen) via direct venipuncture or in-

dwelling catheter. The following haematological variables were determined: RBC, WBC, WBC-DC, Hct, Hb, mean corpuscular haemoglobin, MCHC, MCV and mean platelet volume(MPV). The tested clinical chemistry parameters included the following: albumin, AP, ALT, AST, BUN, amylase, calcium, chloride, cholesterol, CPK, creatinine, gamma-glutamyl transpeptidase, glucose iron, LDH, lipase, phosphorous, potassium, sodium, total bilirubin, total protein, CO_2, triglycerides and uric acid. Plasma and RBC ChE activities were measured using blood samples collected at the following time points: prior to dosing on day zero, and at one, two, four, six, eight, twelve, 24 and 48 hours post dosing. The enzyme activities were determined according to the method of Ellman et al., (1961) with thiocholine as the substrate. Urinalysis (pH, specific gravity, urobilinogen and creatinine) was conducted at one, two and sixteen days post dosing.

Experimental methods for plasma diazinon and urinary metabolite analysis and the findings are summarised elsewhere in this report (Wong and Anderson, 2000, and Hughes and Vaughn, 2000).

Descriptive statistics were calculated for the following parameters: subject demographics, incidence of adverse effects, clinical chemistry and haematology data, urinalysis data, vital signs and electrocardiogram findings. Where appropriate, the following tests were used to analyse the data: one-way ANOVA, Dunnett's and paired t-test.

Blood samples collected at screening and at check-in two days and one day before dosing were analysed on separate occasions and represented the day-to-day pre-dose variations for each subject. This pre-dose variation for each subject was used to determine whether the subject had recovered following dose administration (i.e. reached discharge criteria on day fifteen post dosing). Data collected from Group 1 samples ten, seven and four days before dosing were considered as informational data on baseline variability, and were not included in any analyses. The samples collected at zero, one, two, four, six, eight, twelve and 24 hours after dosing were analysed at the same time. The zero hour plasma and RBC ChE values were regarded as the baseline data for these two variables.

The following protocol deviations for various aspects of the study were reported:

- *Inclusion/exclusion:* Two subjects (subjects number twelve and eighteen) were not fasted prior to screening and two other subjects (subjects number seventeen and 32) were overweight according to the 1996 Metropolitan Height and Weight Tables; caffeine/xanthine test was not performed on one subject (subject no. unspecified); subject number 37 had allergies to pollen and dust that required medication; and subject number 28 took chewable antacid thirteen days prior to study entry.

- *Check-in:* Subject Number 21 (placebo control) voluntarily withdrew from the study for personal reasons and exited on day eight of the study; two subjects did not fast for clinical laboratory tests that were conducted seven and four days before dosing, respectively; and two subjects (subjects number three and eleven) did not meet the criteria for ranges of vital signs.

- *Vital signs:* Subjects were not always seated for at least five minutes prior to when vital signs were taken; the time of the day vital signs were measured for subject thirty was not specified, and his blood pressure at 48 hours post dosing was not specified.

All subjects enrolled in the study were considered healthy as they met all the defined inclusion criteria and did not meet any of the exclusion criteria. A total of 42 adverse events were recorded. Forty-one of the 42 events showed no association with treatment (e.g. accidental injury, asthenia, chills, headache, neck pain, diarrhoea, flatulence, nausea, vomiting, ecchymosis, lymphadenopathy, cough, pharyngitis, rhinitis, rash, skin discolouration, ear pain). All of these events occurred either

pre-dose or in subjects receiving placebo control capsules, and were classified as "mild" in severity. Back pain was reported at seven days post dosing in one subject given 0.3 mg/kg bw diazinon; this event was classified as "possibly related to treatment" by the study authors. The pain was reported to have resolved on the same day without any treatment. No further adverse events attributable to the test substance were reported.

No treatment-related abnormalities were detected in any individual on physical examinations, in vital signs or ECGs during the study. There were no treatment-related effects on any of the tested clinical laboratory parameters.

Dose-related and statistically significant ($p \leq 0.05$) plasma ChE inhibition was observed at and above 0.12 mg/kg bw (see Table 2.103) at the majority of the observation times compared to placebo controls. For all dose groups, toxicologically significant enzyme inhibition was first reached at about four hours post treatment; compared to placebo controls inhibition ranged from 32% to 78%. Maximal inhibition was recorded at about six hours after test substance administration (42% tp 93% inhibition). A similar pattern of inhibition was seen if inhibition was calculated relative to baseline values, rather than to placebo controls. No statistical tests could be conducted for the single individual in the 0.3 mg/kg bw group. Nonetheless, plasma ChE inhibition at this dose level ranged from 2% to 93% in comparison to placebo controls, with the maximal inhibition of 93% occurring at about six hours after dosing.

Plasma ChE levels showed signs of recovery commencing at eight hours post treatment. This recovery was lengthy and seven individuals (two each at 0.12, 0.2 and .021 mg/kg bw, and the single individual at 0.3 mg/kg bw) had not returned below around 20% plasma ChE inhibition by study discharge (at fifteen days post dosing). The following table provides additional details.

