Australian Government

Australian Pesticides and Veterinary Medicines Authority

DICHLORVOS
Toxicology Assessment

The reconsideration of approvals of the active constituent, registrations of products containing dichlorvos and approvals of their associated labels

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The Australian Pesticides & Veterinary Medicines Authority publishes this review report for oral, intramammary and injectable products, which contain dichlorvos and their associated approved labels. For further information about this review or the Chemical Review Program, contact:

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ABBREVIATIONS

Time		**Weight**	
d	Day	bw	Body weight
h	Hour	g	Gram
min	Minute	kg	Kilogram
mo	Month	µg	Microgram
wk	Week	mg	Milligram
s	Second	ng	Nanogram
yr	Year	wt	Weight

Length		**Dosing**	
cm	Centimetre	id	Intradermal
m	Metre	im	Intramuscular
µm	Micrometre	inh	Inhalation
mm	Millimetre	ip	Intraperitoneal
nm	Nanometre	iv	Intravenous
		po	Oral
		sc	Subcutaneous
		mg/kg bw/d	mg/kg bodyweight/day

Volume		**Concentration**	
L	Litre	M	Molar
mL	Millilitre	ppb	Parts per billion
µL	Microlitre	ppm	Parts per million

Clinical chemistry, haematology

A/G	Albumin/globulin ratio
ALT	Alanine Aminotransferase (SGPT)
AP	Alkaline Phosphatase
AST	Aspartate Aminotransferase (SGOT)
BUN	Blood Urea Nitrogen
ChE	Cholinesterase
CPK	Creatine Phosphatase (phosphokinase)
GGT	Gamma-Glutamyl Transferase
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate Dehydrogenase
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Corpuscular Volume
NTE	Neurotoxicity/neuropathy Target Esterase
PCV	Packed Cell Volume (Haematocrit)
PT	Prothrombin Time
RBC	Red Blood Cell (Erythrocyte)
WBC	White Blood Cell/leucocyte
WBC-DC	White Blood Cell – Differential Count

Anatomy

CNS	Central Nervous System
GIT	Gastro-Intestinal Tract

Chemistry

CMC	Carboxymethyl Cellulose
CO_2	Carbon Dioxide
DCA	Dichloroacetaldehyde
DMSO	Dimethyl Sulfoxide
GC	Gas Chromatography
GLC	Gas Liquid Chromatography
HPLC	High Pressure Liquid Chromatography

LSC	Liquid Scintillation Counting
LSS	Liquid Scintillation Spectrometry
MS	Mass Spectrometry
PEG	Polyethylene Glycol
RIA	Radioimmunoassay
TLC	Thin Layer Chromatography
TOCP	Tri-Ortho Cresyl Phosphate

Terminology

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
CI	Confidence Interval
ECG	Electrocardiogram
EEG	Electroencephalogram
gd	Gestational day
GLP	Good Laboratory Practice
LOEL	Lowest Observed Effect Level
MCL	Mononuclear Cell Leukaemia
MRL	Maximum Residue Limit or Level
NOEC	No Observed Effect Concentration
NOEL	No Observed Effect Level
NZW	New Zealand White
OP	Organophosphorus pesticide

Organisations & publications

ACPH	Advisory Committee on Pesticides and Health
APVMA	Australian Pesticides and Veterinary Medicines Authority
CRP	Chemical Review Program
FAO	Food and Agriculture Organisation of the United Nations
FAISD	First Aid Instructions & Safety Directions
IARC	International Agency for Research on Cancer
IPCS	International Programme on Chemical Safety
JECFA	FAO/WHO Joint Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
NCI	National Cancer Institute
NDPSC	National Drugs and Poisons Schedule Committee
NHMRC	National Health and Medical Research Council
NOHSC	National Occupational Health & Safety Commission
NTP	National Toxicology Program
SUSDP	Standard for the Uniform Scheduling of Drugs and Poisons
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

EXECUTIVE SUMMARY

Dichlorvos is an organophosphorus (OP) insecticide used in storage areas in domestic, commercial and industrial premises. Its main food use is for stored grain and potatoes, while it is also applied to animal, green, glass and mushroom houses. Outdoor use includes the treatment of garbage bins, picnic areas and rockeries. Home garden products formulated as peststrips (and ministrips) or solid tablets are available for use in rooms or non-food storage areas such as wardrobes and drawers, or in outside garbage bins.

Dichlorvos was nominated for review under the Australian Pesticides and Veterinary Medicines Authority's (APVMA) Chemical Review Program (CRP) because of its high acute toxicity and concerns over its carcinogenic potential.

The current Acceptable Daily Intake (ADI) for dichlorvos of 0.001 mg/kg bw/d was reaffirmed in the present review. This ADI is based on the No Observed Effect Level (NOEL) of 0.014 mg/kg bw/d in a 28-day human study for plasma cholinesterase (ChE) inhibition at and above 0.021 mg/kg bw/d, and using a 10-fold safety factor. The current Health Value for Australian drinking water should be amended from 0.001 to 0.007 mg/mL to reflect this ADI. The present review identified a suitable acute oral dosing study in humans to allow the refinement of the existing acute reference dose (ARfD) for dichlorvos. The new ARfD of 0.1 mg/kg bw/d was calculated by applying a 10-fold safety factor to the NOEL of 1 mg/kg bw for the inhibition of erythrocyte ChE activity.

No changes to the approval status of dichlorvos have been proposed in this review. However, approval holders should be required to justify on toxicological grounds the existing impurity limit for chloral at 5 g/kg. Based on the results of an inhalational exposure assessment, the registration of Sureguard Pest Strip Household Insecticide (APVMA product code 45596) can no longer be supported as it poses an unacceptable chronic inhalational risk to human health. There is no objection on public health grounds to the continued registration of all other existing dichlorvos products.

The existing poisons schedule for dichlorvos remains appropriate. The review identified a number of additions and amendments to the existing First Aid Instructions and Safety Directions (FAISDs) for Australian dichlorvos products.

Registrants of all dichlorvos products containing a hydrocarbon solvent must provide data on the polycyclic aromatic hydrocarbon content or the mutagenicity index of the chemical. In addition, registrants of all ministrips, should be required to provide data on dichlorvos air levels generated from the use of such products. It would be desirable for approval holders to submit a short-term dermal exposure study for occupational health and safety purposes.

TOXICOLOGY HAZARD PROFILE

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of oral absorption	Almost complete (93-96%), and rapid (T_{max} <0.5 h)
Distribution	All tissues but predominantly concentrated in liver and kidneys
Potential for accumulation	No evidence of accumulation in fat
Rate and extent of excretion	Rapidly excreted in exhaled CO_2 and urine within 24 h
Metabolism	Extensive: transformed to urea, hippuric acid, desmethyl dichlorvos and dichloroethanol glucuronide
Toxicologically significant compounds (animals, plants and environment)	Parent compound

Acute toxicity

Rat oral LD_{50} (mg/kg bw)	46-108
Worst oral LD_{50} in other species	74 (rabbit)
Rat dermal LD_{50} (mg/kg bw)	75-210
Worst dermal LD_{50} in other species	No data
Rat inhalation LC_{50} (mg/m^3)	340-523 (aerosol, droplet size unspecified, 4 h)
Worst inhalation LC_{50} in other species	>218 (mice)
Skin irritation	Slight Irritant
Eye irritation	Moderate Irritant
Skin sensitisation	Sensitiser (humans) Sensitiser (guinea pig maximisation test)

Short-term toxicity

Target/critical effect	ChE inhibition
Lowest relevant oral NOEL (mg/kg bw/d)	0.014 (28-day human study)
Lowest relevant dermal NOEL (mg/kg bw/d)	No data
Lowest relevant inhalation NOEC (mg/m^3)	0.15, highest concentration tested; 5-day human study

Genotoxicity	Not genotoxic *in vivo*. Mutagenic and clastogenic *in vitro*

Long-term toxicity and carcinogenicity

Target/critical effect	ChE inhibition
Lowest relevant NOEL (mg/kg bw/d)	0.008 (2-y dog study)

Carcinogenicity	Evidence of carcinogenicity in gavaged laboratory animals (mouse forestomach tumours).

Reproductive toxicity

Reproduction target/critical effect	Reduced fertility and pregnancy indices, still births, abnormal cycling and reduced pup weights, in the presence of maternotoxicity
Lowest relevant reproductive NOEL (mg/kg bw/d)	2 (rats)

Developmental toxicity

Developmental target/critical effect	None in the absence of maternotoxicity
Lowest relevant developmental NOEL (mg/kg bw/d)	7 (rabbits)#
Delayed neurotoxicity	No evidence of delayed neurotoxicity
Immunotoxicity	No data
Dermal absorption	22-30% at 0.5-30 $\mu g/cm^2$ (rats) – extensive loss of applied material due to evaporation

Summary	NOEL (mg/kg bw/d)	Study	Safety factor
ADI (0.001 mg/kg bw/d) [plasma ChE inhibition]	0.014	Ryder (1967)	10
ARfD (0.1) [RBC ChE inhibition]	1	Gledhill (1996)	10

Health Value in drinking water	Current: 0.001 mg/L Amended: 0.007 mg/L

= highest dose tested

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SUMMARY TOXICOLOGY REPORT

Introduction

Dichlorvos was nominated for review as part of the APVMA's CRP because of its high acute toxicity and concerns over its carcinogenic potential. The current toxicology report consolidates supplementary data received after completion of the OCS's 1999 review. The new data consisted of laboratory animal studies on metabolism, percutaneous absorption, subchronic and chronic toxicity, reproductive and developmental toxicity, genotoxicity, neurotoxicity and forestomach irritation. In addition a number of human volunteer studies not previously evaluated by the OCS were submitted.

Metabolism and Toxicokinetics

The metabolism of radiolabelled dichlorvos in rats, cows and a goat was investigated in a series of experiments performed by Casida et al (1962). Animals were dosed with either [^{32}P-methyl]dichlorvos or [^{14}C-vinyl]dichlorvos via the oral, intravenous or subcutaneous routes. [^{32}P-methyl]dichlorvos was rapidly absorbed and distributed in rats following oral dosing. The distribution reflected rapid metabolism (hydrolysis) to inorganic ^{32}P, which was subsequently incorporated into tissues. The recovery of radioactivity within 7 days in faeces and urine ranged from 68-100% across all species. Urine was the principal excretory pathway in rats following dosing with [^{32}P-methyl]dichlorvos, with mono- and dimethyl phosphate the main urinary metabolites. ^{23}P-metabolites were detected in cow and goat milk, with peak levels detected at 4-12 hours after subcutaneous or intravenous dosing, and at 12-24 hours after oral dosing. In rats dosed orally or intraperitonealy with [^{14}C-vinyl]dichlorvos, the major elimination routes were expired air (16% in 24 hours) and urine (30% in 7 days). Substantial radioactivity remained in the tissues of rats sacrificed 7 days after dosing. More than 90% of administered radioactivity appeared as a conjugate of dichloroethanol in the urine of rats given [^{14}C-vinyl]dichlorvos (probably dichloroethyl glucuronide). A small amount of labelled dichloroacetaldehyde and/or desmethyl dichlorvos (<2% of the total) was evident. Little if any unconjugated dichloroethanol was present and there was no unchanged dichlorvos found in urine.

Hutson et al (1971) investigated the metabolic fate of [^{14}C-vinyl]dichlorvos in rats following oral, inhalational and intraperitoneal exposure. Respired air accounted for the greatest proportion of eliminated ^{14}C and most was recovered within 24 hours following inhalational exposure (head only). After 4 days, the highest tissue levels occurred in the carcass and skin (~38 and 26%, respectively of the total amount of $^{14}CO_2$). Analysis of metabolites following oral dosing with [^{14}C-vinyl]-labelled or [^{36}Cl]-labelled dichlorvos detected free ^{36}Cl and at least 7 metabolites. Identified metabolites included hippuric acid (8.3%), desmethyl dichlorvos (10.9%) and dichloroethanol glucuronide (27%). The presence of dichloroacetaldehyde could not be demonstrated. No parent compound was identified. The majority of ^{14}C in the livers from rats given a single oral dose of [^{14}C-vinyl]dichlorvos was detected in the protein fraction (as glycine and serine). Urinary metabolites detected following intraperitoneal administration of [^{14}C-vinyl]dichlorvos included hippuric acid and desmethyl dichlorvos (2-5%) and dichloroethanol glucuronide (76%). Following inhalational exposure, identifiable urinary metabolites also included hippuric acid (9.3%) and desmethyl dichlorvos (4.3%), and urea (5.3%). No unchanged dichlorvos was found in the urine following dosing by either the oral or inhalational routes.

In a series of experiments, [^{14}C-vinyl]dichlorvos was given by gavage to male and female mice (0.2 mg, approximately 8 mg/kg bw), and Syrian hamsters (0.22-0.56 mg, approximately 4 mg/kg bw), and to 1 man orally (5 mg in 100 mL orange juice, approximately 70 μg/kg bw) and another by inhalation (38 mg/m^3 unlabelled dichlorvos for 105 min). Elimination rates and urinary excretion were compared with results obtained from a second experiment using rats. Overall, the data indicated that the rates and routes of elimination were largely similar (predominantly air and urine) in all species and there was no marked sex difference in laboratory animals. Following oral dosing of mice and rats with [^{14}C-methoxy]dichlorvos, the major urinary metabolite was dimethyl phosphate (~70% of urinary radioactivity and 40% of the dose). Desmethyl dichlorvos constituted 4% of total urinary radioactivity in rats and 28% in mice. Minor metabolites present at less than 2% of total urinary radioactivity were S-methyl-L-cysteine (both species), S-methyl-L-cysteine oxide and methylmercapturic acid (mouse only), and methylmercapturic acid S-oxide (rat only). Two other minor chromatographic peaks could not be identified. Following oral dosing with [^{14}C-vinyl]dichlorvos hippuric acid, desmethyl dichlorvos and urea were detected in the urine of mice and the man, with only hippuric acid measured in hamsters. A dichloroethanol conjugate in the urine of the man exposed to dichlorvos by inhalation was detectable but not quantifiable because of interference from endogenous peaks. (Hutson and Hoadly 1972a & b)

Blair et al (1975) examined the tissue distribution of dichlorvos in mammals following inhalational or intravenous administration. Rats were exposed to dichlorvos vapour at 0.05 or 0.5 mg/m^3 (in open chambers for 14 days), 50 mg/m^3 for 2-4 h, or 10 or 90 mg/m^3 for 4 h (head-only exposure). In addition, two male human subjects were exposed in a 20 m^3 chamber to atmospheres containing 0.25 mg/m^3 dichlorvos for 10 h or 0.7 mg/m^3 for 20 h. A separate group of rats was given a single intraperitoneal injection of 0.83 mg/kg bw dichlorvos. No dichlorvos was detected in either of the two male subjects. At low concentrations (<1.0 μg/g) recovery of dichlorvos from blood and tissues was difficult because the compound was rapidly degraded. In rats and mice inhalationally exposed to 90 mg/m^3 dichlorvos for 4 h, low blood and tissue concentrations were detected. The highest levels were detected in the kidneys (0.54 μg/g in female mice to 2.39 μg/g in male rats). Concentrations of approximately 1 μg/g were detected in the fat of male mice but were lower in female mice and in rats. Low concentrations (generally <0.2 μg/g) were detected in blood, liver, testes, lung and brain of rats and mice. No dichlorvos was found in tissues of rats exposed to 10 mg/m^3 for 4 h, or in blood or tissues exposed to 0.05 or 0.5 mg/m^3 for 14 days. Following intravenous injection, little or no dichlorvos was found in rat liver, fat, testes or brain. Dichlorvos was detected in blood in only one of three rats at 10 and 30 min postdose. Overall, the results indicated that dichlorvos was rapidly metabolised *in vivo*.

Cheng (1989 & 1991) examined the metabolism and tissue distribution of [vinyl-1-^{14}C] dimethyl dichlorovinylphosphate (^{14}C-dichlorvos) in rats. Five rats/sex/group were administered ^{14}C-dichlorvos as a single intravenous dose of 1.0 mg/kg bw, a single gavage dose of 0.8 or 21.0 mg/kg bw, or 15 daily oral gavage doses of 0.8 mg/kg bw unlabelled dichlorvos followed by a single radiolabelled dose of 0.8 mg/kg bw on the 16th day. The control group consisted of 2 untreated rats. The major route of excretion was via exhaled CO_2 (40-58%) followed by the urine (10-15%) and faeces (4-7%). Excretion was rapid, occurring within the first 24 hours after dosing. Marked levels of ^{14}C (13-26%) remained in the carcass seven days after dosing. Relatively low levels of ^{14}C were found in other tissues such as the liver (3.5-4.8%), blood (0.3-0.5%) and kidneys (0.2-0.5%). Excretion and tissue distribution appeared to be independent of dose route and sex. Several radioactive metabolites were detected in urine, including urea (19-33%) and hippuric acid (3.78-19.5%). In faeces, numerous uncharacterised minor metabolites were detected in addition to hippuric acid (<6%) and urea (2.7-29.7%). A number of other urinary and faecal components were detected but were unable to be identified due to their low concentrations and/or volatility.