Table 2.103: Plasma ChE activity (µmol/L)[a] at different observation times

Dose (mg/kg bw)	**Observation time (hours or days after dosing)**					
	0 hours (baseline value)	**1 h**	**2 h**	**4 h**	**6 h**	**8 h**
Control	4211± 610	4226 ± 676	4232 ± 634	4335 ± 631	4289 ± 593	4320 ± 581
0.03	4694 ± 681	4576 ± 675 (2.8%)	4612 ± 705	4456 ± 606 (4.8%)	4388 ± 586 (6.3%)	4389 ± 613 (6.3%)
0.12	4404 ± 1166	4105 ± 1384 (7.9%)	4046 ± 1392 (9.4%)	3049 ± 1292 (32.5%)	2524 ± 586 (41.6%)	2580 ± 625 (40.5%)
0.20	4210 ± 915	4209 ± 852	3688 ± 1087 (12.7%)	1942 ± 574 (52.8%)	1668 ± 262 (58.4%)	1713 ± 247 (57.3%)
0.21	4126 ± 472	3686 ± 581 (9.8%)	3470 ± 480 (14.7%)	1894 ± 561 (54.2%)	1574 ± 571 (62.2%)	1788 ± 614 (57.1%)
0.3[b]	3517	3516 (2%)	3406 (2%)	953 (78%)	268 (93%)	388 (91%)
Continued						
	12 h	**24 h**	**48 h**	**5 days**	**8 days**	**15 days**
Control	4233 ± 595	4317 ± 575	4236 ± 549	4062 ± 585	4148 ± 467	4121 ± 549
0.03	4398 ± 613 (6.3%)	4443 ± 562 (5.1%)	4479 ± 560 (4.2%)	4146 ± 619 (11.7%)	4421± 746 (6.0%)	4189 ± 632 (10.7%)
0.12	2580 ± 625 (38.9%)	2872 ± 666 (33.7%)	2859 ± 709 (34.5%)	3084 ± 823 (29.7%)	3511 ± 1040 (20.6%)	3722 ± 1019 (15.5%)
0.20	1713 ± 247 (55.4%)	2073 ± 201 (48.8%)	2200 ± 215 (46.0%)	2699 ± 281 (34.2%)	3096 ± 370 (24.8%)	3558 ± 460 (13.4%)
0.21	1788 ± 614 (57.4%)	2044 ± 537 (50.8%)	2388 ± 566 (42.5%)	2723 ± 571 (34.3%)	2978 ± 609 (28.2%)	3333 ± 500 (19.2%)
0.3[b]	453 (89%)	810 (81%)	1116 (73%)	1719 (57%)	2083 (50%)	2496 (39%)

[a]Mean ± SD; enzyme activity expressed as µmol/L = µmoles of thiocholine produced/min/L. Values in parentheses represent mean percent inhibition compared to placebo controls. [b]n = 1. Statistically significant findings are discussed in the text above.

The data in relation to RBC ChE activity are presented in the following Table. Mean RBC ChE activity was not inhibited more than 13% relative to pre-dose base line value at any dose level.

- At 0.2 mg/kg bw, RBC ChE activity was inhibited by 8, 3%, 1.5 and 1% on days two, five, eight, and fifteen after dosing respectively, compared to baseline values. When percent inhibition was calculated against the placebo controls, the eight percent inhibition at 48 hours became 2%, with no inhibition at the other observation times. The inhibition was not statistically significant by either method of calculation. Hence the effects seen at 0.2 mg/kg bw at 48 hours after dosing, while probably treatment-related, are not considered to be of sufficient magnitude to be regarded as toxicologically significant.
- At 0.21 mg/kg bw, an analysis using an among group comparison of placebo versus all other treatment groups (one-way ANOVA and Dunnett P-value) revealed that RBC ChE inhibition was statistically significant in comparison to placebo controls at each of the five, eight and fifteen day post-dose sample times. This inhibition was sustained at about 10% during this period.
- At 0.3 mg/kg bw, the level of RBC inhibition ranged from 4 to 13%relative to pre-dose baseline values. The single individual at 0.3 mg/kg bw showed an inconsistent pattern of RBC ChE inhibition with the maximum inhibition (compared to baseline) of 13% at fifteen days post dose. Additionally, enzyme inhibition was greater than that observed at 0.20 mg/kg bw and was generally persistent from day one post-dose onwards.

Although the group mean data at 0.21 mg/kg bw exhibited no marked inhibition, but with statistical significance from day five after dosing onwards, individual data showed more than 12% inhibition in one subject at five days (20.5%) and in another individual on three occasions (at four hours and at five and eight days post dosing; 20 to 21% inhibition) after dosing relative to placebo controls, with concomitant greater than 12% inhibition in plasma ChE activity. As this individual satisfied the criteria for cessation of dose escalation as defined in the study protocol, no additional dose escalations were performed and further subjects were dosed at the lower dose of 0.2 mg/kg bw.

As noted above, at 0.21 mg/kg bw RBC ChE inhibition was statistically significant ($p \leq 0.05$) at five, eight and fifteen days after dosing and was slightly greater and more consistent than that observed at 0.2 mg/kg bw over these observation times. This indicates that 0.21 mg/kg bw may be a borderline LOEL for this toxicological endpoint (RBC ChE inhibition). This conclusion is supported by the conditions for cessation of dose escalation being met at this dose.

Table 2.104: RBC ChE activity (µmol/g) at different observation times[a]

Dose (mg/kg bw)	**Observation time (hours or days after dosing)**					
	0 hours (baseline value)	**1 h**	**2 h**	**4 h**	**6 h**	**8 h**
Control	8426± 1039	8511 ± 1049	8426 ± 965	8489 ± 978	8532 ± 707	8435 ± 884
0.03	8439 ± 1237	8412 ± 1671	8412 ± 1397	8808 ± 1584	8449 ± 1385	8686 ± 1630
0.12	9698 ± 911	9756 ± 1101	9676 ± 947	9711 ± 802	9758 ± 1316	9494 ± 1021
0.20	8709 ± 612	8734 ± 750	9016 ± 754	9015 ± 607	8689 ± 521	8676 ± 707
0.21	8776 ± 763	8607 ± 901	8412 ± 658 (4%)	7883 ± 481 (10%)	8975 ± 908	8165 ± 742 (7%)
0.3[b]	9145	8552 (6%)	8517 (7%)	8795 (4%)	8691 (5%)	7976 (13%)
Continued						

Dose (mg/kg bw)	Observation time (hours or days after dosing)					
	12 h	24 h	48 h	5 days	8 days	15 days
Control	8654 ± 1008	8538 ± 854	8236 ± 927	8282 ± 635	8550 ± 800	8481 ± 929
0.03	8614 ± 1233	8459 ± 1410	8566 ± 1258	8881 ± 1696	8574 ± 1547	9845 ± 1447
0.12	9928 ± 1104	9666 ± 1305	10242 ± 1687	9633 ± 1573	10304 ± 1746	10097 ± 1764
0.20	8726 ± 771	8743 ± 842	8043 ± 702 (8%)	8407 ± 894 (3%)	8574 ± 747 (1.5%)	8611 ± 815 (1%)
0.21	8853 ± 785	8840 ± 400	8275 ± 821 (5%)	7816 ± 907 (11%)*	7963 ± 787 (9%)*	7896 ± 526 (10%)*
0.3	9285	9756	8709 (5%)	8587 (6%)	8988	7924 (13%)

[a]Mean ± SD; *Significantly different from controls ($p \leq 0.05$, Dunnett's test). Values in parentheses represent mean percent inhibition compared to corresponding baseline values. [b]n = 1).

Based on these findings, where there was a statistically significant effect observed at and above 0.12 mg/kg bw, the NOEL for plasma ChE inhibition was 0.03 mg/kg bw for this study. Further, the NOEL for RBC ChE inhibition was 0.2 mg/kg bw. The occurrence of statistically significant RBC ChE inhibition at a dose only 5% higher may necessitate a cautious interpretation of this value.

2.3.11.3. 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-Giegy 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 hours) to three adult male volunteers (77-95 kg bw) such that the total daily ingestion was 0.05 mg/kg bw/day. Originally it was intended that the dosing regimen proceed for thirteen weeks with doses being progressively increased from 0.05 to 5 mg/kg bw/day, however, because significant plasma ChE inhibition was observed at 0.05 mg/kg bw/day after only five days of treatment, the dosing regimen was modified so that this five 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 fourteen days. Plasma and RBC ChE activities were determined (by an electrometric method, ΔpH/h) before the AM dosing (i.e. sixteen hours after the previous dose) on five occasions pre-test, then on the first, third and fifth test days of each treatment phase and on days three, six, ten, seventeen, and 21 of the first recovery phase and days three, seven and fourteen of the second. Bodyweight and clinical signs were determined daily. Haematology (Hb, RBC count, total and differential leucocyte count and PT), 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 one, eight and fifteen 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 hours 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 Table 2.105, plasma ChE activity was significantly inhibited on day five of the first ($p<0.01$) and second ($p<0.05$) treatment cycles. Significant inhibition ($p<0.05$) persisted for six days after treatment in the first

cycle, while substantial inhibition of up to 22% was observed for six days after treatment in the second. No concurrent changes in RBC ChE activity were observed during treatment.

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.

Table 2.105: 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
Volunteer	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 pre-test value

Statistically significant results marked as * ($p \leq 0.05$); or ** ($p \leq 0.01$).

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 four 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 ai) and volunteers were given either four or five capsules depending on whether their bodyweight was closer to 75 or 100 kg bw respectively, the actual administered doses varied slightly, (i.e. 0.03, 0.027, 0.022/0.027 (alternate day treatment with four and five capsules) and 0.026 mg/kg bw/day, respectively). Each dose was divided so that two of the four capsules were taken between 0800 and 0900 hours and then again between 1300 and 1400 hours; for the five capsule treatment, the fifth was taken between 0600 and 0700 hours. After complete plasma ChE inhibition in the two volunteers was observed after one day of treatment, dosing was suspended from days five to ten of the 42-day dosing regimen to ascertain reversibility of effects. The other two volunteers commenced treatment a month later and were treated uninterrupted for 34 days.

The recovery of diazinon (measured by ultraviolet absorption) from two different batches of capsules ranged between 92% and 96% two months after preparation and TLC analysis did not reveal any substantial change in the number or concentration of impurities during this time. Plasma and RBC ChE activities were measured by colorimetric assay on two or three occasions before treatment and then before breakfast (approximately 0730 hours) at three to seven day intervals during treatment. Thus the interval between dosing and ChE activity measurements was (approximately) either thirteen or eighteen hours. Haematology measurements involving Hb, Hct, RBC count, total and differential leucocyte count and clotting time were performed six days before treatment and then again on days ten and 43 for the 42-day dosing regimen and sixteen days before treatment and then again on day 34 for the 34-day regimen. Serum alkaline and acid phosphatase were also measured six days before treatment and on days ten, 26 and 43 for the 42-day dosing

regimen and only twice during treatment for the 34-day regimen, i.e. days five 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 pre-test levels in two volunteers during the 42-day dosing regimen. No pre-test levels were measured for the other two volunteers, but both were likely to have been reduced during treatment by comparison with pre-test activities as determined for the first two volunteers 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 Table 2.106, though it was not entirely clear what control was used to determine the 'percentage activity' in plasma. Hence, the activities reported and shown in Table below are not relative to pre-test or controls but to some undefined benchmark.