Percutaneous absorption

^{14}C-dichlorvos (in water) was applied to the shaved backs of 12 rats/dose at 3.6, 36 or 360 μg/animal (rates of 0.5, 3 and 30 μg/cm^2, respectively). The application site was 12 cm^2 and the total volume applied to each rat was 100 μL. A substantial proportion of ^{14}C-dichlorvos (38-55%) evaporated from the skin surface following application. The total level of dermal absorption was 22-30% and was consistent over time and dose (0.5-30 g/cm^2). Absorption occurred within the first 10 hours of sample application, with the actual amount of ^{14}C-dichlorvos absorbed increasing with dose. The excretion routes were via exhaled air (CO_2) (2.2-4.9%), urine (1-2%) and faeces (0.1-0.6%). Blood levels decreased over time. Analysis of the stability of the test material lacked transparency and was somewhat difficult to follow in the study report. (Jeffcoat 1990).

Acute Studies

Active constituent

In lethal-dose studies, oral LD_{50} values in rats range from 46-108 mg/kg bw, and dermal LD_{50} values from 75-210 mg/kg bw. The oral LD_{50} in rabbits and dogs is 74 and 100-316 mg/kg bw, respectively. The LD_{50} in mice following intraperitoneal administration is 24 mg/kg bw. In rats, the inhalational LC_{50} was >206 mg/m^3 for vapour exposures and in the range of 340-523 mg/m^3 for aerosol exposures. The effects of acute dichlorvos intoxication were consistent with those seen for other OP insecticides, and included exophthalmia, prostration, lacrimation, salivation, tremors, spasms, altered gait, and death. Dichlorvos was a slight skin irritant and a moderate eye irritant in rabbits (Pauluhn 1985). Dichlorvos was a skin sensitiser in both humans (patch-test) and guinea pigs (maximisation test) (Ueda 1994).

When a single dose of dichlorvos of between 0.1 to 80 mg/kg bw was administered to rats in an acute range-finding study, the estimated time to peak effect ranged from 15-45 minutes after dosing. The predominant clinical signs included gait alterations, whole body tremors, reduced limb grasp, constricted pupils and exophthalmus. All rats other than that at 80 mg/kg bw which died, had recovered by 24 hours. (Lamb 1992)

Products

There were no acute toxicity studies submitted on any of products registered in Australia.

Short-Term Repeat-Dose Studies

Oral administration - rats

In a range-finding study, dichlorvos was administered orally by gavage to 5 female rats/group at 0, 0.1, 10 or 20/40 mg/kg bw/d for up to 7 days. At 40 mg/kg bw/d, one rat died on the first day of dosing, with the 4 remaining rats appearing hypoactive and displaying tremors. Consequently, this dose was reduced to 20 mg/kg bw/d following a 2-day washout period. Rats from the control and 0.1 mg/kg bw/d groups appeared normal. Plasma and RBC ChE activities were significantly lower than the control group at and above 10 mg/kg bw/d. The level of inhibition was approximately 73% for plasma ChE activity and 30% for RBC ChE activity. (Kleeman 1988a)

Inhalational administration –Dogs, cats and rabbits

Dogs, cats and rabbits were continuously exposed to dichlorvos via the air for 8 weeks. The dichlorvos was generated from two commercial resin strip formulations containing 20% dichlorvos. Mean dichlorvos air levels ranged from 0.05 to 0.3 µg/L (mg/m^3) after equilibrium was reached within 3 days. No effects were observed on the general health of animals or on electroencephalogram (EEG) recordings obtained from selected animals of each species. Plasma and RBC ChE activities, determined in blood samples taken weekly, showed no treatment-related effects. (Walker et al 1972)

Subchronic Studies

Rats

Dichlorvos was administered orally by gavage to 10 rats/sex/group over 5 days/week for 13 weeks at 0, 0.1, 1.5 or 15 mg/kg bw/d. The NOEL was 0.1 mg/kg bw/d, based on the inhibition of plasma ChE activity in males at week 7, and the inhibition of RBC ChE activity in both sexes at week 7 and at termination (week 14), at 1.5 mg/kg bw/d. At the highest dose of 15 mg/kg bw/d, inhibition of plasma, RBC and brain ChE activities, and clinical signs (salivation, presence of urine stains), occurred. There was slight anaemia in males at and above 1.5 mg/kg bw/d, and in females at 15 mg/kg bw/d. Kidney tubular mineralisation in females and an equivocal occurrence of hyperplasia and pigmentation of the pancreatic islet cells in males occurred at 15 mg/kg bw/d. (Kleeman 1988b)

Dogs

Dogs (3/sex/group) were dosed orally with dichlorvos in gelatine capsules for 90 days at 0, 0.3, 0.9 or 1.4 mg/kg bw/d. As no physiological signs of toxicity were evident, dosing of the 0.3 mg/kg bw/d group was increased to 3.0 mg/kg bw/d and an additional group of 3 dogs/sex were dosed at 0.3 mg/kg bw/d. All dogs at 3.0 mg/kg bw/d and 2/6 receiving 1.4 mg/kg bw/d showed signs of increased excitation, aggression and urinary output, indicating autonomic stimulation. However, no effects on pupil size or response to light, muscle tone or muscle fasciculations were noted. There were no deaths or treatment-related effects on bodyweight gain or food consumption. Although there was considerable variability in plasma and RBC ChE activities, a dose-dependent suppression in mean ChE activity was evident. There were no treatment-related effects on haematology or clinical chemistry parameters, or on gross pathology and organ weights. The NOEL was 0.3 mg/kg bw/d based on the inhibition of RBC and plasma ChE activities at and above 0.9 mg/kg bw/d, whilst brain ChE activity was only inhibited at 3.0 mg/kg/d. (Hine 1962)

Chronic Studies

Oral administration

Mice

Dichlorvos was admixed in the diet and fed to groups of 50 mice/sex at 0, 1000 or 2000 ppm, with these levels decreased to 300 and 600 ppm after two weeks as the initial concentrations were poorly tolerated. Treatment continued for a total of 80 weeks and all surviving mice were sacrificed for necropsy after an additional 12-14 weeks without treatment. Time-weighted average dietary concentrations of dichlorvos were 318 and 635 ppm (equivalent to a calculated intake of 47.7 and 95.3 mg/kg bw/d, respectively). Clinical signs (tremors, rough coat, diarrhoea and poor general appearance) occurred during the first 2 weeks of treatment at the higher doses. The only clinical sign noted after the first weeks were alopecia and rough coats, particularly in males, which developed from approximately week 20. Survival rates were

unaffected by treatment. The only noteworthy neoplastic lesions were squamous cell carcinoma of the oesophagus in low-dose males (1/47) and high-dose females (1/41), and a single oesophageal papilloma observed in high-dose females (1/41). Epithelial hyperplasia of the oesophagus was evident in 3/47 low-dose males but not in any other groups. Based on historical control data, the lack of a dose-response relationship and the lack of statistical significance, the low incidence of these forestomach lesions was considered to show an equivocal relationship with treatment. The LOEL was 48 mg/kg bw/d (the lowest dose tested), based on the occurrence of clinical signs (alopecia and rough hair coats) and oesophageal hyperplasia (males). (NCI 1977)

Mice were given freshly prepared drinking water *ad libitum* containing 0, 400 or 800 mg/L dichlorvos for 102 weeks (estimated oral doses of 56 and 112 mg/kg bw/d, respectively). There was no treatment-related effect on mortality but there was a dose-dependent decrease in bodyweight gain. The main tumour types observed were lung adenomas and tumours of the liver, spleen, thymus and salivary gland, which occurred in all three groups. There was no statistically significant difference in tumour incidences at any site in any group. (Konishi et al 1981) *[This study was not evaluated by the OCS. The text is based on the 1989 IPCS monograph on dichlorvos (EHC 79)]*

Dichlorvos in water was administered by oral gavage to mice at 0.2 mg/animal either 2- or 3-times per week for 50 weeks followed by a 60-week observation period. These doses were equivalent to 2.9 and 4.3 mg/kg bw/d, respectively. Control mice were either untreated or gavaged with water 3-times per week. Survival was unaffected by treatment. There was a marginal increase in the incidence of focal hyperplasia of the bladder in treated mice compared to the controls (5-12% *versus* 0-9%), which was not considered treatment-related. There were no other non-neoplastic lesion, and no neoplastic lesions, which could be attributed to treatment. While this study had limited regulatory value due to the poor study design and lack of reporting detail, it indicated that chronic oral administration of dichlorvos to mice was not carcinogenic. (Horn et al 1987)

Dichlorvos (in corn oil) was administered by oral gavage to mice at 0, 10 or 20 mg/kg bw/d (males) or 0, 20 or 40 mg/kg bw/d (females) 5 days/week for 103 weeks. ChE activity was analysed in a satellite study using the same mouse strain and route of administration at doses of 0, 5, 10, 20 or 40 mg/kg bw/d, 5 d/wk for 1 month. Plasma ChE activity was significantly and dose-dependently reduced in both sexes, by 50% at 5 mg/kg/d to 85% at 40 mg/kg/d. No marked effects on RBC ChE activity were evident at any dose level in either sex. Non-neoplastic lesions occurred with similar frequency in all groups and no effects of treatment on incidences were observed. The only treatment-related increase in tumour incidence was of the forestomach squamous epithelium. Forestomach squamous cell papillomas were significantly elevated in high-dose males and females (10 and 36%, respectively, compared to the control incidences of 2 and 10%, respectively) above the historical control range of approximately 0-4%. At 40 mg/kg bw/d, forestomach carcinomas were evident in 2/50 females (4%) but not in any other group. This finding was also above the historical control value but was considered to show an equivocal relationship with treatment. There was no significant effect on the incidence of forestomach hyperplasia (a lower grade lesion on the morphological, proliferative continuum leading to papilloma). The LOEL following dietary administration to mice for one month was 5 mg/kg bw/d, based on the inhibition of plasma ChE activity in the satellite study. The NOEL for forestomach lesions (specifically papillomas) following chronic dietary administration was 10 mg/kg bw/d for males and 20 mg/kg bw/d for females. The occurrence of forestomach carcinomas in females at 40 mg/kg bw/d showed an equivocal relationship with treatment. Mechanistic studies discussed in Section 12, demonstrate that dichlorvos is irritating to the mouse GIT, as it is to rabbit eyes and skin. Given its irritancy and consistently negative results in *in vivo* genotoxicity studies, the increase in forestomach papillomas seen in this study are concluded to be irrelevant to human dietary risk assessment. (Chan 1989)

In a study by Horn et al (1990), dichlorvos was administered to mice by oral gavage 3 times/week at 0.2 mg/mouse (~4.3 mg/kg bw/d) in conjunction with a subcutaenous injection of 50 µg N-nitrosodiethylamine. Mice were treated for 50 weeks and then observed for up to 110 weeks. There was no evidence that dichlorvos was co-carcinogenic. *[This study was not evaluated by the OCS. The text is based on the 1993 JMPR evaluation report on dichlorvos]*

Rats

Dichlorvos was admixed in the diet and fed to rats at 0, 0.1, 1, 10, 100 or 500 ppm for 2 years (equivalent to 0, 0.005, 0.05, 0.5, 5 and 25 mg/kg bw/d). Dietary analysis revealed considerable loss of dichlorvos concomitant with a gradual increase in the concentration of DCA. There were no treatment-related mortalities, clinical signs or effect on bodyweight gain. There were no perturbations in haematology or urinary parameters. At 100 ppm, plasma and RBC ChE activities were reduced to 50-90% of controls, while at 500 ppm, they were reduced to 20-70%. Brain ChE activity was significantly decreased only at 500 ppm, by 45-47% in rats sacrificed after 6 months, declining to 5-15% in those sacrificed after 24 months. There

was no effect on organ weights and no treatment-related macroscopic lesions. Histopathology revealed hepatocellular fatty vacuolisation in all rats at 500 ppm and in approximately 80% of females and 62% of males at 100 ppm, after 18-24 months of treatment. No treatment-related effects were observed on the incidence or timing of tumours. The NOEL was 10 ppm (mean analytical concentration of 4.67 ppm; equivalent to 0.23 mg/kg bw/d) based on the inhibition of plasma and RBC ChE activities at and above 100 ppm (mean analytical concentration of 46.7 ppm; equivalent to 2.3 mg/kg bw/d). (Witherup et al 1967)

Dichlorvos was admixed in the diet and fed to rats at 0, 500 or 1000 ppm (equivalent to 25 and 50 mg/kg bw/d, respectively, using a dietary conversion factor of 20). As 1000 ppm was poorly tolerated it was reduced to 300 ppm after 3 weeks (equivalent to 15 mg/kg bw/d). The low-dose was also reduced to 150 ppm to comply with the study protocol (equivalent to 7.5 mg/kg bw/d). Treatment continued for a total of 80 weeks followed by a 30-week recovery period. Cholinergic signs were evident at 1000 ppm during the first 3 weeks of treatment but did not occur when the dose was reduced to 300 ppm. Alopecia, epistaxis, haematuria, dark urine, palpable masses, and abdominal distension developed in control and treated groups as the study progressed, increasing in incidence in the treated groups and predominating in high-dose females. Average body weights of high-dose males and females were consistently lower than controls during the treatment phase, recovering during the post-treatment period. There was no obvious effect of treatment with dichlorvos on non-neoplastic proliferative or inflammatory lesions, and there were no treatment-related effects on the total number or time of onset of neoplastic lesions. A significant dose-related increase in malignant fibrous histiocytoma occurred in males at 7.5 and 15 mg/kg bw/d. The relevance of this neoplasm to the hazard assessment of dichlorvos is unclear as other studies using different rat strains and higher doses failed to cause a similar effect. (NCI 1977)

Rats were given freshly prepared drinking water *ad libitum* containing 0, 140 or 280 mg/L dichlorvos for 104 weeks (estimated oral doses of 14 and 28 mg/kg bw/d, respectively). Slight inhibition of body weight gain was observed in high-dose males, but there was no effect on mortality. The overall tumour incidences were 100, 96 and 98% in males and 37, 31 and 33% in females in the control, low dose and high dose groups, respectively. High incidences of interstitial cell tumours of the testes were seen in males across all groups without dose-dependency (49/51, 41/48 and 47/48 in control, low- and high-dose groups). Mononuclear cell leukaemia was found in all groups at 4-12%. There was no statistically significance difference in tumour incidence at any site in any group. (Enomoto 1981) *[This study was not evaluated by the OCS. The text is based on the 1989 IPCS monograph on dichlorvos (EHC 79)]*.