Table 2.106: '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), i.e. 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, (e.g. pre-test ChE activities for the same volunteer can vary by up to three fold). Therefore trying to interpret the physiological relevance of a ChE activity ranging between 4 and 13% in pre-test 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 pre-test levels in series 2 (i.e. as a surrogate control) for the corresponding days. Three assay dates were common to both series, i.e. 25/4, 2/5 and 9/5, and these correspond to day 29, 36 and 43 of the first dosing regimen and all three pre-treatment assay days in series 2. The ChE activities measured on those days are shown in the following Table.

Table 2.107: 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 RBCs 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 four volunteers. However, although ChE activity in RBCs was apparently unaffected by the 34- or 36-day treatment, ChE activity in plasma in two 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 five-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 (approximately forty percent) of ChE in plasma occurred. Thus, in the presence of a reported 60% reduction in acid phosphatase activity and 40% reduction in plasma ChE activity, a NOEL could not be established because of limits in the study design and uncertainties in the way results were determined.

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 three healthy adult male volunteers (numbered 4, 5 and 6) the final dose was 0.025 mg/kg bw. A concurrent control group of three volunteers (numbered 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, i.e. at 0800, 1200 and 1800 hours for 43 days. In a second dosing regimen, three different volunteers (numbered 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 RBC ChE activities in all eight volunteers were measured on five separate occasions before dosing and then at one to five day intervals throughout treatment and recovery using an electrometric method (ΔpH/h). Bodyweight and clinical signs were monitored daily. Haematological parameters, including Hb, RBC count, total and differential leucocyte count and PT were determined at four to seven day intervals during treatment and at four to fourteen day intervals during recovery, as were clinical chemistry parameters, i.e. ChE (RBCs and plasma), BUN, AP and ALT, and urinalysis, i.e. 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 Table 2.108.

Table 2.108: 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; Statistically significant result marked as ** ($p \leq 0.01$).

The plasma ChE inhibition observed in the three volunteers was calculated relative to their individual mean pre-test activities. A comparison with pre-test plasma ChE inhibition seems reasonable since group numbers were low (n=3) and the interassay variability for controls relative to their respective mean pre-test activities was only 3%. Therefore the significant change observed for the combined mean ChE plasma inhibition relative to the combined mean pre-test values for the treated volunteers indicates a clear treatment-attributable effect. By contrast, mean RBC 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 RBC 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 eight percent and recovery appeared complete after sixteen days. Similarly, RBC 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.

Beilstein P (1998) Tolerance study in Novartis managers upon repeated oral administration of diazinon. Study No. 972019, Lab: Novartis Crop Safety/Human Safety Assessment, CH-4002 Basel, Switzerland. Sponsor: Novartis Crop Protection AG, BU Insect Control, CH-4002, Basel, Switzerland. Study duration: October-December, 1997. Report No. 972019, Report date: March 13, 1998.

Non-quality assured study.

Diazinon (purity: 99.5%, batch: AMS 140/7, source: Swiss Caps, Switzerland) in gelatin capsules was administered to four healthy, male subjects at 0.03 mg/kg bw/d, once daily for 28 to 31 days. The dose level was apparently chosen based on an assessment of previous human studies and the purity of currently manufactured technical grade diazinon. It was expected that the dose level of 0.03 mg/kg bw/d would be a NOEL for the study. The capsules were analysed for the diazinon content before and after the dosing period by the study sponsor. Body weight of the subjects ranged from 79.5 kg to 95 kg (age: unspecified). Prior to commencement of the study, all subjects were screened for eligibility.

Screening included recording of personal data, medical history, twelve-lead electrocardiogram, comprehensive physical examination (blood pressure, heart rate, heart rhythm, pulmonary system, lymph nodes, skin, abdomen including liver spleen, height, weight and oral temperature), neurological tests (motor and sensory reflexes, optical mobility, Romberg test, finger-nose test and Babinski test), haematology (RBC, Hb, Hct, packed cell volume, MCHC, mean corpuscular haemoglobin, WBC and WBC-DC), clinical chemistry tests (glucose, C-reactive protein, ALT, γ-GT, AP, creatinine, uric acid and cholesterol), plasma and RBC ChE assay, urinalysis (protein, glucose, ketones, urobilinogen, bilirubin, pH and blood), and serological tests for HIV and hepatitis B. These tests were performed 28 days (subjects 1, 2 & 3) and 27 days (subject 4) prior to commencement of the study. During the course of the study, an inhibition of RBC ChE activity to

70% or below of pre-test values was considered as the criteria to discontinue or interrupt the test substance administration.

After completion of the screening, the baseline values for all of the above study parameters (except serology) were determined at day 27 (subjects 1 & 2) or day 7 (subjects 3 & 4) prior to initiation of dosing, and on day 1 (all subjects) of the study. On the day before the first dosing day, the subjects were clinically examined for any acute disorders or any changes that might have occurred since the screening and baseline determinations. The time point of the first administration of the test compound was designated as day zero.