Rats were dosed with 0.1 mg dichlorvos in 0.2 mL water by oral gavage 2 or 3 times per week for 60 weeks (doses were equivalent to 0.07 and 0.11 mg/kg bw/d, respectively). Thereafter, rats were observed for another 51 weeks before they were sacrificed for necropsy. Mortality was unaffected by treatment. There was a marginal increase in focal hyperplasia of the forestomach at both doses and in both sexes, with forestomach papillomas also detected in a small number of rats at the high dose. These findings were not statistically significant and did not follow a dose-response relationship (recognising that the latter was possibly the result of the closeness of the dose selection). While the occurrence of hyperplasia and papillomas are consistent with studies conducted in mice, in the absence of suitable historical control data for this particular rat strain, the current findings are considered equivocal. There was an apparent dose-related increase in focal oval cell or bile duct proliferation males, which was statistically significant at the high dose. However, the interpretation of this finding was complicated by the lack of suitable historical control data. The incidence of focal bladder hyperplasia was increased in males, but was not statistically significance. No neoplastic lesions were detected that could be attributed to treatment. While this current study had a number of deficiencies, which limited its regulatory value, such as the low and poor dose selection and lack of reporting detail, it indicated that dichlorvos is unliklely to be carcinogenic in this particualr rat strain. The occurrence of proliferative lesions of the forestomach and bile duct showed an equivocal relationship with treatment. (Horn et al 1988)

Rats were given dichlorvos (in corn oil) by oral gavage at 0, 4 or 8 mg/kg, 5 days per week for 103 weeks. Plasma and RBC ChE activities were analysed in a separate experiment where 10 mice/sex/dose were dosed orally by gavage at 0, 2, 4, 8 or 16 mg/kg bw/d 5 times per week for 37 days. Mild diarrhoea was reported to be treatment-related but there were no other adverse clinical signs. RBC ChE activity was slightly (<20%) but significantly reduced in males at and above 4 mg/kg bw/d but was unaffected in females. Plasma ChE was significantly reduced (>30%) in both sexes at all doses. Cytoplasmic vacuolisation of the liver and adrenal cortex was observed at increased frequency in males. Adrenal cortical cytoplasmic vacuolisation was also observed in both sexes. Non-neoplastic and neoplastic pancreatic lesions were observed in both sexes. The incidence of pancreatic adenomas was significantly higher in males at both doses compared to the concurrent control. However, the interpretation of this finding was complicated by the high background incidence in the concurrent control (which was significantly greater than the mean historical control incidence) and the large historical control range (0-28%). A further complication is the fact that corn oil gavage is known to increase the incidence of acinar cell adenomas in

male rats (Haseman 1985). Therefore, the occurrence of pancreatic acinar adenomas was considered to show an equivocal relationship with treatment. The increased incidences of MCL in males and mammary tumours in females were not considered biologically significant due to the lack of a clear dose-response relationship and as the incidences fell within the NTP's respective historical control range. No NOEL was established in this study because plasma ChE activity was inhibited at every dose. Overall, dichlorvos was considered to show equivocal evidence of carcinogenicity in rats. (Chan 1989)

Dogs

Dichlorvos was admixed in the diet and administered to 3 dogs/sex/group at 0, 0.1, 1.0, 10.0, 100 or 500 ppm for 2 years (equivalent to 0, 0.0025, 0.025, 0.25, 2.5 and 12.5 mg/kg bw/d, respectively). Volatilisation reduced the concentration of dichlorvos present in the feed. There was dose-dependent depression of RBC ChE activity in both sexes, particularly during the earlier periods of treatment. In males, this was evident at 10-100 ppm and in females at 100-500 ppm, with RBC ChE activity reduced by 50%-90% in both sexes over these concentrations. Plasma ChE followed a similar trend but was only depressed in dogs from the 100 and 500 ppm groups. ChE activity had returned to pretreatment levels at the end of the 2-year treatment period. Absolute and relative liver weights in male dogs of the 100 and 500 ppm group and females of the 500 ppm group were marginally higher than those of controls. This was reported as of 'borderline significance' but no details of statistical methodologies were provided. Rarefaction of the cytoplasm of hepatic cells, with cellular enlargement and thickening of the cell membrane, increased in incidence and severity with dose; graded as slight in 1/3 females at 10 ppm and 1/3 males and 3/3 females at 100 ppm, and as moderate in all 500 ppm animals. Based on the inhibition of RBC ChE activity in males at and above 3.2 ppm (10 ppm group), the NOEL was 0.32 ppm, equivalent to approximately 0.008 mg/kg bw/d. (Jolley et al 1967)

Dichlorvos was administered orally to 4 dogs/sex/group in gelatine capsules at 0, 0.05 (0.1 for the first 3 weeks of the study), 1.0 or 3.0 mg/kg bw/d for 52 weeks. The NOEL was 0.05 mg/kg bw/d based on the inhibition of plasma and RBC ChE activities at and above 1.0 mg/kg bw/d. There was an increase in the incidence of emesis in males at 3.0 mg/kg bw/d predominantly due to a single animal. Classic cholinergic signs were only observed when one of the high-dose males was inadvertently overdosed. (Markiewicz 1990)

Inhalational exposure

Rats

Rats were exposed to dichlorvos by inhalation for at least 23 h/d for 2 years at mean analytical concentrations of 0, 0.05, 0.48 or 4.70 mg/m^3. Trimethyl phosphate was also present, at 0, 0.007 and 0.04 mg/m^3 in the low-, mid- and high-dose atmospheres, respectively, as was DCA at 0.007, 0.013 and 0.028 mg/m^3, respectively. The method of exposure meant that the rats were exposed dermally, orally (via contamination of food and water, cage interior and grooming fur), as well as by inhalation. Body weight gain was slightly but significantly reduced in both sexes at the highest dose, with terminal body weight depressed by 10-13%. The low survival in controls caused the early cessation of treatment in males during week 99; female treatment continued to week 104. At termination, there was a slight though significant increase in plasma aspartate amino transferase (AST) and alanine amino transferase (ALT) activities and decreased chloride concentrations in high-dose males. Mean plasma and RBC ChE activities were dose-dependently and significantly reduced at the mid- (17-32%) and high-dose (78-96%) in both sexes. RBC ChE activity was also slightly but significantly reduced in low dose females (12%). In brain tissue, ChE activity was reduced by approximately 10 and 80% in mid- and high-dose rats, respectively. This study showed no evidence for carcinogenicity at dichlorvos air levels up to 4.70 mg/m^3. The authors reported that test atmospheres of 5 mg/m^3 resulted in a retained dose of 0.5 mg/d in rats when only the head was exposed. The estimated daily ingestion of dichlorvos was 6.2 mg via food, 3.6 mg via grooming and less than 0.1 mg via drinking water (percutaneous absorption was unknown). Thus, the actual daily intake of dichlorvos was estimated at be approximately 10 mg/rat, or 25 mg/kg bw/d at the highest dose. The NOEC was 0.05 mg/m^3 (approximately equal to a NOEL of 0.25 mg/kg/ bw/d), based on the inhibition of plasma and RBC ChE activity at 0.5 mg/m^3. (Blair et al 1974 & 1976)

Reproduction Studies

Rats

Dichlorvos was administered to rats via the drinking water at 0, 5.0, 20.0 or 80.0 ppm (approximately equal to 0, 0.5, 2 and 8 mg/kg bw/d, respectively) for 2 parental generations of animals and their offspring

throughout all phases of the study. Due to the low breeding performance of F1 rats, females were subjected to a 3-week prebreed vaginal cytology examination to examine oestrous cyclicity and then remated with a separate group of untreated males. In addition, F1 males were subjected to a more extensive assessment for reproductive toxicity. Water consumption (g/kg bw/d) was significantly reduced in parental males and females during the various phases of the study by up to 20%. It was unclear whether this finding was due to compound-related toxicity or to the reduced palatability of the drinking water. The NOEL for parental toxicity was 5 ppm (~0.5 mg/kg bw/d) based on the occurrence of toxicologically- and statistically-significant inhibition of plasma, RBC and brain ChE activities at 20 ppm (~2 mg/kg bw/d). The NOEL for pup toxicity was 20 ppm (~2 mg/kg bw/d) based on lower pup weights in the F1 and F2 generations at 80 ppm (~8 mg/kg bw/d). The NOEL for reproductive toxicity was 20 ppm (~2 mg/kg bw/d) based on reduced fertility and pregnancy indices, increased stillbirths in the F2 generation, and abnormal cycling in F1 maternal rats, at 80 ppm (~8 mg/kg bw/d). (Tyl et al 1992 & 1993)

Developmental Studies

Mice

Mated mice were dosed by vapour inhalation for 7 hours/day or by oral gavage (in corn oil), during gestational (gd) days 6-15. Mean inhalational exposure levels were 4.06 ($\pm$0.63) mg/m^3, and oral doses were intended to be the maximum tolerated dose for the species (60 mg/kg bw/d). There were no adverse clinical signs in animals exposed orally or by inhalation. Body weight gain was significantly depressed compared to controls on day 16 (but not gd 10 or 18) in mice given 60 mg/kg bw/d dichlorvos by gavage, but bodyweight was unaffected following inhalational exposure. There were no significant differences between treated and control groups in the number of live foetuses, resorptions, foetal weight and length, or sex ratio, or in the incidence and type of visceral or skeletal anomalies or malformations. (Schwetz et al 1979)

Rats

A developmental toxicity study was conducted using up to 15 pregnant rats per group, which were inhalationally exposed to dichlorvos at 0, 0.25, 1.25 or 6.25 mg/m^3 from gd 0-20. Exposure was for 23 hours/day, 7 days/week. There were no treatment-related effects on pregnancy rates, the number of resorptions, foetal deaths, live litter sizes and foetal weights. There were no visceral or skeletal abnormalities that were attributable to dichlorvos. The NOEL for maternal toxicity was 0.25 mg/m^3, based on the inhibition of plasma, RBC and brain ChE activities at and above 1.25 mg/m^3. The NOEL for foetal and developmental toxicity was 6.25 mg/m^3, the highest dose tested. (Thorpe et al 1971)

Dichlorvos (in water) was administered by oral gavage to timed-pregnant rats (25/group) on gd 6-15 at 0, 0.1, 3.0 or 21.0 mg/kg bw/d in water. There was no evidence that dichlorvos was teratogenic. The NOEL for maternal toxicity was 3.0 mg/kg bw/d, based on the occurrence of cholinergic signs (tremors and prone positioning) and reduced bodyweight and food consumption at 21.0 mg/kg bw/d. Tremors were reported to occur within 10-60 minutes of dosing. The NOEL for foetal and developmental toxicity was 21.0 mg/kg bw/d, the highest dose tested. (Tyl et al 1990a)

Rabbits

Mated female Dutch rabbits (up to 20/group) were exposed to atmospheres of dichlorvos at 0, 0.25, 1.25 or 6.25 μg/L (equivalent to 0, 0.25, 1.25 and 6.25 mg/m^3, respectively) from gd 7-19. In a second experiment, mated female rabbits were exposed to 0, 2 or 4 μg/L dichlorvos (equivalent to 0, 2 and 4 mg/m^3, respectively). Exposure was for 23 hours per day, 7 days per week. Mortalities occurred at and above 2 mg/m^3 and these were preceded by clinical signs (anorexia, lethargy, muscle tremors, mucous nasal discharge and diarrhoea). Toxicologically- significant inhibition of plasma, RBC and brain ChE activity occurred at and above 1.25 mg/m^3 in maternal rabbits. Dichlorvos was not teratogenic. The NOEL for maternal toxicity was 0.25 mg/m^3, based on the inhibition of plasma, RBC and brain ChE activities at and above 1.25 mg/m^3. The NOEL for foetal and developmental toxicity was 2 mg/m^3, based on the reduction in average foetal weight at 4.0 mg/m^3. (Thorpe et al 1971)

Rabbits were given dichlorvos (in corn oil) by oral gavage at 0 or 5 mg/kg bw/d, or exposed to dichlorvos vapour at 4 mg/m^3 for 7 h/d, during gd 6-18. There were no adverse clinical signs and no effects on bodyweight gain. No difference in the incidence of litters with resorptions was noted, however there was a 3-fold increase in the number of resorptions/litter following oral dosing, which was not statistically significant. There were no significant differences between treated and control groups in the number of live foetuses, foetal weight and length, or sex ratio, or in the incidence and type of visceral or skeletal

anomalies or malformations. Therefore, an oral dose of dichlorvos at 5 mg/kg bw/d or an inhalational exposure of 4 mg/m^3 was without maternal or developmental toxicity (Schwetz 1979).

Dichlorvos (in water) was administered by oral gavage to artificially inseminated rabbits (16/group) on gd 7 to 19 at 0, 0.1, 2.5 or 7.0 mg/kg bw/d. The NOEL for maternal toxicity was 0.1 mg/kg bw/d, based on the occurrence of mortalities at and above 2.5 mg/kg bw/d. Cholinergic signs and reduced food consumption occurred at 7.0 mg/kg bw/d. The NOELs for foetotoxicity and developmental toxicity were 7.0 mg/kg bw/d, the highest dose tested. There was no evidence that dichlorvos is teratogenic. (Tyl et al 1990b)

Genotoxicity Studies

Dichlorvos has been extensively tested for genotoxicity in a variety of *in vitro* and *in vivo* assays, with the majority of findings published in the open scientific literature. In the current submission, six unpublished genotoxicity studies were evaluated. Dichlorvos was mutagenic in the L5178Y TK+/- mouse lymphoma forward mutation assay in the presence and absence of metabolic activation. However, the mutation frequency in the absence of metabolic activation was approximately 2-fold higher than in the presence of metabolic activation (Ford et al 1986). A forward mutation assay conducted in *E.coli* WP2 suggested that dichlorvos was not mutagenic, however, the study report lacked adequate detail to allow an independent assessment of the findings (Dean 1971). *In vivo* studies indicated that dichlorvos was negative in the mouse dominant lethal assay (Ford et al 1985a; Ford & Killeen 1987), negative for cytogenicity in mouse bone marrow and sperm (Putman & Shadly 1992), did not induce sister chromatid exchanges (SCE) in mice (Putman 1985) and was negative in the mouse micronucleus test (Ford et al 1985b).

Neurotoxicity Studies

Hens

In an acute delayed neurotoxicity study, a single oral gavage dose of dichlorvos (in water) was administered to 10 fasted domestic chickens/group at 0 or 16.5 mg/kg bw. A positive control group was administered a single oral gavage dose of 600 mg/kg bw tri-o-tolyl phosphate (TOCP) in corn oil. All birds treated with dichlorvos were given a single intramuscular injection of 5 mg/kg bw atropine at the time of dosing, and thereafter at 2 mg/kg bw as required. Dichlorvos caused cholinergic signs and significantly reduced food consumption. Histopathological examination revealed sciatic nerve degeneration in one hen in the absence of any clinical signs of delayed neurotoxicity. It was equivocal whether this finding was attributable to dichlorvos treatment. Deficiencies noted in this study were the absence of statistical analysis and macroscopic examination. Furthermore, no biochemical analysis was performed on any birds, such as the measurement of neuropathy target esterase (NTE) or brain ChE activity. (Beavers et al 1988)

In a 28-day repeat dose neurotoxicity study, dichlorvos (in water) was administered orally by gavage to 12 adult female domestic hens per group at 0, 0.3, 1.0 or 3.0 mg/kg bw/d for 28 days. An additional group of birds was dosed at 0.1 mg/kg bw/d for measurement of brain ChE analysis. A positive control group was dosed with 7.5 mg/kg bw/d TOCP in corn oil. The NOEL was 0.1 mg/kg bw/d, based on the inhibition of brain ChE activity at and above 0.3 mg/kg bw/d. Cholinergic signs occurred at and above 1.0 mg/kg bw/d. There was no evidence that dichlorvos caused delayed neurotoxicity. A number of supplementary studies were undertaken to investigate the questionable ability of TOCP to induce delayed neurotoxicity, given the absence of clinical signs in the positive control group. This was subsequently attributed to a substandard batch of TOCP. The absence of statistical analysis and the results of the locomotor assessment were deficiencies of this study. (Redgrave et al 1994a & b; Redgrave & Mansell 1994; Jortner 1994; Hardisty 1998)

Rats

A single dose of dichlorvos (in water) was administered by oral gavage to non-fasted rats (12/sex/group) at 0, 0.5, 35 or 70 mg/kg bw. Mortalities occurred in both sexes at 70 mg/kg bw. Transient neurotoxicity was evident during the functional observation battery (FOB) at and above 35 mg/kg bw, which occurred within 15 min of dosing. Homecage observations revealed abnormal posture, clonic convulsions and tremors. Handling observations included salivation, respiratory abnormalities, pale skin, and exophthalmus and decreased muscle tone. Open field observations showed impaired mobility, abnormal gait, stupor, reduced rearing and confirmed the presence of clonic convulsions and tremors. Sensory observations included reduced approach, touch, tail pinch and pupil response, in addition to a loss of the air righting relex. Neuromuscular abnormalities included reduced hindlimb extensor strength, forelimb and hindlimb grip strength and rotarod performance. Catalepsy was increased and there was a reduction in body temperature. Locomotor activity was reduced. The majority of abnormal FOB findings at and above 35 mg/kg bw were different (higher or lower) than historical control values for age, sex and time-matched rats

from the performing laboratory. The NOEL was 0.5 mg/kg bw, based on clinical signs of neurotoxicity (FOB) at and above the next highest dose of 35 mg/kg bw. There was no evidence that dichlorvos caused delayed neurotoxicity. (Lamb 1993a)

In a subchronic neurotoxicity study, dichlorvos (in water) was administered orally by gavage to non-fasted rats (15/sex/group) at 0, 0.1, 7.5 or 15 mg/kg bw/d for 13 weeks. Treatment-related clinical signs occurred at and above 7.5 mg/kg bw/d, but predominated at 15 mg/kg bw/d and included cholinergic signs (tremors, salivation and lacrimation), the presence of a wet clear material on the forelimbs, rales, exophthalmus and chromodacryorrhea. A few females had a wet red, orange or yellow material, and a dried red material, around the mouth at 15 mg/kg bw/d. Clinical signs were reported to occur at 15 min postdose (the time of peak effect) throughout the 13-week dosing period. Females appeared to be marginally more sensitive than males to dichlorvos treatment in terms of clinical signs. Female bodyweight gain was significantly reduced at 15 mg/kg bw/d. Toxicologically- and statistically-significant inhibition of plasma ChE activity occurred at above 7.5 mg/kg bw/d, with toxicologically-significant inhibition of RBC ChE also occurring. There was no effect on brain ChE activity. The NOEL was 0.1 mg/kg bw/d, based on the occurrence of cholinergic signs and inhibition of plasma and RBC ChE activity at and above 7.5 mg/kg bw/d. There was no evidence that dichlorvos caused delayed neurotoxicity. (Lamb 1993b)

Human Studies

Oral Exposure

Dichlorvos was administered to healthy young men in gelatine capsules at 1.0, 1.5, 2.0 or 2.5 mg/d for 28 days (equivalent to 0.014, 0.021, 0.029 and 0.036, respectively assuming an average bodyweight of 70 kg). At each dose, 4 or 5 subjects received the capsules containing dichlorvos, while 2 control subjects received capsules containing only corn oil. Additional groups of subjects received 0 or 1.5 mg/d dichlorvos over 60 days followed by a recovery period of 74 days. There were no treatment-related symptoms and no effect on any haematology, clinical chemistry or urinary parameter, or on RBC ChE activity. The NOEL was 1.0 mg/d (0.014 mg/kg bw/d), based on the inhibition of plasma ChE activity at and above 1.5 mg/d (0.021 mg/kg bw/d). (Rider 1967)

A single 70 mg dose of dichlorvos formulated in corn oil was administered orally to six healthy male volunteers in gelatine capsules. This dose was equivalent to approximately 1 mg/kg bw for a 70 kg male. No control group was used. No treatment-related symptoms were reported by the subjects and there was no effect on body temperature. Mean RBC ChE activity was significantly lower than pretreatment activity at days 5 or 6, 7 and 14, with subjects 1, 2, 3 and 6 showing significantly lower RBC ChE activity on single occasions at 3-14 days postdose. However, as the level of inhibition was below 20%, none of these findings were considered toxicologically-significant. The NOEL was 1 mg/kg bw, based on the absence of RBC ChE inhibition, clinical symptoms and effects on body temperature at this dose. (Gledhill 1996; Morris 1996a; Gledhill 1997a)

Dichlorvos, formulated in corn oil, was administered orally to six healthy fasted male volunteers in gelatine capsules at 7 mg for 21 days (equivalent 0.1 mg/kg bw/d assuming an adult bodyweight of 70 kg). A control group of three healthy males received 21 daily doses of the placebo (gelatine capsules containing corn oil). There was a time-related increase in the inhibition of RBC ChE activity, which was not evident in the placebo group. Mean RBC ChE activity was significantly lower than the placebo at days 7, 11, 14, 16, 18 and 21 postdose. Given the high statistical significance of this result and that the pattern of inhibition was consistent with the dosing regime, the inhibition of RBC ChE activity was attributable to dichlorvos treatment. Therefore, the LOEL for inhibition of RBC ChE activity was 0.1 mg/kg bw/d. Plasma ChE activity was not measured. (Gledhill 1997b & c; Morris 1996b)

Inhalational or dermal exposure

Experiments were conducted to examine the effect of dermal or inhalational exposure to Vapona Resin Vaporizer (20% dichlorvos). No effect on plasma or RBC ChE activities occurred in subjects exposed to 2.5 x 5-inch resin strips by direct contact of the forearm for 30 min each day for 5 consecutive days. Similarly, there was no effect on plasma or ChE activities in residents exposed to resin strips installed in their homes for up to 6 months according to label directions. Air monitoring in two residences approximately 1 month after the last strip had been installed, detected dichlorvos air levels of 0.097 and 0.087 µg/L (97 and 87 $\mu g/m^3$, respectively). (Zavon & Kindel 1966)

Blood samples were collected from 47 hospital patients who were inhalationally exposed to dichlorvos via vapona strips installed at the recommended rate of one strip/28 m^3. No effect on plasma or RBC activities

was reported. In a separate study, 2 subjects were exposed to dichlorvos for 20 hours/day for 2 days in a room containing 10 vapona strips/51.5 m^3 (ie. 5-times the recommended level). There was no effect on RBC ChE activity, while plasma ChE activity was reduced to approximately 80% of pre-exposure activity. A separate group of two subjects showed greater levels of ChE inhibition after spending 48 hours confined to a room containing 17 strips/46.8 m^3 (ie. 10-times the recommended dosage). Plasma ChE activity was suppressed to 80, 70 and 60% of pre-exposure activity after 12, 24 and 48 hours of confinement, respectively. Pre-exposure levels of activity returned after 7 days. Less marked effects were observed for RBC ChE but no data were presented. Air levels of dichlorvos were 2.2 and 0.8 µg/L (mg/m^3) after 13 and 48 hours, respectively, in the room containing 10 strips. Air levels of dichlorvos were 7.1 and 2.4 µg/L (mg/m^3) after 3 and 48 hours, respectively, in the room containing 17 strips. (Ueda & Nishimura 1967)

Cavagna et al (1970) analysed plasma and RBC ChE activities in healthy newborn babies exposed to dichlorvos via commercial vapona strips installed in hospital nurseries. Exposure was for approximately 18 h/d for 5 days at average air levels of approximately 0.053 or 0.15 mg/m^3. There was no effect on plasma or RBC ChE activities at these concentrations, with the highest concentration of 0.15 mg/m^3 estimated by the authors to be equivalent to a dose of 0.036 mg/kg bw/d.

Hunter (1970a) conducted studies in laboratory personnel to examine the effect of exposure to dichlorvos vapour. In the first study, 26 males and 6 females were exposed to dichlorvos vapour (approximately 1 mg/m^3) in an inhalation chamber for 2-7.5 h. Some subjects considered they could detect dichlorvos odour in the air but no symptoms were reported. Plasma ChE activity was depressed by >20% in some individuals after >6 h exposure. However, mean values remained within 20% of pre-exposure activity. There were no marked effects on RBC ChE activity. In a second study, 3 males were exposed for 5.5-8 h/day on 4 consecutive days; two showed no effects on plasma ChE activity but the third showed 21, 23 and 37% inhibition on days 2, 3 and 4 respectively. Graphical representation of mean single-exposure data showed a strong dose-response relationship for plasma ChE activity, declining as dose (expressed as mg min/m^3) increased. At 20% inhibition, the dose was approximately 580 mg min/m^3, whilst at zero inhibition, the dose was 200 mg min/m^3, which was considered to be the NOEC for the inhibition of plasma ChE activity.

In a follow-up study by Hunter (1970b), 7 male laboratory staff were exposed to dichlorvos vapour at 1-53 mg/m^3 for 1-4 h. One subject (a smoker) complained of upper airway irritation and tightness of the chest and consequently exposure was terminated after 9 minutes at 52 mg/m^3. This subject showed no signs of airway obstruction. Other subjects reported throat dryness and occasional rhinorrhoea, which were not considered treatment-related. RBC ChE activity was somewhat reduced (9-16%) at exposures greater than 1450 mg/min/m^3 relative to pre-exposure activity, however, this was not considered treatment-related. Graphically-presented data illustrated a linear relationship between exposure (mg/min/m^3) and the inhibition of plasma ChE activity. Exposures greater than approximately 2000 mg/min/m^3 reduced plasma ChE activity by more than 20% (measured at the end and 16 hours after exposure) relative to pre-exposure activity. The level of inhibition was approximately 90% in the subject exposed to the highest level of 5100 mg min/m^3.

Over a period of two years, three studies were conducted in a number of residences in Tucson Arizona to determine the safety of PVC resin strips containing 18.5% dichlorvos. In total, 84 adults and 55 children were involved and the period of exposure ranged from 1.5-12 months. Resin strips were generally used at 1/28.32 m^3, with between 4-18 strips used per residence. There were no treatment-related symptoms or effects on RBC ChE. In one study using 1 strip /14.16 m^3, there was a slight decrease in plasma ChE activity over 6 months, which was not considered toxicologically-significant. In one study, the concentration of dichlorvos in air reportedly reached a maximum of 0.12-0.13 mg/m^3 within several days, declining to a steady state level of 0.08-0.09 from days 13 to 28. When exposure was increased to 2 strips/14.16 m^3 a maximum level of 0.16 mg/m^3 was reached in 2 days, decreasing to 0.11 mg/m^3 13 days later. It was reported that no dichlorvos was detected in the air 17 days after the removal of all strips. (Leary et al 1974).

In a published study by Gold and Holcslaw (1984), two commercial applicators were monitored for dichlorvos exposure during treatment of 20 single-family residences with a 0.5% water-emulsion spray prepared from Vaponite. Total dermal exposure was 0.028 mg/kg bw/h, while potential inhalational exposure was 0.037 mg/h or 0.0004 mg/kg bw/h. The toxic dose of dichlorvos was 0.028$\pm$0.021 %/h, with a worst case estimate for an unprotected applicator of 0.11 %/h. There was evidence that dichlorvos penetrated the clothing and rubber gloves of applicators. One of applicators was forced to stop applying dichlorvos due to illness, which may have been treatment-related. He reportedly had a 59% reduction in plasma ChE activity at 7 hours post-treatment but this recovered by 30 hours. The second applicator had a 21% reduction in plasma ChE activity at 2 h, which returned to the baseline activity at 48 hours. A proportion of residents (15%) experienced a headache following re-entry into their premises. Residents who spent a mean of 15.8 hours in their treated residences were calculated to be exposed to 0.08 mg/kg

bw. The potential respiratory exposure to residents was an order of magnitude greater than the applicators. There was a 7.9% reduction in plasma ChE activity in residents at 24 hours post application, which was statistically significant. RBC ChE activity was reportedly decreased by 5.3-37.5%, a result that was not statistically significant. No DHA was detected in the urine of either applicators or residents. This study suggested that toxicologically-significant exposure of applicators and residents can occur during and immediately following the application of dichlorvos.

Other Studies

Methylation of nucleic acids

In two replicate experiments, each involving 2 groups of 5 male CFE rats, animals were exposed for 12 h to [^{14}C-methyl]dichlorvos (113 Ci/mol) at 0.064 μg/L (equivalent to 0.064 mg/m^3). Following exposure, rats were decapitated and soft tissue was processed for extraction of DNA and RNA. Analysis revealed methylation of the $\underline{N}_7$ atom of quinine moieties. The limits of detection of methylation were one methyl group per 5.7x10^{11} and per 2.0x10^9 nucleotide units for DNA and RNA respectively. Thus, dichlorvos did not appear to methylate rat nucleic acids following inhalation at low (practical use) concentrations. (Wooder et al 1976)

Wright et al (1979) reviewed the chemical structure, reactivity and metabolic fate of dichlorvos, particularly in relation to its possible genotoxicity. On the basis of a comparison of mammalian and bacterial assays reported in the literature, the authors concluded that the mutagenicity of dichlorvos in bacterial assays was due to methylation of DNA under the conditions of the tests. However, the methylation of mammalian DNA could not be demonstrated *in vivo*. Conversely, methylation by the known alkylating mutagen, methyl methansulfonate, was evident. The failure to detect methylation by dichlorvos *in vivo* was attributed to highly efficient enzyme-catalysed biotransformation relying on the phosphorylating reactivity of dichlorvos. The metabolic pathways for dichlorvos had been characterised largely in the rat but were also reported to be common to pig, mouse, hamster and humans.

Forestomach irritation studies

In a study designed to investigate the genotoxic and/or irritant effects on mouse forestomach, dichlorvos (in corn oil) was administered orally to fasted mice by gavage at 0, 10, 20, 40 or 100 mg/kg bw. At each dose, 4 separate groups of 5 mice/sex were assigned to one of 4 sacrifice times of 2, 4, 12 or 48 hours. Positive control groups of 5 mice/sex were treated with either 300 mg/kg bw butylated hydroxyanisole (BHA) in aqueous gum tragacanth or 200 mg/kg bw *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in corn oil. BHA is a non-genotoxic promoter of forestomach tumours, while MNNG is a genotoxic forestomach carcinogen. Mortalities were confined to high-dose males (3 found dead; one sacrificed in a moribund condition). Dichlorvos did not induce unscheduled DNA synthesis (UDS) or replicative DNA synthesis (RDS) in the mouse forestomach up to 100 mg/kg bw, a dose where mortalities and macroscopic stomach abnormalities (dilation of blood vessels and haemorrhage) occurred in males. Dichlorvos caused a range of histopathological forestomach effects including oedema, epithelial cell hypertrophy and hyperplasia at and above 10 mg/kg bw. The induction of hyperplasia was similar to that caused by the BHA, a known non-genotoxic promoter of forestomach tumours in mice. (Benford 1991; Benford et al 1994)

A similar experimental protocol was used in a follow-up study by Benford (1992) to further investigate the irritant effects of dichlorvos on mouse forestomach. The same doses of dichlorvos were used except that sacrifice times were 8, 10 and 48 hours. In addition, UDS was not measured. Histopathological effects on the epithelium of the forestomach were consistent with the previous study and were similar to that caused by BHA (oedema, epithelial cell hypertrophy and hyperplasia). However, the severity of the effects seen with dichlorvos was less than with BHA. There was an equivocal increase in RDS and cell proliferation.

HAZARD ASSESSMENT

The toxicological database for dichlorvos is extensive and consists of unpublished reports generated by industry, in addition to a range of published studies. There were no studies conducted on any products registered in Australia.

Mechanism of Mammalian Toxicity

In common with all organophosphate compounds the primary mode of action of dichlorvos is via the inhibition acetylcholinesterase activity, which causes over-stimulation of those parts of the nervous system that use acetylcholine to transmit nerve impulses. Signs of intoxication are consistent with acetylcholinesterase inhibition and include salivation, lachrymation, vomiting, diarrhoea and laboured breathing. If intoxication is severe, muscle twitching, loss of reflexes, convulsions and death can eventuate. The onset of these signs and subsequent recovery from non-lethal doses is relatively rapid; the time to peak effect in rats following oral dosing is 15-60 minutes (Tyl et al 1990a; Lamb 1992; Lamb 1993b), with recovery occuring by 24 hours (Durham et al 1957; Lamb 1992).

Metabolism and Toxicokinetics

Dichlorvos is absorbed via all exposure routes and does not require activation to inhibit acetylcholinesterase activity. Dichlorvos is immediately inactivated in the liver, which has important implications for the consideration of its carcinogenic potential. Various studies have shown little interspecies variation in the metabolism, distribution and excretion of dichlorvos, which also appear to be independent of the dose route or sex of the animal (Hutson & Hoadly 1972a & b; Blair et al 1975; Cheng 1989 & 1990). Perhaps a most remarkable feature was the elimination of radioactivity in the expired air, which accounted for approximately 50% of the administered dose.

Dichlorvos is deactivated either by ester hydrolysis to yield dimethylphosphate and dichloroacetaldehyde or by oxidative O-demethylation. Hydrolysis of the O-demethylated metabolite yields methylphosphate and eventually phosphoric acid and methanol. The hydrolytic pathway is generally the predominant metabolic pathway, with the oxygen-vinyl bond split to generate dimethyl phosphate and DHA. The latter is further metabolised to dichloroethanol or possibly dichloroacetic acid, and eventually to dichloroethanol glucuronide, hippuric acid, urea and CO_2. As mentioned, dichlorvos is rapidly metabolised *in vivo*, with no parent compound detected in any laboratory animal studies. Following administration of [^{14}C-vinyl]dichlorvos, urinary metabolites have included urea, hippuric acid, desmethyl dichlorvos and dichloroethanol glucuronide (Hutson 1971; Hutson & Hoadly 1972a & b; Cheng 1989 & 1990). Hippuric acid and urea have also been detected as faecal metabolites (Cheng 1989 & 1990).

Metabolism in humans appears to be consistent with other mammals. Oral dosing of a single male subject with [^{14}C-vinyl]dichlorvos generated hippuric acid, desmethyl dichlorvos and urea in urine (Hutson & Hoadly 1972a & b). In a separate study, no dichlorvos was detected in two male subjects who were inhalationally exposed to dichlorvos vapour at 0.25 mg/m^3 for 10 hours or 0.7 mg/m^3 for 20 hours (Blair et al 1975).

The major excretory pathways following oral, intravenous, intraperitoneal, inhalational or dermal administration of [^{14}C-vinyl]dichlorvos to rats was via expired air and urine (Casida 1962; Hutson 1971; Hutson & Hoadly 1972a & b; Cheng 1989 & 1990; Jeffcoat 1990). Excretion was rapid, occurring within the first 24 hours after dosing (Hutson 1971; Cheng 1989 & 1990). Marked levels (13-38%) of radioactivity were detected in the carcass 4 or 7 days after dosing (Casida 1962; Hutson 1971; Cheng 1989 & 1990), which was possibly due to the incorporation of ^{14}C into tissue protein. Hutson (1971) detected ^{14}C radioactivity in rat livers as glycine and serine residues. Relatively high levels of radioactivity were also detected in rat skin (Hutson 1971). In terms of tissue distribution, the highest levels of radioactivity have been detected in the liver, blood and kidneys 7 days after dosing (up to 4.8, 0.5 and 0.5% of the administered dose, respectively) (Cheng 1989 & 1990). There is no evidence that dichlorvos or its metabolites accumulate in tissues.

Percutaneous absorption

The level of percutaneous absorption in rats was 22-30% when dichlorvos was applied to 12 cm^2 of skin at 3.6, 36 or 360 μg in a total volume of 100 μL (equal to 0.5, 3 or 30 $\mu g/cm^2$) (Jeffcoat 1990). Absorption occurred within the first 10 hours of exposure and a substantial proportion (38-55%) of dichlorvos was found to evaporate from the skin surface following application. In the absence of such evaporation, it is plausible that close to 100% of the applied dose would have been absorbed. However, this scenario is unlikely under actual use conditions.