Blood samples were collected for plasma and RBC assay at six hours, and at 1, 2 or 3, 8, 10 (only 3 subjects), 13 or 14 and 20 days during the treatment phase, and at 28, 29 or 30, and 48 or 57 days during the post treatment phase. Plasma ChE activity was assayed by the method of Ellman et al., (1961), while RBC ChE activity was measured by the method of Augustinsson et al., (1978). All other clinical assessments (as above) and urinalysis were performed at 8, 13 or 14, 20, 28, 29 or 30 days. The final examination, which included all clinical chemistry tests (except serology), assessment of neurological status and physical examination, was conducted one day after the last intake of the test compound.

It was reported that 24-hours urine samples were collected from one subject at seven and four days prior to commencement of the study and then on study days zero, one, three, seven, fourteen and 28 for optional kinetic and metabolic analyses. However, none of the study parameters related to these study areas were specified, and no statistical tests were conducted to analyse the data.

The following results were reported:

- *Physical examination, neurological status and electrocardiogram:* No abnormalities were detected at any of the observation points.

- *Clinical chemistry and haematology:* Administration of diazinon had no effect on any of the clinical chemistry parameters tested. In haematology, marginally elevated leucocytes (12.4 K/µL; *normal range: 3.0-10 K/µL*) and segmented neutrophils (74%; *normal range: 45-70%*) were noted in one subject at fourteen days during treatment. No toxicological significance was attributed to these isolated findings, given that they were not repeated, and were limited to one subject.

- *Plasma ChE activity:* Individual values of the four subjects are presented in Table 2.109. In comparison to pre-test values, treatment-related and toxicologically significant plasma ChE activity inhibitions (22-48%) were observed in all four subjects from study day eight onwards. The inhibition was seen to persist in all four subjects, showing signs of enzyme activity recovery by day 31 after cessation of treatment.

- *RBC ChE activity*: Some fluctuations in RBC ChE activity (generally increases) were noted in all four subjects, with a mean coefficient of variation of 9.5% (range 6 to 13%), similar to the normal coefficient of variation of 10% reported in the literature (Jokanovic & Maksimovic, 1997).

Table 2.109: Plasma ChE activity (U/L)[a]

Study day	Subject 1	Subject 2	Subject 3	Subject 4
Pre-test				
-28	8950	8894	11597	ND

Study day	Subject 1	Subject 2	Subject 3	Subject 4
-27	8740	8943	ND	10890
-7	ND	ND	11392	10918
-1	8386	7592	10792	9575
Mean	8692	8476	11262	10461
Treatment period				
0	8085 (7%)	7415 (13%)	10692 (5%)	9739 (7%)
1	8168 (6%)	7711 (9%)	10442 (7%)	10387 (1%)
2	8362 (4%)	8308 (2%)	ND	ND
3	ND	ND	10315 (8%)	9262 (11%)
8	5103 (41%)	6593 (22%)	7353 (35%)	6061 (42%)
13	4557 (52%)	ND	ND	ND
14	ND	5390 (36%)	5500 (51%)	5719 (45%)
20	4653 (46%)	4454 (47%)	5077 (55%)	5895 (44%)
Post-treatment				
28	ND	ND	5696 (49%)	5631 (46%)
29	ND	5052 (40%)	ND	ND
31	3958 (54%)	ND	ND	ND
48	7639 (12%)	ND	9953 (12%)	9579 (8%)
57	ND	7287 (14%)	ND	ND

[a]Values in parentheses represent percent inhibition. ND = not determined.

Thus oral administration of diazinon in gelatine capsules to human subjects at 0.03 mg/kg bw/d for 28- 31 days resulted in 22-48% inhibition in plasma ChE activity. The enzyme activity showed some signs of recovery by four weeks after cessation of treatment. Some fluctuations in RBC ChE activity were noted, but the observations were consistent with normal individual variations. It is not stated why no observations or ChE assays were performed during the last eight days of dosing. This study was clearly a dose-safety confirmation test for a subsequent clinical study and is considered to be of limited regulatory value, as only four male subjects were tested without reference to GLP standards.

2.3.11.4. 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'-^{14}C]-diazinon (specific activity 66 µCi/mg) was applied to a ten sq. cm area of the ventral forearm or abdomen to groups of six volunteers each. A third group of six 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 hours. Any remaining diazinon was washed off after 24 hours and the surface of the application site stripped repeatedly (ten times) with adhesive cellophane tape seven days later. For each volunteer the radioactivity, in the surface wash, attached to the cellophane tape, and present in 24 hours pooled urine collected daily over seven days, was measured using liquid scintillation spectrophotometry.

After adjusting for the residual radioactivity at the application site (24 hours wash, 0.5%, 1.40%, 0.35%; tape strip, 0.01%, 0.01%, 0.04%), the cumulative seven 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

(Wester et al. 1993) had shown that approximately 56% of an intravenously administered dose was excreted in the urine over seven days, it was reasoned that the human percutaneous adsorption rate for the three groups, after correcting for incomplete or other route excretion, was 3.8%, 3.2% and 2.9% respectively. It was assumed that the pharmacodynamics in monkeys were similar to humans. 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 hours is uncertain, then the reliability of the calculated absorption rates is in doubt.

The same study also reported an *in vitro* study, in which abdominal skin (500 μm thick) cut from two male human cadavers (23 and 56 years old) with a dermatome and stored in Eagle's minimum essential medium at 4 ^{0}C for no more than five days, was used to estimate the percutaneous absorption rate of diazinon in an *in vitro* flow-through cell. [2'-^{14}C]-diazinon (specific activity 66 μCi/mg) in acetone at a concentration of 0.25 μg/cm^2 was applied (volume not reported) onto the one square centimetre skin samples in six separate cells for each donor. After 24 hours without occlusion and a buffered saline flow rate of 1.25 mL/h (one 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 (approximately 40%) had evaporated over the duration of the study.