The concentration range tested in this study of 0.0036%-0.36% is an accurate reflection of the working concentration of the 8 emulsifiable concentrate products currently registered for use (0.1-1%). Therefore, the above level of percutaneous absorption is considered a reliable figure for use in the occupational health and safety assessment for dichlorvos.

Acute Toxicity

Dichlorvos has high acute toxicity in experimental animals. Clinical signs of toxicity occur soon after dosing and are typical of OP poisoning (exophthalmus, salivation, lachrymation, tremors, dyspnoea, convulsions and death). Survivors recover completely within 24 hours (Durham et al 1957; Lamb 1992). The time to peak effect in rats following oral dosing is 15-60 minutes (Tyl et al 1990a; Lamb 1992; Lamb 1993b).

Oral LD_{50} values in rats range from 46-108 mg/kg bw, and dermal LD_{50} values from 75-210 mg/kg bw. The oral LD_{50} in rabbits and dogs is 74 and 100-316 mg/kg bw, respectively. The LD_{50} in mice following intraperitoneal administration is 24 mg/kg bw. In rats, the inhalational LC_{50} was >206 mg/m^3 for vapour exposures and in the range of 340-523 mg/m^3 for aerosol exposures.

Dichlorvos was a slight skin irritant and a moderate eye irritant in rabbits (Pauluhn 1985), and a skin sensitiser in humans (patch-test) and guinea pigs (maximisation test) (Ueda 1994).

Repeat-dose toxicity

Dose-related inhibition of plasma, RBC and brain ChE activities was the most common manifestation of dichlorvos toxicity in short-term, subchronic and chronic studies in mice, rats and dogs. Cholinergic signs and occasional mortalities occurred in rats and dogs at the same doses as the inhibition of brain ChE activity. Plasma and RBC ChE activities were also inhibited following chronic inhalational exposure in rats (LOEC = 0.5 mg/m^3; Blair et al 1974 & 1976).

There was little indication that repeated oral or inhalational exposure had any effect on haematology, clinical chemistry or urinary parameters, or on organ weights or gross pathology. In some rat and dog studies, histopathology revealed cytoplasmic vacuolisation of the liver (Jolley at l 1967; Witherup el 1967; Chan 1989).

Genotoxicity

Numerous *in vitro* and *in vivo* experiments have tested the genotoxic potential of dichlorvos, with the majority of data generated between 1972 and 1990 and published in the open scientific literature. In the current submission, seven unpublished genotoxicity studies were evaluated, which showed that dichlorvos was genotoxic *in vitro* but not *in vivo*. These findings are consistent with the extensive genotoxicity database for dichlorvos (see Appendix I).

Dichlorvos is mutagenic and DNA-reactive in bacteria and other microorganisms *in vitro*, in both the presence and absence of exogenous metabolic activation (although effects were commonly reduced in the presence of exogenous metabolic activation). Dichlorvos is also mutagenic and clastogenic in a range of mammalian cells exposed *in vitro*, including induction of UDS but not chromosomal aberrations or SCE in cultured human cells. In contrast, the majority of *in vivo* studies, including tests for induction of UDS, SCE, micronucleus formation, chromosomal aberrations and dominant lethal mutations, have yielded negative results. These negative *in vivo* genotoxicity results can be explained by the rapid metabolism and inactivation of dichlorvos, which appears to prevent systemic exposure to intact dichlorvos at concentrations likely to lead to direct molecular interactions.

Tungul et al (1991) described micronucleus formation in mouse epidermal keratinocytes after direct application of dichlorvos to the skin, confirming that dichlorvos has genotoxic potential at localised high concentrations and in the absence of metabolism. Pletsa (1999) reported a 3-fold increase in the mutation frequency in the liver of transgenic mice following repeated intraperitoneal injection of dichlorvos. In this

same study, a single injection failed to have an effect, while neither single nor repeated administration generated methylated DNA adducts or increased the frequency of mutations in other tissues. These findings are consistent with increased tumours (mostly papillomas) observed in the forestomach of mice, where tissue was exposed to high concentrations of unchanged dichlorvos (NCI 1977; Chan 1989).

The extensive genotoxicity database indicates that in the absence of metabolism, dichlorvos is mutagenic and clastogenic at the point of contact, where unchanged dichlorvos may be in direct contact with tissue. There is no evidence that dichlorvos has any systemic genotoxic potential. Scenarios of prolonged exposure in the absence of metabolic activity are unlikely in the general population given the current patterns of use. Chronic inhalational exposure (the most likely exposure route in humans) failed to cause tumours (Blair et al 1974) or to methylate nucleic acids in rats (Wooder et al 1976). The failure of dichlorvos to methylate DNA or RNA *in vivo* has been attributed to its phosphorylating reactivity, leading to highly efficient biotransformation (Wright et al 1979). Furthermore, the consistently negative *in vivo* genotoxicity findings consequent with rapid metabolism indicate that dichlorvos is unlikely to pose a genotoxic risk to humans.

Carcinogenicity

The carcinogenic potential of dichlorvos has received considerable attention in previous human health risk assessments undertaken by various countries and international agencies, including Australia. Successive Australian health advisory committees have independently examined the carcinogenic and genotoxic potential of dichlorvos on several occasions (see Section 1.1 and Appendix I). These previous risk assessments have concluded that dichlorvos is unlikely to pose a carcinogenic risk to humans.

Summary of carcinogenicity findings

Strain	Route	Dose	Duration	Findings	Reference
MICE					
B6C3F1	Oral (diet)	48 or 96 mg/kg bw/d	80 weeks; 12-14 week observation period	Equivocal increase in oesophageal tumours	NCI (1977)
B6C3F1	Oral (drinking water)	56 or 112 mg/kg bw/d	102 weeks	Negative	Konishi et al (1981)
C57B1/6/Bln	Oral (gavage in water)	2.9 or 4.3 mg/kg bw/d	50 weeks; 60 week observation period	Negative	Horn et al (1987)
B6C3F1	Oral (gavage in corn oil)	10, 20 or 40 mg/kg bw/d 5 d/wk	103 weeks	Forestomach papillomas. Equivocal increase in forestomach carcinomas	Chan (1989)
C57B1/6/Bln	Oral (gavage)	4.3 mg/kg bw/d[1]	50 weeks; 60 week observation period	Negative	Horn et al (1990)
RATS					
CD	Oral (diet)	0.005, 0.05, 0.5 5 or 25 mg/kg bw/d	2-years	Negative	Witherup et al (1967)
Osborne-Mendel	Oral (diet)	25 or 50 mg/kg bw/d	80 weeks; 30 week observation period	Negative	NCI (1977)
F344	Oral (drinking water	14 or 28 mg/kg bw/d	104 weeks	Negative	Enomoto (1981)

Strain	Route	Dose	Duration	Findings	Reference
BD IX/Bln	Oral (gavage in water)	0.07 or 0.11 mg/kg bw/d	60 weeks; 51 week observation period	Equivocal occurrence of proliferative forestomach lesions	Horn et al (1988)
F344	Oral (gavage in corn oil)	4 or 8 mg/kg bw/d 5 d/wk	103 weeks	Equivocal increase in proliferative lesions of the pancreas	Chan (1989)
Carworth Farm	Inhalation	0.05, 0.5 or 5 mg/m^3	2 years	Negative	Blair et (1974)

1 = co-administration with 50 μg N-nitrosodiethylamine (sc)

While the majority of laboratory animal studies conducted in mice and rats have been negative, studies performed by the National Cancer Institute (1977) and the National Toxicology Program (Chan 1989) reported forestomach tumours in mice and a variety of tumours in rats. Historically, it is the results of these studies, in addition to positive *in vitro* genotoxicity findings, that have contributed to concern over the possible carcinogenic risk to humans from dichlorvos exposure. The Table above summarises the findings of a range of carcinogenicity assays conducted in mice and rats.

Mice

Carcinogenicity findings in mice suggested a possible relationship between dichlorvos treatment and the occurrence of forestomach lesions in B6C3F1 mice, namely papillomas and carcinomas, following gavage administration (Chan 1989). Studies employing a different mouse strain or dosing via the drinking water at approximately equivalent doses were negative (Konishi et al 1981; Horn et al 1987 & 1990).

Forestomach squamous papillomas are a progression from hyperplasia, and an increase in the incidence of papillomas or the progression from hyperplasias to papillomas and eventually to carcinomas, are thought to be from the localised irritative action of high concentrations of dichlorvos introduced by gavage. Studies by Benford (1991 & 1992) showed that dichlorvos was irritating to the forestomach of B6C3F1 mice following a single oral gavage dose, causing oedema, epithelial cell hypertrophy and hyperplasia, but not unscheduled DNA synthesis (a genotoxic endpoint). These irritant effects were similar though less severe to those caused by butylated hydroxyanisole (antioxidant in human food), a non-genotoxic promoter of forestomach tumours in mice.

In conclusion, dichlorvos is considered to have a localised irritant effect on the mouse forestomach following gavage dosing, leading to hyperplasia and possible tumour formation. The role of the mouse forestomach as a storage organ means that dichlorvos would be in prolonged contact with the epithelium thereby increasing the possibility of irritation. As there is no analogous structure in humans such conditions of prolonged exposure to high concentrations of unchanged dichlorvos are unlikely and therefore the forestomach findings in mice are not considered relevant for human risk assessment.

Rats

Evidence for the carcinogenicity of dichlorvos in rats was inconsistent, because although the incidence of some tumours was increased in individual lifetime studies, no two studies showed an increase in the same tumour in the same tissue. Studies employing dietary or drinking water administration were negative (Witherup et al 1967; NCI 1977; Enomoto; Enomoto 1981). Equivocal increases in proliferative forestomach or pancreatic lesions occurred in some studies following gavage dosing (Horn et al 1988; Chan 1989). While the occurrence of forestomach hyperplasia could be viewed as consistent with the two mouse studies, the equivocal nature of the finding does not allow any definitive conclusion to be made.

Assessment of the carcinogenic risk to humans

The above oral dosing studies in mice and rats suggest little in the way of a carcinogenic risk to humans following dietary or drinking water exposure. The occurrence of forestomach tumours in B6C3F1 mice following gavage dosing suggests that repeated exposure to high localised concentrations coupled with some irritancy potential are necessary conditions for dichlorvos to be carcinogenic. Importantly, such a scenario is unlikely in humans and the main potential exposure of the general population is by inhaling low concentrations of dichlorvos vapour when used indoors. The most relevant study in the database for assessing the long-term risk posed by inhaling dichlorvos vapour was a 2-year rat inhalation study

conducted by Blair et al (1974 & 1976). In this study, no treatment-related effects on the type, distribution or incidence of non-neoplastic or neoplastic lesions occurred up to 5 mg/m^3 (achieved concentration of 4.7 mg/m^3).

Given the equivocal evidence of carcinogenicity in mice and rats following oral administration it is worth considering the carcinogneic (and genotoxic potential) of other OPs, such as trichlorfon and naled, which are transformed to dichlorvos *in vivo*.

Trichlorfon or metrifonate is an OP insecticide, which is converted non-enzymically to dichlorvos in water, biological fluids and tissues at pH values greater than 5.5. Trichlorfon has been evaluated by Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1972, 1976 and 1979, the International Program on Chemical Safety (IPCS) in 1992 (Environmental Health Criteria 132) and the Joint WHO/FAO Expert Committee on Food Additives (JECFA) in 2000 (WHO Food Additive Series 45). The International Agency for Research on Cancer (IARC) assessed the carcinogenicity of trichlorfon in 1983 and 1987 classifiying it in Group 3 ("The agent is not classifiable as to its carcinogenicity in humans"). Furthermore, the OCS has evaluated trichlorfon in 1986, 1993, 1994, 1995 and 2000. Collectively these evaluations found no evidence of any carcinogenicity following dosing by various routes in rats, mice or hamsters. Trichlorfon had some genotoxic potential and was mutagenic in bacteria and mammalian cells, however, there was some variability in the results which was attributed to the purity of the test material and/or the possible formation of dichlorvos. While the majority of genotoxicity assays were negative, trichorfon was found to induce chromosomal damage at relatively high doses. On the weight-of-evidence it was concluded that trichlorfon would be unlikely to pose a genotoxic risk to humans.

Naled is an OP insecticide that converts to dichlorvos *in vivo*. Naled has previosly been evaluated by the OCS in 2000 and 2003, with no evidence of carcinogenicity found in mice following gavage administration. Naled showed evidence of mutagenicity in bacterial but not mammalian *in vitro* assays.

Conclusion

Forestomach tumours were observed in B6C3F1 mice that received dichlorvos by oral gavage. Dichlorvos was considered to have a localised irritant effect on the mouse forestomach following gavage dosing, leading to hyperplasia and possible tumour formation. While forestomach tumours are not considered directly relevant to humans, and scenarios of high oral exposures are unlikely, these findings indicate that repeated exposure to high concentrations of dichlorvos is undesirable. There is equivocal evidence of carcinogenicity in rats following long-term oral dosing. A long-term inhalational study in rats, which simulated the most relevant exposure route in humans, showed no evidence of carcinogenicity. In addition, related compounds that are metabolised to dichlorvos *in vivo* showed no carcinogenic potential. On the weight-of-evidence, dichlorvos is not considered to pose a carcinogenic risk to humans.

Reproductive and Developmental Toxicity

Dichlorvos affected reproduction at maternotoxic doses in rats following administration for two generations via the drinking water (Tyl et al 1992 & 1993). However, as the NOEL for reproduction and pup toxicity is well above the NOEL for parental toxicity (2 mg/kg bw/d *versus* 0.5 mg/kg bw/d), dichlorvos is not considered to pose a reproductive hazard to humans.

There was no evidence that dichlorvos was teratogenic based on a range of studies conducted in mice, rats and rabbits following oral or inhalational exposure (Schwetz et al 1979; Thorpe 1971; Tyl 1990a & b).

Neurotoxicity

Dichlorvos is acutely neurotoxic in chickens and rats by virtue of its ability to inhibit brain ChE activity (Beavers 1988; Lamb 1993a). This is typified by the occurrence of cholinergic signs and abnormal FOB (rats) following single or repeated oral dosing. There was no evidence that dichlorvos causes delayed neuropathy (Beavers 1988; Redgrave et al 1994a & b, Redgrave & Mansell 1994, Jortner 1994 and Hardisty 1998; Lamb 1993b).

Human Toxicity

Like other mammals, the inhibition of plasma ChE activity is the most sensitive toxicological endpoint in humans following repeated exposure [the NOEL following repeated oral dosing is 0.014 mg/kg bw/d (Rider 1967)]. For acute or short-term exposures, the inhibition of RBC ChE activity is the most sensitive toxicological endpoint [the NOEL following a single oral dose is 1 mg/kg bw/d (Gledhill 1996; Morris 1996a; Gledhill 1997a)].

As mentioned previously, inhalational exposure to dichlorvos is considered the main exposure pathway in humans based on its current use patterns. A number of studies have investigated the inhalational exposure of humans in residential and public premises under normal or exaggerated use conditions. Inhalational exposure to dichlorvos resin strips installed according to the recommended directions for use did not result in the inhibition of plasma or RBC ChE activities (Zavon & Kindel 1966; Ueda & Nishimura 1967; Leary et al 1974). In contrast, exaggerated exposure (10 or 17 strips per room) caused the inhibition of plasma ChE activity in adult males (RBC ChE activity was not inhibited in the 10-strip study, while no RBC ChE data were presented for the use of 17 strips; Ueda & Nishimura 1967). Air levels of dichlorvos in this study were up to 2.2 and 7.1 mg/m^3, respectively. Newborn babies exposed to air levels of 0.095-0.25 mg/m^3 for 18 h/d showed no effects on plasma or RBC ChE activities (Cavagna et al 1970). Two studies by Hunter (1970a & b) examined the effect of dichlorvos vapour on laboratory staff following exposure for up to 7.5 hours. There was no treatment related effect on RBC ChE activity, while plasma ChE was inhibited (≥20%) at and above approximately 580 mg min/m^3.

Few studies have examined the dermal toxicity of dichlorvos in humans. Repeated dermal exposure to resin strips for 5 days failed to perturb plasma or RBC ChE activities (Zavon & Kindel 1966). Plasma ChE was reportedly inhibited in two commercial pesticide applicators following the spraying of up to 20 residences (Gold & Holcslaw 1984). In this study, the level of dermal exposure was estimated to be 0.499 μg/cm^2/h (total dermal exposure of 0.028 mg/kg bw/h).

DOSE LEVELS RELEVANT FOR RISK ASSESSMENT

Dose levels relevant for dietary risk assessment

To identify the lowest NOELs for the establishment of an ADI and ARfD, a summary of the NOELs determined in those studies considered adequate for regulatory purposes are shown in the following Tables.