2.3.11.5. 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.

2.3.11.6. 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 eighteen months, and were accustomed to applying 1200 to 1500L diluted diazinon (0.1%) once weekly by backpack

spray. The study noted that neither mask nor gloves were used during application. 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 1mg atropine sulphate IM; a dose repeated two hours later. He was discharged the next day. Relative to a control group of unexposed males his plasma ChE activity was inhibited more than 20% on day eight after poisoning, with recovery at day fifteen. RBC ChE activity was inhibited more than 20% on day 18, but 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 of spraying. This progressed to burning eyes, blurred vision and difficulty breathing, as well as a severe headache persisting for three days. He did not seek any medical advice, but recovered by three days after exposure. Plasma ChE activity was inhibited more than 20% on day ten after the incident, with recovery on day seventeen. RBC ChE activity was inhibited more than 20% on day seventeen after the incident with recovery on day twenty.

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-ethyoxy-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. S,S-TEPP (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 thirty-minute 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 thirty-minute 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 RBC ChE activity was determined twice pre-test, two to four hours and four days after the test. No volunteers reported any effects following exposure. No bodyweight losses observed. Plasma ChE activity was inhibited from 17% to 27% within four hours of test completion, in comparison to pre-exposure values. Activity four days later was returning to normal, with inhibitions of 9 to 14% of pre-exposure values. There was no inhibition of RBC ChE activity detected.

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 three 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 fourth day he suffered a second cardiac arrest and died.

An autopsy was conducted four hours 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 post-mortem 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 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 fifteen minutes, seventeen 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 RBC ChE activity was at the low end of the normal range. Two of these workers developed nausea and vomiting after returning to work two days later. Eight other workers sought advice within 48 hours of exposure, and ChE activity was determined. All of these workers had a follow-up ChE test fifteen days later. In all cases, plasma and RBC ChE activity increased between these tests. If the levels found fifteen days after exposure were taken to be the normal levels for these workers, the mean plasma ChE inhibition seen was 29% and mean RBC 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 fifteen 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 three to five days later. If there is an increase in ChE activity between the tests, a further test should be done three to five 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.

The diazinon dose was assessed by measuring the excretion of the diazinon metabolites DETP and dimethylthiophosphate in the urine of workers, 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 eighteen symptoms possibly related to pesticide exposure.

Seven behavioural tests were completed by each worker tested, both pre- and post-shift. The tests were selected on the basis of their sensitivity, reliability, ease of administration, subject acceptance, and the inclusion of central nervous system functions that are known to be affected by pesticide exposure. The tests are detailed in Table 2.110.

Table 2.110: Behavioural Tests

Test	Psychological 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 nine symbols paired with digits, and is required to enter the correct digit on being presented with a symbol.
Pattern Comparison Test	Visual perception	Three patterns presented, two of which were identical; subject required to select odd pattern.
Pattern Memory Test	Visual memory	Subject presented with pattern for 5 seconds. After three-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 four choices.
Finger Tapping	Motor speed	Subject instructed to tap keys for 10 second with L and R index fingers, then alternate between two 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 then 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 eighteen 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, after they became torn or 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 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 were 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-hour 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 DETP levels ranged from less than

0.1 to 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) was 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, despite the 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 sheep farmer 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 multiple-organ failure and he died two days after hospital admission.

At autopsy, 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 gall bladder 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 centrivenous 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. It is possible that toxic breakdown products of diazinon contributed to this death.

2.3.11.7. 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 in this paper. There were cases affecting fourteen males and eleven females, with ages ranging from 18 months to 60 years, although the majority of cases were aged from fifteen to 24 years. Each had ingested approximately fourteen to 57 g of diazinon (presumable formulation); the exact quantities ingested were not generally known. 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. Autopsy examinations of the two patients who died 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 mention in this report of any changes in the pancreas associated with these cases.

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 over twelve months (October 1964 to Oct 1965) in Ahmedabad, India were reviewed in this paper. There were seventeen males and eight females admitted to the hospital with 21 of the poisoned patients being between ten and thirty years old. Most of the cases ingested less than 21 mL of diazinon; eleven 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) was also seen. Treatment included stomach washing, atropine treatment and airway maintenance. Three patients died despite treatment; no autopsy 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 nineteen-year-old male ingested approximately four ounces (113 g) of Tik-20 (diazinon; formulation not otherwise specified), and was admitted to hospital approximately one to two hours 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) There were 42 males and eighteen females involved, aged between eleven and sixty years. The common clinical signs were vomiting, giddiness, constricted pupils and signs of bronchoconstriction with pulmonary congestion. Urinalysis showed mild albuminuria in four cases and haematuria in ten cases. Five patients died despite treatment, but treatment with atropine was successful in the remaining cases. In all cases where death occurred, the patients had ingested at least fifteen mL of diazinon and treatment had been delayed by more than eight hours after ingestion. Autopsy examination of the patients that died revealed congestion of viscera with or without pulmonary oedema and subendocardial haemorrhage.

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. Autopsy, including 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 (300 μg/g) was higher than that in the liver (80 μg/g), kidney (40 μg/g) or lung (15 μg/g), but all were markedly lower than that in the stomach (7560 μg/g) and small intestine contents (2620 μg/g).