Studies relevant for the establishment of an ADI

Species	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Toxicological Endpoint	Reference
Subchronic Studies				
Rats 13-wk po gavage	0.1	1.5	Inhibition of plasma and RBC ChE activities	Kleeman (1988b) [QA, GLP]
Dogs 90-day po, gelatine capsules	0.3	0.9	Inhibition of plasma and RBC ChE activities	Hine (1962)
Chronic studies				
Rats 2-y dietary	0.23	2.3	Inhibition of plasma and RBC ChE activities	Witherup et al (1967)
Dogs 2-y dietary	0.008	0.08	Inhibition of RBC ChE activity in males	Jolley et al (1967)
Dogs 52-wk po, gelatine capsules	0.05	1.0	Inhibition of plasma and RBC ChE activities	Markiewicz (1990) [GLP]
Reproduction studies				
Rats 2-generation	0.5	2	Parental toxicity: inhibition of plasma, RBC & brain ChE activities	Tyl et al (1992 & 1993)
	2	8	Pup toxicity: decreased bw	
	2	8	Reproductive toxicity: reduced fertility & pregnancy indices, increased stillbirths (F2), reduced cycling and increased abnormal cycling (F1 maternal rats)	
Neurotoxicity studies				
Rats 13-wk, po gavage	0.1	7.5	Inhibition of plasma and RBC ChE activities, and cholinergic signs	Lamb (1993b)
Human studies				
28-d po, gelatine capsules	0.014	0.021	Inhibition of plasma ChE activity	Rider (1967)
21-d po, gelatine capsules	-	0.1	Inhibition of RBC ChE activity	Gledhill (1997b & c) Morris (1996b) [QA, GLP]

QA = quality assured study; GLP = statement of compliance with principles of good laboratory practice

Studies relevant for the establishment of an ARfD

Species	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Toxicological Endpoint	Reference
Acute studies				
Rat po gavage	0.1	0.5	Clinical signs (exophthalmus, absent hindlimb grasp, reduced forelimb grasp). Other signs observed at higher doses (10-80 mg/kg bw) & occurring within 15-45 min of dosing	Lamb (1992) [GLP]
Developmental studies				
Rat po gavage	3.0	21.0	Maternal rats: cholinergic signs (within 10-60 min of dosing) & reduced food consumption	Tyl et al (1990a) [QA, GLP]
	21.0	-	Foetuses: no toxicity	
	21.0	-	Developmental toxicity: none	
Rabbit po gavage	0.1	7.0	Dams: mortalities	Tyl et al (1990b) [QA, GLP]
	7.0	-	Foetuses: no toxicity	
	7.0	-	Developmental toxicity: none	
Neurotoxicity studies				
Rat Acute, po gavage	0.5	35	Clinical signs of neurotoxicity in the FOB	Lamb (1993a) [QA, GLP]
Human studies				
Single-dose, po Gelatine capsules	1	-	Inhibition of RBC ChE activity	Gledhill (1996), Morris (1996a), Gledhill (1997a) [QA, GLP]

QA = quality assured study; GLP = statement of compliance with principles of good laboratory practice

Dose levels relevant for the risk assessment of home garden products

The only dichlorvos products available for home garden use are impregnated resin strips used indoors to control insects in confined spaces or rooms, and naphthalene/dichlorvos blocks for use in outdoor garbage bins. Exposure via the use of the naphthalene/dichlorvos blocks is considered negligible, as they are only used outdoors. Therefore the main potential residential exposure of the general population is by inhaling dichlorvos vapour from impregnated resin strips. On theoretical grounds, there is a slight potential for dermal exposure to dichlorvos vapour. However, given that the likely air concentration of dichlorvos would be low and the duration of exposure short, dermal exposure to dichlorvos vapour is not considered to pose a significant risk to residents.

At present, there are two strengths of impregnated resin strips available for home garden use, which contain either 186 or 328 g/kg dichlorvos. These strips are used predominantly in confined storage areas, such as in drawers and wardrobes, with only one product used to treat entire rooms. These types of home garden products are unlikely to pose an oral or dermal hazard due to the encasement of the resin strip within a plastic/cardboard housing and because they are not recommended for use in food preparation or storage areas.

The characteristics of the dichlorvos pest strips available for home garden use in Australia are summarised in the following Table. While there are differences in the concentration, size and application rate (No. strips/m^3) between the 5 strips, the amount of dichlorvos applied per cubic metre of space is similar.

Characteristics of dichlorvos pest strips

Product	Dichlorvos	Net strip weight (g)	Dichlorvos per strip (g)	Application rate	Total amount dichlorvos (g) per m^3 space*
Mortein Moth and Insect Strips	328 g/kg	5	1.64	1 strip/m^3	1.64
Mortein Moth Guard for Wardrobes and Drawers	328 g/kg	5	1.64	1 strip/m^3	1.64
Sureguard Pest Strip	186 g/kg	103	19.2	1 strip/30 m^3 (room)	0.64
Sureguard MiniStrip	186 g/kg	20	3.72	1 strip/3 m^3	1.24
Scuttle bug pest strip	186 g/kg	20	3.72	1 strip/3 m^3	1.24

* either cupboard, drawer, wardrobe or room space

To perform a residential inhalational risk assessment, suitable NOECs from laboratory animal or human inhalational studies are compared with the average air level of dichlorvos. The acceptable margin of exposure (MOE) is $\geq$10 for a NOEC based on human data (resulting from the application of a 10-fold uncertainty factor for intra-species variability) and $\geq$100 for a NOEC based on laboratory animal data (resulting from the application of a 10-fold intra- and 10-fold interspecies uncertainty factors). In the absence of a suitable inhalational study and/or a NOEC, a NOEL from an oral dosing study can be converted to an inhalational NOEL following correction for inhalational absorption (ie. route-to-route extrapolation). The average air level can then be converted to an inhaled dose (mg/kg bw/d) and compared to this inhalational NOEL.

As mentioned previously, the inhibition of plasma ChE activity is the most sensitive toxicological endpoint in humans following repeated exposure, and as such, should be used as the basis for the inhalational exposure assessment. There are two inhalational studies in the toxicological database for dichlorvos that are considered appropriate for the establishment of a repeat-dose NOEC. The 5-day study by Cavagna et al (1970) established a NOEC of approximately 0.15 mg/m^3 in new-born babies, based on the absence of plasma and RBC ChE inhibition at and below this concentration. The 2-year rat study by Blair et al (1974) established a NOEC of 0.05 mg/m^3, based on the inhibition of plasma and RBC ChE activities at 0.5 mg/m^3.

An alternative approach to the use of the above NOECs is to use route to route extrapolation (inhalational to oral dose extrapolation). The most suitable study for this approach is the 28-day oral dosing study of Rider (1967), in which the NOEL was 0.014 mg/kg bw/d. The study was deemed highly suitable because it demonstrated a NOEL and a LOEL for plasma ChE inhibition and was performed with human subjects, thereby eliminating uncertainty associated with inter-species extrapolation. Adjusting for the inhalation absorption factor of 70%[1], the resulting inhalational NOEL becomes 0.02 mg/kg bw/d.

[1] A study (Kirkland, 1971) evaluated by the WHO (1988) demonstrated that at dichlorvos concentrations of 0.1–2.0 mg/m^3, pigs retained 15–70% of the inhaled dichlorvos. A 70% inhalation absorption factor is therefore used for risk assessment purposes.

HUMAN EXPOSURE

In Australia, sources of potential public exposure to dichlorvos include residues in food and drinking water (oral exposure) and residential exposure (inhalational exposure).

Residues in food and drinking water

Dichlorvos is applied as a spray to food commodities such as stored grain and potatoes, to vegetables grown in green houses (eg. tomatoes and cucumbers) and as a fog to mushrooms grown in mushroom houses. Label directions also state that it can be used in combination with chlorpyrifos on avocadoes. Besides these 'direct' food applications, dichlorvos is also used to treat a number of commercial food preparation or storage areas, which could potentially contribute to total food residues. These include dairy cattle sheds, stables, piggeries, abattoirs and meat works, wineries, food warehouses, mills and empty grain silos.

Maximum Residue Levels (MRLs) for dichlorvos have been established for cereal grains, cocoa beans, coffee beans, edible offal, eggs, fruits, lentils, lettuce, meat, milks, mushrooms, peanut, poultry meat and offal, rape seed, rice bran, soya bean, tomato, tree nuts, vegetables (other than lentils, lettuce and soya bean), wheat bran and wheat germ.

Chronic Dietary Intake

Both the 19th and 20th Australian Total Diet Surveys (ATDS) (2002 and 2003, respectively) performed under the auspices of Food Standards Australia New Zealand (FSANZ) detected no dichlorvos in any of the foods surveyed. Therefore, the dietary exposure for the population (including infants, children and adults) was estimated by FSANZ to be zero as the concentration of dichlorvos was less than the limit of detection.

Residues in Drinking Water

Based on its current pattern of use, exposure of the general population to dichlorvos residues in drinking water is considered negligible.

Air levels generated from the use of dichlorvos pest strips

There were no data on the levels of dichlorvos released from pest strips marketed in Australia. Therefore, surrogate data were obtained from various published and unpublished studies conducted using 20% room strips or vaporisers. These surrogate data are only relevant to a single Australian product, namely Sureguard Pest Strip, which is used for insect control in entire rooms. The majority of other resin strip products are available as "ministrips" and used in confined areas such as wardrobes, bookcases, linen cupboards, under sinks and in toilets.

The data summarised in the Table below and the subsequent exposure calculations are not necessarily relevant for the ministrip products, which have a different use pattern; it is unlikely that persons would be continuously exposed to dichlorvos at the levels seen with the room strips. However, based on evidence indicating that a lack of ventilation (ie. containment) results in relatively high levels of dichlorvos (Shell Chemicals 1965; Elgar & Mathews 1968), the concentration of dichlorvos within the confined space is likely to be relatively high. This could pose an acute risk, for example, every time someone opened a wardrobe or drawer or used the toilet. In the absence of data on the air levels generated from the use of dichlorvos ministrips, the probability and risk of such exposures can not be ascertained. Therefore, it is important that such data be made available to the OCS for evaluation.

The following Table summarises various published and unpublished studies, which measured dichlorvos air levels generated by the use of the 20% room strips or vaporisers. There was no information available on the formulations of these products.

Air levels of dichlorvos generated from the use of 20% pest strips

Sample	Conditions	Air Levels (mg/m^3)	Reference
20% vapona insecticide resin strips	1 strip/28.32 m^3 Closed, air conditioned hotel room with no air exchange 23°C, 55% RH	0.43 (4 h), 0.44 (8h), 0.34 (12 h), 0.34 (1 d), 0.36 (2 d), 0.30 (3 d), 0.25 (6 d), 0.09 (8 d), 0.16 (10 d), 0.12 (14 d), 0.13 (29 d), 0.09 (30 d); mean = 0.23	Shell Chemicals (1965)
Vapona resin vaporiser (20% dichlorvos)	1 vaporiser/144 m^3 1 vaporise/227 m^3 Installed over 6 months. Replaced monthly for 4 months	0.097 residence 1 0.087 residence 2 Detected 1 month after installation of last vaporiser	Zavon & Kindel (1966)[1]
Vapona strips (details unspecified)	1 strip/20.39 m^3 No ventilation (~1 air change per hour)	22°C/40% RH; max = 0.57 (d 2) min = 0.06-0.07 (d 72-84) 20°C/79% RH; max = 0.26-0.30 (d 1-10) min 0.05-0.07 (d 53-87) 34°C/46% RH; max = 1.10 (d 1) min 0.01-0.02 (d 76-90) 34°C/80% RH; max = 0.74 (d 2) min = 0.01-0.02 (d 36-43)	Elgar & Mathews (1968)
6.5 inch Vapona strip (details unspecified)	1 strip/18.4 m^3 24°C, 60% RH 5 air changes/h	0.098, 0.068, 0.080, 0.066, 0.056, 0.042, 0.031, 0.024, 0.014, 0.015 at 1, 8, 15, 22, 29, 43, 57, 71, 99 and 120 d, respectively	Shell International Chemical Company (date unspecified)
Vapona strips (details unspecified)	House trials in the UK, Holland and Southern France	0.015-0.079 from 3 days to 4 weeks after hanging strips	Shell Research Ltd (1968a & b; 1969a, b &c); Elgar & Steer (1972)
Dichlorvos slow-release strips (details unspecified)	1 strip/30m^3 House trial in Melbourne 24°C, 46% RH	Average concentrations: 0.02 (wk 1) 0.04 (wk 6) 0.01 (wk 11)	Elgar & Steer (1972)
	1 strip/30m^3 House trial in Brisbane 21-27°C, 55-61% RH	Average concentrations: 0.02 (wk 1) <0.01 (wk 3) 0.005 (wk 4)	
No-Pest® Insecticide Strips 25 cm (details unspecified)	1 strip per 20.39-192.3 m^3 Both air conditioned & non air conditioned	Mean (range) at 1, 7, 14, 28, 56 and 91 days was 0.06 (0.02-0.11), 0.04 (0.01-0.09), 0.03 (0.01-0.06), 0.02 (<0.01-0.05), 0.01 (<0.01-0.02) and <0.01 (<0.01-0.02), respectively	Collins & DeVries (1973)
25 cm commercial pest strips (20% vapona)	1 strip/28.32 m^3 Minimum ventilation Strips removed after 2 weeks	0.12-0.13 d 0-12 0.08-0.09 d 13-28	Leary et al (1974)[1]

1 = studies evaluated as part of the current review; RH = relative humidity

The above data indicates some variability in dichlorvos air levels between the different studies. Some of the earlier studies conducted in the absence of ventilation found relatively high levels of dichlorvos (ie. >0.3 mg/m3) within the first few days (Shell Chemicals 1965; Elgar & Mathews 1968). In contrast, house trials conducted in a number of countries found much lower levels (<0.1 mg/m3) most likely due to ventilation.

Besides ventilation, other variables such as humidity and temperature are likely to affect dichlorvos air levels. Elgar and Mathews (1968) (see Table above) found that at high temperature (34°C), initial dichlorvos concentrations were approximately 2-fold higher than at low temperature (20-22°C) under both low and high conditions of humidity (~40 and 80%, respectively). A more rapid rate of decline then occurred at the high compared to the low temperature. High humidity was found to increase the rate of decline in air levels of dichlorvos only at the high temperature, with little effect seen at the low temperature.

It is likely that within Australia, a degree of variability would occur in dichlorvos air levels due to differences in temperature and humidity between different climatic zones. Further, there is likely to be seasonal differences in ventilation rates within households. For example, homes in Melbourne were found to have higher average air levels than Brisbane and these levels were maintained for a longer period of time (see Table above) (Elgar & Steel 1972), which was attributable to differences in ventilation rates.

Taking into consideration all of the above data, it is likely that when a 20% pest strip is used to treat a 30 m3 room as recommended, the maximum level of dichlorvos would not exceed 0.15 mg/m3 within the first few days of installation, dropping to levels of below 0.05 mg/m3 for the duration of the 4 month use period. It is acknowledged that higher levels than these have been reported in some studies when the number of strips used was greater than the recommended number and when there was inadequate ventilation. For the inhalational exposure calculations performed below, the above figure of 0.05 mg/m3 has been used as the average air concentration of dichlorvos over a 4-month period.

HUMAN RISK ASSESSMENT

Dietary risk assessment

The dietary risk assessment for dichlorvos, utilising the ARfD and ADI, will be conducted by FSANZ and the APVMA.

Residential risk assessment

To perform a residential inhalational exposure assessment, the above average air level of 0.05 mg/m3 has been converted to an inhaled dose and then compared to the pivotal inhalational NOEL of 0.02 mg/kg bw/d (see Tables below). Calculations were based on the US EPA's Exposure Factors Handbook (1996) (see http://www.epa.gov/ordntrnt/ORD/WebPubs/ exposure/). Exposure periods of 24 and 8 hours were used to simulate the worse case scenario of someone remaining in a treated room continuously and also someone spending a proportion of their day at home, for example, to sleep.

Chronic inhalational exposure assessment

Age Group	Bodyweight (kg)	Ventilation rate (m^3/day)*	Dose* (mg/kg bw/d)		MOE**	
			24 h	8 h	24 h	8 h
Children <1 y	8	4.5	0.0281	0.009	0.7	2.1
Children 1-12 y	23	8.7	0.0189	0.006	0.9	2.7
Adult female	60	11.3	0.0094	0.003	2.1	6
Adult male	70	15.2	0.0109	0.004	1.8	5.4

* Dose (mg/kg bw/d) = dichlorvos concentration in air (0.05 mg/m^3) × ventilation rate (m^3/day) ÷ [bodyweight (kg) × exposure duration (1 or 0.33 days); ** margin of exposure [NOEL (0.02 mg/kg bw/d) ÷ Dose (mg/kg bw/d)]

Based on the above calculations, continuous daily exposure to dichlorvos at the average air concentration generated during the use of a 20% pest strip results in MOEs of <10 of the pivotal inhalational NOEL for each population subgroup. While these findings suggest an unacceptable risk to human health, it is recognised that continuous 24-hour exposure is unlikely for most people, excluding the infirm or infants, who may be confined to a treated room for long periods of time. When a more common exposure scenario was used (ie. 8 h) the MOE is still <10 for each population subgroup and is therefore considered unacceptable.