Reichert EF, Yauger 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 thirty minutes 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 three children in this family also presented with acute signs of OP poisoning after eating an oatmeal breakfast. Plasma and RBC ChE activity measured seventeen 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, Yauger 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 successfully treated cases involving intentional ingestion of 25% diazinon were reported in this paper. Patients ingested between sixty and 180 mL of the pesticide formulation (between fifteen and 45 mg diazinon). Four patients were seen at a hospital within thirty minutes while the male who ingested sixty mL did not present at the hospital until five hours 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 whether serum amylase activity was determined in this case. 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 four to ten 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 two 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 post-mortem 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 mental fat (15 mg/g), and the liver (4 mg/g), brain (2 mg/g) and kidney (0.1 mg/g). 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 one hour after consuming ten 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 four 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 two hours, 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 rapid treatment of the case. In the addendum, the authors noted that there had been four 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 one hour after ingesting thirty mL of an unknown poison (later identified as diazinon). Her pupils were small (one 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, two grams of pralidoxine and three mg atropine IV. Plasma ChE activity remained around 100 mIU/mL until day eleven, with atropine therapy required until day seven. Clinical improvement was noted on days eight to nine, which was associated with a slight rise in plasma ChE activity. Plasma ChE activity increased relatively rapidly from day thirteen, returning to normal on approximately day twenty.

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 thirty-year-old female was admitted to hospital one hour 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 hours of treatment (41 mg atropine administered in total). On day three after admission, the patient reported as 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 found to be 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 hours 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 two hours 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 five mg atropine IV every five minutes until the pupils were fully dilated (forty mg atropine within thirty minutes). 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 one hour 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 urination reduced frequency. On the second day of treatment, there were amorphous crystals in the urine that were not identifiable by the hospital laboratory. IV fluids were increased, and treatment with pralidoxime commenced. Amorphous crystalluria persisted until day nine 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 twenty-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 three hours 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 hyper-reflexia but without limb weakness. A case history of diazinon ingestion (unknown quantity) was subsequently obtained. Plasma ChE activity was depressed for four days. After this time the neurologic examinations returned to normal 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 five 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 hypersalivation 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 fifteen minutes. When vital signs stabilised and secretions decreased, the patient was extubated. He recovered without incident and was discharged six days after admission. The plasma ChE activity at the time of admission was 1 IU/mL (normally seven to nineteen 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 one month and eleven 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 hours of the home being sprayed or fogged.

Signs and symptoms on admission included miosis in 73%, hypersalivation in 70%, muscle weakness in 68%, respiratory distress in 59%, lethargy in 54% and tachycardia in 49% of cases. Tonic-clonic convulsions were seen in 22% of cases; this is not a common sign in adults (around 2% was suggested by these authors), but it was felt that hypoxia may have contributed to the increased frequency of this effect, particularly in young children. The difficult of diagnosis in the young child was highlighted, since increased urination and defecation may be difficult to determine where the child is not toilet trained, and 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. RBC ChE was determined in 24/37 cases: in sixteen of these RBC ChE 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 storeroom spilled onto the back of one of the workers. As she changed her clothes, her companion mopped up the spilt pesticide with rags. Approximately three hours later the worker who cleaned up the spill became giddy and had diarrhoea and vomiting. She was admitted to hospital and was frothy at the mouth, cyanotic, tachypnoiec and drowsy. The history of pesticide exposure was not explained, and despite a clear chest x-ray the patient 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 the respirator was removed. Later in the evening she vomited and had epigastric pain. Serum amylase levels were increased approximately ten fold over normal levels. She was treated for acute pancreatitis and recovered, with enzyme levels returning to normal over the next three days.

The second worker developed nausea, vomiting, diarrhoea, abdominal pain and dizziness approximately seven hours after exposure. She was admitted to hospital four hours after first showing signs, and was treated for pesticide poisoning with atropine. Plasma ChE levels was decreased by approximately 75% in comparison to normal values, increasing over the next four 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. In this case it is also possible that toxic breakdown products of diazinon were present in the open bottle at the time of exposure.

Samal KK & Sahu CS (1990) Organophosphorus poisoning and intermediate neurotoxic syndrome. Department of Medicine, Medical College, Burla, India. JAPI 38: 181-182

A twenty-year old male ingested an unknown volume of diazinon, was admitted into hospital six hours 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 one g IV every twelve hours. He was apparently recovering until the second day after admission when he developed difficulty in swallowing and became unable to move any limbs. A neurological examination revealed deficits of function in the sixth and seventh cranial nerves with some effects on the ninth and tenth 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 twenty hours after the first signs of weakness were noted. The patient died of respiratory failure approximately five hours 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 developed vomiting and a runny nose, and the four and a half-year-old child developed 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 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 four months, were reduced relative to the normal range 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, including washing the walls, plasma ChE activity of family members returned to normal apparently normal levels, 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/m^2. The air levels were also high (2.5 μg/m^3). After the cleaning procedure surface levels of diazinon decreased to 0.01-0.08 mg/m^2, and air levels were below the level of detection. The spraying practices of the exterminator company were subsequently

investigated, given the high residual levels still present approximately four 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 authors investigated the occurrence of acute pancreatitis in children who had been exposed to OP or carbamate pesticide, and showed clinical signs of such exposure, including decreased plasma ChE levels. All admitted patients with poisoning signs were treated with atropine and obidoxime chloride. Blood samples were taken for determination of glucose, electrolytes, calcium, urea and creatinine levels, serum amylase and immunoreactive trypsin. Arterial blood gases and plasma ChE levels were also determined in each case.