In support of the above findings utilising route-to-route extrapolation, when the NOEC of 0.15 mg/m3 from Cavagna (1970) is compared with the average dichlorvos concentration of 0.05 mg/m3, the resulting MOE of 3 indicates an unacceptable inhalational risk. Further, when the NOEC of 0.05 mg/m3 from Blair et al

(1974) is compared with the average dichlorvos concentration of 0.05 mg/m3, the resulting MOE of one also indicates an unacceptable inhalational risk.

On the basis of the above calculations performed using the NOECs and inhalational NOEL, it is concluded that pest strips containing 20% dichlorvos, which are used to treat entire rooms, pose an unacceptable chronic inhalational risk to human health (in terms of the inhibition of plasma and RBC ChE activities). Consequently, the registration of these products can no longer be supported.

Conclusion

The use of pest strips containing 20% dichlorvos to treat entire rooms is not considered an acute or short-term risk to human health. However, chronic exposure calculations indicate an exceedence of safe levels for infants, children and adults. On this basis, registration of these types of pest strips is no longer supported. It should be noted that this recommendation only affects a single product [Sureguard Pest Strip Household Insecticide (APVMA Product code 45596)].

While it is unlikely, on theoretical grounds, that the use of dichlorvos ministrips poses an unacceptable chronic risk to human health, this is not necessarily true for acute or short-term exposures. Therefore, it is recommended that sponsors be asked to provide data on the air levels generated from the various uses of dichlorvos ministrips.

CONSIDERATION OF PUBLIC HEALTH STANDARDS

Approval Status

There is no objection on toxicological grounds to the ongoing approval of dichlorvos active constituent sourced from the following manufacturers:

Amvac Chemical Corporation
4100 East Washington Boulevard
Los Angeles, California 90023-4406 USA

United Phosphorus Ltd
117 GIDC
Ankleshwar Gujarat 393002 INDIA

Syngenta Crop Protection Monthey AG
Monthey Plant
Usine de Monthey
CH-1870 Monthey SWITZERLAND

Impurity Limits

An integral part of the safety assessment of an active constituent is a consideration of the chemical composition of the material. Technical-grade active constituents will contain measurable levels of impurities, which can arise during manufacture and/or from subsequent degradation during storage. The chemical identity of these impurities is generally well characterised. The impurities present in the technical-grade material are usually of no particular concern since health standards are established on the basis of toxicology studies conducted using the mixture. However, for those which have high acute toxicity, genotoxicity or teratogenic potential, concentration limits need to be set, so that the toxicological profile of the technical-grade active constituent does not appreciably alter in the event of slight changes in the proportions of the impurities.

Chloral (5 g/kg) is listed in the APVMA's minimum compositional standard for dichlorvos. Chloral is used as an intermediate in the production of dichlorvos and is rapidly converted to chloral hydrate in aqueous solutions. Neither the OCS nor any current/past Committee within the DoHA have raised any toxicological concerns regarding this impurity. Declarations of composition for two of the three sources of dichlorvos indicate that chloral levels of <0.1% are achievable and therefore approval holders should be asked to justify the continued listing of chloral in the APVMA's compositional standard.

Acceptable Daily intake

The ADI for humans is the level of intake of a chemical that can be ingested daily over an entire lifetime without appreciable risk to health. It is calculated by dividing the overall NOEL for the most sensitive toxicological endpoint from a suitable study (typically an animal study) by an appropriate safety factor. The magnitude of the safety factor is selected to account for uncertainties in extrapolation of animal data to

humans, intraspecies variation, the completeness of the toxicological database and the nature of the potential toxicologically-significant effects.

The current Australian ADI for dichlorvos is 0.001 mg/kg bw/d. It was set in 1999 using the NOEL of 0.014 mg/kg bw/d in a 28-day human study for the inhibition of plasma ChE activity (Rider et al 1967) and using a 10-fold intraspecies safety factor. As this is the most suitable study in the database for establishing the overall NOEL for chronic dichlorvos toxicity, the current ADI remains appropriate.

A more recent human study, conducted according to Good Laboratory practice (GLP), was evaluated as part of the current submission. This study by Gledhill (1997b & c) established a LOEL for the inhibition of RBC ChE activity at 0.1 mg/kg bw/d, following repeated oral dosing for 21 days. In the absence of a NOEL and the measurement of plasma ChE activity (a more sensitive toxicological endpoint suitable for establishing an ADI), this study was not considered suitable for refining the current ADI.

Acute Reference Dose (ARfD)

The ARfD is the estimate of the amount of a substance in food or drinking water, expressed on a milligram per kilogram body weight basis, that can be ingested over a short period of time, usually one meal or one day, without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation.

The current Australian ARfD for dichlorvos is 0.003 mg/kg bw and was set in 2000 using the same 28-day human study used as the basis for the current ADI (Rider 1967). It was calculated by applying a 10-fold safety factor to the NOEL of 0.033 mg/kg bw for the inhibition of RBC ChE activity 24 hours after the first dose. A more recent human study was identified in the current submission to allow the refinement of the ARfD. In the study of Gledhill (1996), there was no inhibition of RBC ChE activity following an acute oral dose of 1 mg/kg bw (the only dose tested). An ARfD of 0.1 mg/kg bw/d was calculated by applying a 10-fold safety factor (to account for intraspecies variation) to this NOEL of 1 mg/kg bw. Therefore it is recommended that the current ARfD of 0.003 mg/kg bw be amended to 0.1 mg/kg bw.

Health Value for Australian Drinking Water

The Australian Drinking Water Guidelines (ADWG) are a joint publication of the National Health and Medical Research Council (NHMRC) and the Agricultural and Resource Management Council of Australia and New Zealand. The ADGW are not legally enforceable but rather provide a standard for water authorities and State health authorities to ensure the quality and safety of Australia's drinking water.

The guideline value (mg/L) is analogous to an MRL in food and is generally based on the analytical limit of determination. If a pesticide is detected at or above this value then the source should be identified and action taken to prevent further contamination. The health value (also expressed as mg/L) is intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The health values are derived so as to limit intake from water alone to approximately 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg having a daily water consumption of 2 L over a lifetime.

Given that the ADI for dichlorvos is 0.001 mg/kg bw/d, the Health Value may be calculated as:

$$\frac{0.001 \text{ mg/kg bw/d} \times 70 \text{ kg} \times 0.1}{2 \text{ L/d}}$$

$$\sim 0.004 \text{ mg/L}$$

Hence, the current Health Value for dichlorvos of 0.001 mg/L should be amended to 0.004 mg/L.

Poisons Schedule

At present, dichlorvos is listed in Schedule 7 of the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP). There are two lower schedule entries to accommodate dilute preparations: a Schedule 6 entry for preparations containing 50% or less of dichlorvos; and a Schedule 5 entry for dichlorvos impregnated in plastic resin strip material containing 20% or less of dichlorvos, in sustained release pellets containing 20% or less of dichlorvos for the treatment of animals and in pressurised spray packs containing 10 g or less of dichlorvos. These Schedules remain appropriate.

First-Aid Instructions

Existing first aid instructions for dichlorvos as they appear in the First Aid Instruction and Safety Directions (FAISDs) Handbook are as follows:

In sustained release resin pellets for veterinary use containing 20 per cent or less dichlorvos	a
In pressurised spray packs	o
In plastic resin strips	a
In compressed liquid carbon dioxide	a, x
In other preparations for agricultural or veterinary use	a, h

Where 'a' is "If poisoning occurs contact a doctor or poisons information centre"; 'o' is "If sprayed on skin , wash thoroughly. If sprayed in mouth, rinse with water"; 'x' is " If poisoned by skin absorption or through lungs, remove any contaminated clothing, wash skin thoroughly and give one atropine tablet every 5 minutes until dryness of the mouth occurs. Get to a doctor or hospital quickly"; and 'h' is "If swallowed, give one atropine tablet every 5 minutes until dryness of the mouth occurs - if poisoned by skin absorption or through lungs, remove any contaminated clothing, wash skin thoroughly and give atropine tablets as above. Get to a doctor or hospital quickly". These first aid instructions as they relate to currently registered dichlorvos products remain appropriate.

It should be noted that the label for one dichlorvos product, Insectigas®-D states, "if swallowed, induce vomiting, preferably using Ipecac Syrup APF". Following a review of First Aid Instructions by the Working Party of the NDPSC, there was a recommendation that a number of statements relating to the induction of vomiting and the immediate treatment of poisoning with milk or raw egg be deleted. This recommendation followed an assessment of the clinical value of the induction of vomiting with the attendant risk of aspirating the vomitus. This recommendation was accepted by the NDPSC. Hence, as from June 2000, such entries no longer appear in the FAISD handbook. Therefore, the presence of such a first aid instruction on this particular dichlorvos product is considered inappropriate.

Existing first aid instructions for naphthalene as they appear in the FAISD Handbook are as follows:

For domestic uses, all forms	i, d
For other uses	i, d

Where 'i' is "If poisoning occurs get to a doctor or hospital quickly" and 'd' is "Avoid giving milk or oils".

These first aid instructions as they relate to currently registered dichlorvos products containing naphthalene remain appropriate.

Existing first aid instructions for hydrocarbons as they appear in the FAISD Handbook are as follows:

Hydrocarbons, liquid	a, c

Where 'a' is "If poisoning occurs contact a doctor or poisons information centre"; and 'c' is "If swallowed do NOT induce vomiting. Give a glass of water."

These first aid instructions as they relate to currently registered dichlorvos products containing hydrocarbon solvent remain appropriate, noting that statement 'a' is duplicated in the first aid instructions for dichlorvos.

Warning Statements and General Safety Precautions

There are no warning statements or general safety precautions for dichlorvos or hydrocarbon (liquid) that appear in the FAISD handbook. Existing warning statements for naphthalene are as follows:

For domestic uses, all forms	09, 44
For other uses	09

Where '09' is "can be fatal to children if sucked or swallowed" and '44' is "Do not use on the clothing of infants or in the bedrooms of young children". Given that the two dichlorvos products containing naphthalene are used for insect control in outdoor garbage bins, statement '44' is not considered relevant and does not need to appear on product labels.

Safety Directions

The existing safety directions for Australian products containing dichlorvos, as recommended in the FAISD handbook, are shown in the Table below.

Existing Safety Directions

AE 50 g/kg or less	
210 211	Avoid contact with eyes and skin
BA 10 g/kg or less	
130 133	Poisonous if swallowed
210 211	Avoid contact with eyes and skin
250	Do not touch bait
251	Use scoop or measure
252	If on skin and after each baiting, wash thoroughly with soap and water
EC 1140 g/L or less	
120 121 130 131 132 133	Product and spray are poisonous if absorbed by skin contact, inhaled or swallowed
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	Avoid contact with eyes and skin
220 223	Do not inhale spray mist
373	Obtain an emergency supply of atropine tablets 0.6 mg
279 280 281 284 290 292 294	When opening the container, preparing and using in enclosed areas, wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow length PVC gloves, goggles, half facepiece respirator with combined dust and gas cartridge
297 300 303	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 days use, wash gloves, goggles, respirator and if rubber wash with detergent and warm water and contaminated clothing.
360 361 363 364 366	
LD 50 g/kg or less in compressed liquid carbon dioxide	
130 131 132	Poisonous if absorbed by skin contact and inhaled
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	Avoid contact with eyes and skin
220 223	Do not inhale spray mist
279 280 290 292 294 301 303	When opening the container, wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow length PVC gloves, full facepiece respirator with combined dust and gas cartridge or canister
320	Thoroughly ventilate treated areas before reoccupying
349	Avoid re-entry for (time to be inserted by registering authority) after use in glass houses or other confined spaces. If re-entering, wear all protective clothing including respirator
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water
360 361 364 366	After each days use, wash gloves, respirator and if rubber wash with detergent and warm water and contaminated clothing.
SR	
380	Do not open inner (envelope) (pouch) until ready for use
351	Wash hands after use

AE = aerosol; BA = bait; EC = emulsifiable concentrate; LD = liquid; SR = slow release generator

The existing safety directions for Australian products containing naphthalene, as recommended in the FAISD handbook, are shown in the Table below.

HG all forms and strengths

130 132 133	Poisonous if inhaled or swallowed
161 162 164	Will irritate the eyes and skin
180	Repeated exposure may cause allergic disorders
220 222	Do not inhale vapour
210 211	Avoid contact with eyes and skin
351	Wash hands after use

HG = home garden

There are currently 18 dichlorvos products registered for use in Australia including 8 emulsifiable concentrates (ECs), 5 slow-release generators (SRs), 2 solid (SO) formulations, 1 veterinary oral paste (PA) and 2 liquid (LD) products. There were no toxicology studies on any Australian dichlorvos products that were submitted for evaluation as part of the current review. Therefore, safety directions for all currently registered products were reviewed based on the toxicology profile of each product constituent. The estimation of the toxicological hazard of these products and any consequent amendments or additions to the current FAISDs are discussed below.

It should be noted that a number of dichlorvos products contain a hydrocarbon solvent, which constitutes a significant proportion of the formulation (390-780 g/L). In all cases, the identity of this hydrocarbon solvent has not been provided (ie. the CAS Number), including the level of polycyclic aromatic hydrocarbons (PACs). The health concern with regard to PACs is their dermal carcinogenicity potential. Further, any fraction of liquid aromatic hydrocarbons that boils above 350°C is classifiable in Schedule 7 of the SUSDP except: (a) when in solid polymers; (b) when containing 1 per cent or less of total polycyclic aromatic compounds as measured by IP 346; or (c) when having a Mutagenicity Index of Zero as measured by ASTM E1687-95. Therefore, registrants of all dichlorvos products containing a hydrocarbon solvent must provide data on the polycyclic aromatic hydrocarbon content (measured by IP 346) or the mutagenicity index (measured by ASTM E1687-95) of the chemical. This information is necessary to ensure the appropriate schedule for products and therefore the appropriate signal heading on the label.

Emulsifiable concentrates (EC)

There are 8 ECs available only to licensed pesticide applicators. The toxicological hazard posed by these products varies somewhat due to differences in the presence of hydrocarbon solvent and/or surfactants. In particular, these products vary in their skin and eye irritancy, and their potential to irritate mucous membranes and the respiratory tract.

There are two ‘high-strength’ products containing 1140 g/L dichlorvos as the active constituent, which are in Schedule 7 of the SUSDP (Nufarm Dichlorvos 1140 Insecticide and Divap 1140 Insecticide). These are diluted in water to 0.5-1% and are applied as a spray to control insects infesting stored cereal grain. Both have witholding periods of 7 or 28 days (depending on the application rate) for grain used for processing into human food. Based on a consideration of the toxicity for each constituent in the products, they are considered to have high acute oral and dermal toxicity, moderate inhalational toxicity (aerosols and vapour) and to be moderate skin and eye irritants. These products are likely to be skin sensitisers. On this basis, the following amended hazard-based safety directions are appropriate:

Amended entry

EC ~~1140~~ 1200 g/L or less	
100	Very dangerous
~~120 121~~ 130 131 132 133	~~Product and spray are~~ Poisonous if absorbed by skin contact, inhaled or swallowed
161 162 164	Will irritate the eyes and skin
180	Repeated exposure may cause allergic disorders
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	Avoid contact with eyes and skin
220 **222** 223	Do not inhale **vapour or** spray mist
373	Obtain an emergency supply of atropine tablets 0.6 mg
330 331 332	If clothing becomes contaminated with product or wet with spray, remove clothing immediately
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water

NOTE: An evaluation of the personal protective equipment (PPE) necessary for the safe use of Nufarm Dichlorvos 1140 Insecticide, Divap 1140 Insecticide and Barmac Dichlorvos 500 Insecticide will be performed by the National Occupational Health and Safety commission (NOHSC).

There are 5 EC products containing 500 g/L dichlorvos as the active constituent and 455 g/L hydrocarbon solvent, and are in Schedule 6 of the SUSDP (David Grays DDVP 500 Insecticide; Divap 500 EC Insecticide; Chemag Dichlorvos Insecticide; Garrard's DDVP 500 EC Insecticide; and Barmac Dichlorvos 500 Insecticide). These products can be used undiluted, or diluted to 0.1-1% in water (as an emulsion) or kerosene (as a solution), and applied as a fog, coarse wet spray, mist or as a liquid bait. Indoor uses include dairies and cattle sheds, stables, piggeries, poultry manure, abattoirs, wineries, factories, stores, mills, food warehouses, stored grain, empty grain silos, infested grain, bagged and stored potatoes, avocadoes, tobacco stores, greenhouses, mushroom houses and in domestic households. Outdoor uses cover garbage dumps, beach, picnic and recreation areas, wasp nests in trees, uncultivated ground, rockeries and buildings. A 7-day witholding period applies to stored grain and avocadoes, and a 2-day witholding period applies to all other edible crops. For indoor applications, some label directions recommend that persons not re-enter treated areas until completely dry (~4 hours) and when well ventilated.