Seventeen children were involved in the study (59% males), ranging in age from one to eight years. The pesticide was identified in twelve of the patients (parathion – four cases, malathion – three cases, diazinon – two cases, and unspecified carbamates – three cases). One of the children who was known to have been affected with diazinon was diagnosed with pancreatitis, while overall 5 out of the seventeen cases were diagnosed with pancreatitis. Common signs of poisoning included vomiting and diarrhoea. Abdominal pain was also seen; this was severe in two of the five children diagnosed with pancreatitis.

In the five 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 other patients had raised serum amylase levels, suggestive of pancreatic toxicity, but with no abdominal pain or increase in serum trypsin levels. All of these nine patients recovered without incident.

The study demonstrates that acute pancreatitis can occur in children who have ingested OP or carbamate pesticides. The study also indicates that a raised serum amylase level can occur without further 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 the diagnosis of pancreatitis. It was also proposed that the hyperglycaemia seen relatively frequently following OP poisoning may result from damage to the pancreatic endocrine islets.

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 ingestion of 200 mL of a pesticide containing diazinon. On admission he had mild bradycardia, and hypersalivation. 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. The day after admission, his serum creatinine began to rise, peaking on the seventh day. It returned to normal within two 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. Kidneys appeared to be normal on ultrasound examination. The renal failure was not attributable to any nephrotoxic substances or haemodynamic effects, i.e. the pathogenesis was not defined. Previous OP poisoning experiments in rats have indicated that the predominant effect is an increased urinary flow with low osmolality, suggesting there may be a direct effect on tubular function that is 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 twelve-week-old infant girl developed persistent hypertonicity of the extremities, and, at the age of eight months (i.e. after five months), it was discovered that her home had been treated with an excessive and inappropriate application of diazinon three weeks prior to the onset of symptoms (i.e. when the child was nine weeks old). At the time that diazinon was first identified as a possible cause of the condition (six months after diazinon application in the home), the remaining diazinon residue on the floor was 230 ng/cm^2, in comparison to the expected maximum residue level of 38 ng/cm^2, normally detectable immediately after application. Vacuum cleaner dust contained 1700 mg/kg diazinon, while air contained 2.8 ng/m^3. The infant's urine contained 60 ppb diethyl phosphate and twenty ppm diethylthiophosphate, which was calculated to be a diazinon dose of approximately 0.02 mg/kg bw/day. However, there was no depression of ChE level in comparison to normal values for an infant of this age. When the infant was removed from the home muscle tone returned to normal within six weeks and development proceeded normally from this point. Motor abilities and speech were developing normally when the in fact was twenty months old, 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 (later 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 electroconvulsive therapy, which she had previously received without incident. For the therapy, she was anaesthetised and treated with methohexital, *d*-tubocurare, atropine and succinylcholine. Following electroconvulsive therapy, the patient was slow to resume spontaneous ventilation, requiring assisted ventilation for twelve minutes. She remained weak for the next 25 minutes, 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 admission to hospital.

ACRONYMS AND ABBREVIATIONS

Time	
d	day
h	hour
min	minute

Weight	
bw	body weight
g	grams
kg	kilograms
µg	micrograms
mg	milligrams
ng	nanograms
wt	weight

Length	
cm	centimetre
m	metre
µm	micrometre
mm	millimetre

Dosing	
im	intramuscular
IP	intraperitoneal
IV	intravenous
PO	oral

Volume	
L	litre
mL	millilitre
µL	microlitre

Concentration	
ppb	parts per billion
ppm	parts per million
IU	international units

Chemistry	
FOB	Functional Observational Battery
GC	Gas chromatography
HPLC	High pressure liquid chromatography
TLC	Thin layer chromatography

Clinical chemistry, haematology			
A/G	Albumin/globulin ratio	ETU	ethylenethiourea
ALT	Alanine aminotransferase	Hb	Haemoglobin
AP	Alkaline phosphatase	Hct	Haematocrit
AST	Aspartate aminotransferase	LDH	Lactate dehydrogenase
BUN	Blood urea nitrogen	MCHC	Mean corpuscular haemoglobin concentration
CMC	Carboxymethyl cellulose	MCV	Mean corpuscular volume
CPK	Creatine phosphokinase	PT	prothrombin time
DETP	diethylthiophosphate	RBC	red blood cell
DMSO	Dimethyl sulfoxide	WBC	White blood cell/leucocyte
ESO	Epoxidised soybean oil		

Terminology			
ai	active ingredient	LOEL	Lowest Observed Effect Level
ADI	Acceptable Daily Intake	MTD	Maximum tolerated dose
ARfD	Acute Reference Dose	NOAEL	No Observed Adverse Effect Level
AUC	Area under the concentration–time curve	NOEL	No Observed Effect Level
ChE	cholinesterase	OP	organophosphorus
CL	Clearance	OPIDN	organophosphorus induced delayed neuropathy
Cmax	maximum plasma concentration after administration	SCE	sister chromatid exchange

GLP	Good Laboratory Practice	SPF	Specific pathogen free
LC_{50}	Median lethal concentration	Tmax	Time to maximum plasma concentration
LD_{50}	Median lethal dose		

Organisations & publications

APVMA	Australian Pesticides and Veterinary Medicines Authority	JMPR	Joint Meeting on Pesticide Residues
DoHA	Department of Health and Aging	OCS	Office of Chemical Safety
FAISD	First Aid Instructions & Safety Directions	US EPA	United States Environmental Protection Agency
FAO	UN food and agriculture organisation	WHO	World Health Organisation

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