There is a single combinational EC product containing 250 g/L dichlorvos and 225 g/L chlorpyrifos as the active constituents (Permakill Insecticide), and 390 g/L hydrocarbon solvent. It is in Schedule 6 of the SUSDP. The product is diluted in water to approximately 5-6% dichlorvos and chlorpyrifos and applied as a low pressure spray to the point of run-off to cracks and crevices in residential, commercial and industrial premises. Label directions recommend that persons not re-enter treated areas until completely dry (~4 hours) and when well ventilated.

Based on a consideration of the toxicity for each constituent in these products, they are considered to have high acute oral and dermal toxicity, moderate inhalational toxicity (aerosols and vapour), to be moderate skin irritants, and moderate to severe eye irritants (dependening on whether both hydrocarbon solvent and surfactant are present). Product vapours are likely to irritate mucous membranes and the respiratory tract due to the presence of the hydrocarbon solvent. These products are likely to be skin sensitisers. On this basis, the following new hazard-based safety directions are appropriate:

New entry

EC 550 g/L or less with surfactant in hydrocarbon solvent 500 g/L or less	
100	Very dangerous
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 162	Will damage the eyes
161 163 164	Will irritate the nose and throat and skin
180	Repeated exposure may cause allergic disorders
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	
220 222 223	Avoid contact with eyes and skin
373	Do not inhale vapour or spray mist
330 331 332	Obtain an emergency supply of atropine tablets 0.6 mg
340 342	If clothing becomes contaminated with product or wet with spray remove clothing immediately
340 343	If product on skin, immediately wash area with soap and water
350	If product in eyes, wash it out immediately with water
	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water

NOTE: An evaluation of the PPE necessary for the safe use of David Grays DDVP 500 Insecticide, Divap 500 EC Insecticide and Chemag Dichlorvos Insecticide will be performed by the NOHSC.

New entry

EC 550 g/L or less in hydrocarbon solvent 500 g/L or less

100	Very dangerous
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
161 162 163 164	Will irritate the eyes, nose and throat, and skin
180	Repeated exposure may cause allergic disorders
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	
220 222 223	Avoid contact with eyes and skin
373	Do not inhale vapour or spray mist
330 331 332	Obtain an emergency supply of atropine tablets 0.6 mg
	If clothing becomes contaminated with product or wet with spray
340 342	remove clothing immediately
340 343	If product on skin, immediately wash area with soap and water
350	If product in eyes, wash it out immediately with water
	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water

NOTE: An evaluation of the PPE necessary for the safe use of Garrard's DDVP 500 EC Insecticide will be performed by the NOHSC.

New Entry

EC 300 g/L or less with chlorpyrifos 250 g/L or less with surfactant in hydrocarbon solvent 420 g/L or less	
100	Very dangerous
130 131 132 133	Poisonous if absorbed by skin contact, inhaled or swallowed
207 162	Will damage the eyes
161 163 164	Will irritate the nose and throat and skin
180	Repeated exposure may cause allergic disorders
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	Avoid contact with eyes and skin
220 222 223	Do not inhale vapour or spray mist
373	Obtain an emergency supply of atropine tablets 0.6 mg
330 331 332	If clothing becomes contaminated with product or wet with spray remove clothing immediately
340 342	If product on skin, immediately wash area with soap and water
340 343	If product in eyes, wash it out immediately with water
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water

NOTE: An evaluation of the PPE necessary for the safe use of Permakill Insecticide will be performed by the NOHSC.

Slow-release generators (SRs)

There are 5 SRs available for home garden use, which are formulated as dichlorvos-impregnated resin strips and are contained within a housing, typically made of plastic. Of the 5 product, 3 contain 186 g/kg dichlorvos and are in Schedule 5 of the SUSDP, while 2 higher strength products contain 328 g/kg dichlorvos and are in Schedule 6. Four SRs are available as “ministrips” and are used in confined areas (1 or 3 m3) such as wardrobes, bookcases, linen cupboards, under sinks and in toilets (Mortein Moth and Insect Strips, Mortein Moth Guard for Wardrobes and Drawers, Sureguard Ministrip Household Insecticide and Scuttle Bug Pest Strip). There is one SR used in rooms up to 30 m3 (Sureguard Pest strip Household Insecticide). Cautionary statements on product labels cover the avoidance of contact with clothes, not using the products in food storage cupboards or in infants rooms or sick rooms where people may be continually exposed. Product labels recommend replacing strips every 4 months.

Safety directions for these 5 SRs are already covered within the existing FAISD handbook. Based on a consideration of the toxicity for each constituent in these products, they are considered to have high oral and dermal toxicity, moderate inhalational toxicity and to be slight skin and eye irritants. These products are considered to be skin sensitisers. However, as the dichlorvos is impregnated in to a resin strip, which is contained within a plastic or cardboard housing, there is no potential for oral, skin and eye contact under normal conditions of use. While on theoretical grounds there is the possibility of dermal exposure to the vapour, such exposures are considered to be low and pose no significant risk to humans, based on the anticipated low air concentrations of dichlorvos and the short exposure durations. On this basis, the following amended hazard-based safety directions are appropriate:

Amended entry

SR 350 g/kg or less	
380	Do not open inner (envelope) (pouch) until ready for use
381	Do not remove the insecticidal strip from the plastic case
382	Do not allow children to play with the strip
351	Wash hands after use

Solid (SO) formulations

There are two SO formulations available for home garden use to control flies and maggots in outdoor garbage containers (Binkill and Baygon Outdoot Bin Guard). These products contain 80 g/kg dichlorvos and 800 g/kg naphthalene as the active constituents. Based on a consideration of the toxicity for each constituent in these products, they are considered to have high oral and dermal toxicity, moderate inhalational toxicity and to be slight skin and eye irritants. These products are considered to be skin sensitisers. However, the solid “block of naphthalene and dichlorvos” is contained within a plastic housing, which under normal conditions of use, prevents oral, dermal and ocular exposure. Their use outdoors also minimises any potential of inhalational exposure to vapours. There are currently no specific safety

directions in the FAISD handbook for these products. Based on a consideration of the toxicity for each constituent in the products, the following new entry is recommended.

New entry

SO 100 g/kg or less with Naphthalene 850 g/kg or less in plastic housing	
351	Wash hands after use

Liquid (LD)

Two LD products are available to licensed pesticide applicators. Insectigas®-D is formulated in carbon dioxide and is applied as a spray mist for insect control in domestic and commercial premises, stored product facilities (eg. warehouses, silos, farm machinery and storage bins) and in greenhouses. This product is in Schedule 6 of the SUSDP and contains dichlorvos at 50 g/L, with the remainder consisting of carbon dioxide (950 g/L). Based on a consideration of the toxicity for each constituent in the product, this product is considered to have high oral and dermal toxicity and moderate inhalational toxicity. It is considered to be a severe skin and eye irritant as contact with liquid carbon dioxide can cause cryogenic burns to the skin and eyes upon evaporation. This product is likely to be a skin sensitiser. On this basis, the following amended hazard-based safety directions are appropriate:

Amended entry

LD ~~50~~ 60 g/kg or less in compressed liquid carbon dioxide	
130 131 132 **133**	Poisonous if absorbed by skin contact or inhaled or swallowed
207 162 164	Will damage the eyes and skin
180	Repeated exposure may cause allergic disorders
190	Repeated minor exposure may have a cumulative poisoning effect
210 211	Avoid contact with eyes and skin
220 **222** 223	Do not inhale vapour or spray mist
373	Obtain an emergency supply of atropine tablets 0.6 mg
330 331 332	If clothing becomes contaminated with product or wet with spray remove clothing immediately
340 341 342	If product or spray on skin immediately wash area with soap and water
340 341 343	
350	If product or spray in eyes, wash it out immediately with water
	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water

NOTE: An evaluation of the PPE necessary for the safe use of Insectigas®-D will be performed by the NOHSC.

Knock-Down Residual Spray Insecticide contains dichlorvos (7 g/L), piperonyl butoxide (6 g/L), pyrethrins (1 g/L) and de-aromatised mineral solvent (780 g/L) as active constituents and is in Schedule 5 of the SUSDP. It is applied as a spray to control crawling insects in houses, hospitals, factories and restaurants. There is currently no entry in the FAISD handbook to cover the safety directions for this particular product. Based on a consideration of the toxicity for each constituent in the product, it is considered to have very low oral, dermal and inhalational toxicity, to be a moderate skin and eye irritant and a skin sensitiser. The high liquid hydrocarbon content of this product is likely to generate vapours, which may irritate mucous membranes and the respiratory tract. On this basis, the following new hazard-based safety directions are appropriate:

New entry

LD 10 g/L or less with pyrethrins 2 g/L or less in hydrocarbon solvent 800 g/L or less	
161 162 163 164	Will irritate the eyes and nose and throat and skin
180	Repeated exposure may cause allergic disorders
190	Repeated minor exposure may have a cumulative poisoning effect
373	Obtain an emergency supply of atropine tablets 0.6 mg
210 211	Avoid contact with the eyes and skin
220 222 223	Do not inhale vapour or spray mist
272	Ensure adequate ventilation during use
350	After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with sopa and water

NOTE: An evaluation of the PPE necessary for the safe use of Knock-Down Residual Spray Insecticide will be performed by the NOHSC.

Oral Paste (PA)

There is a single PA formulation used for the treatment of adult roundworms, blood worms, red worms, pin worms and bots in horses (Oximinth Plus Boticide Oral Worm amd Bot Paste for Horses). This product contains 2.5 g/25 mL dichlorvos (ie. 100 g/L) and 5 g/25 mL oxibendazole as the active constituents and is in Schedule 6 of the SUSDP. It is supplied in a 25 mL syringe (for the treatment of a 500 kg horse), which is placed on the horses tongue and the plunger pressed to administer the required dose. The label directions indicate that the horses mouth should be kept closed until the paste has been swallowed.

Based on a consideration of the toxicity for each constituent in the product, it is considered to have high oral, moderate dermal and very low inhalational toxicity. It is also likely to be a slight skin and eye irritant and a skin sensitiser. However, the manner in which the product is supplied (ie. in a plastic syringe) and used, mitigates the risk of oral, inhalational and ocular exposure. However, there is some potential for dermal exposure of the hands during application to the horses tongue and through restraining the horses mouth to ensure all the paste is swallowed. Consequently, there is also the potential for hand-to-mouth and hand-to-eye transfer. Another consideration is the presence of the toffee flavour - although obviously designed to improve the palatability of the product to horses it also increases the potential of misuse by humans because of the pleasant toffee flavour. On this basis, the following new hazard-based safety directions are appropriate:

New Entry

PA 120 g/L or less with oxibendazole 230 g/L or less	
130 131 133	Poisonous if absorbed by skin contact or swallowed
160 162 164	Will irritate the eyes and skin
180	Repeated exposure may cause allergic disorders
190	Repeated minor exposure may have a cumulative poisoning effect
373	
210 211	Obtain an emergency supply of atropine tablets 0.6 mg
340 342	Avoid contact with eyes and skin
351	If product on skin, immediately wash area with soap and water
	Wash hands after use

Existing safety directions for dichlorvos as they appear in the FAISD handbook contain entries for AE 50 g/kg or less and BA 10 g/kg or less (aerosol and bait formulations, respectively). As there are no longer any of these types of dichlorvos products registered for use in Australia, these entries are no longer considered appropriate and should be deleted from the FAISD handbook.

MAIN TOXICOLOGY REPORT

1. INTRODUCTION

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is an organophosphorus (OP) insecticide, and like other OPs, its insecticidal activity is due to its ability to inhibit nerve conductance. A similar mechanism of toxicity occurs in mammals involving the inhibition of acetylcholinesterase at nerve terminals, which causes accumulation of acetyl choline that in turn over stimulates nicotinic and muscarinic receptors in the central and peripheral nervous system.

First described as an impurity of trichlofon, dichlorvos was first synthesised in the late 1940s and began commercial production in 1961. Dichlorvos has been available in Australia for many years. It is used in storage areas in domestic, commercial and industrial premises. Its main food use is for stored grain and potatoes, while it is also applied to animal, green, glass and mushroom houses. Outdoor use includes the treatment of garbage bins, picnic areas and rockeries. Home garden products formulated as peststrips (and ministrips) or solid tablets are available for use in rooms or non-food storage areas such as wardrobes and drawers, or in outside garbage bins.

1.1 History of Public Health Considerations of Dichlorvos in Australia

A detailed history of the public health considerations of dichlorvos by Australian regulatory committees is detailed in Appendix I.

The Australian Department of Health and Ageing (DoHA) has reviewed the toxicology of dichlorvos on a number of occasions. In 1985, the National Health and Medical Research Council (NHMRC) convened a working party to review the toxicology of dichlorvos in response to concerns raised by the Australian Conservation Foundation over the DNA methylating ability of dichlorvos and its mutagenicity in bacteria. This Working Party reported its findings to the 103rd Session of Council in June 1987 which noted that the review did not identify any significant health hazards with the then approved uses of dichlorvos, including its use in household pest strips.

The former NHMRC Standing Committee on Toxicity (SCOT) evaluated dichlorvos in 1988, 1989 and 1991. The SCOT reviewed cancer and genotoxicity data and, in particular, the results of chronic oral dosing studies (gavage) conducted in rats and mice by the National Toxicology Program (NTP), within the US Department of Health and Human Services. Reports of these studies were released in 1989 following several rounds of peer review by the NTP since the first draft was released in 1987. The outcome of these reviews was the conclusion that dichlorvos is carcinogenic in experimental animals and therefore should be regarded as a potential carcinogen in humans if taken orally. However, the SCOT considered that there was insufficient data to determine the carcinogenicity hazard posed via dermal or inhalational administration in humans (the most likely routes of exposure) and that the NDPSC should review use patterns to minimise dermal and inhalation exposure. These routes of exposure were considered to be important in the assessment of human risk as dichlorvos is widely used in vapour pest strips because of its relatively high volatility compared with other OPs (2100 mPa at 25°C compared to 18 mPA for fenitrothion, 12 mPA for diazinon, 2.7 mPA for chlorpyrifos, 0.74 for fenthion and 0.41 for parathion-methyl).
More recently, the Advisory Committee on Pesticides and Health (ACPH) has considered toxicological and public health assessments conducted by toxicologists within the DoHA. In 1996, the ACPH concluded that dichlorvos is unlikely to adversely affect humans or pose a hazard if used in accordance with label directions. Although dichlorvos was genotoxic in vitro, chronic exposure studies simulating the most likely route of exposure for humans, namely by inhalation, did not result in an increased tumour incidence. Hence the risk for humans was considered to be minimal. This position was reaffirmed at the 1998 meeting where it was decided that the carcinogenicity findings in rodents following oral gavage administration of dichlorvos were not necessarily relevant to the assessment of risk to human health. The resolutions from 1996 and 1998 ACPH discussion on dichlorvos are as follows:

At the 9th Meeting (16th February 1996), the Committee:

NOTED that the UK MAFF also considered that the weight of evidence does not suggest that dichlorvos poses a carcinogenic risk for humans;

CONSIDERED that as a basic public health principle, people should not be continuously exposed to pesticides in and around homes; and

RECOMMENDED that the Environmental Health and Safety Unit in collaboration with the National Registration Authority for Agricultural and Veterinary Chemicals review the labels of dichlorvos products registered for non-agricultural use with a view to ensuring the 'directions for use' discourage situations in which continuing (long-term) exposure could occur.

At the 16th Meeting (29th October 1998), the Committee

CONSIDERED that equivocal carcinogenicity findings in rodents following oral gavage administration of dichlorvos were not necessarily relevant to the assessment of risk to human health;

SUPPORTED a revision to the (then) existing acceptable daily intake (ADI) of 0.0005 to 0.001 mg/kg bw/day on the basis of a No-Observed-Effect-Level (NOEL) of 0.014 mg/kg bw/day for plasma cholinesterase (ChE) inhibition in human volunteers from a 28-day oral administration study;

REVIEWED whether there is a need to alter the previous recommendation to limit long term human exposure to dichlorvos in light of the revised toxicological assessment; and

ASKED that the available exposure data for household dichlorvos products be examined and overall risk assessed to determine whether there is a need to modify the previous recommendation to the NRA.

ADI and ARfD

The current Australian ADI of 0.001 mg/kg bw/d was set in 1999. It is based on the NOEL of 0.014 mg/kg bw/d in a 28-day oral dosing study in humans for the inhibition of plasma ChE activity (Rider et al 1967) and using a 10-fold intraspecies safety factor.

The current Australian ARfD for dichlorvos is 0.003 mg/kg bw and was set in 2000 using the same study used as the basis for the ADI (Rider 1967). It was calculated by applying a 10-fold intraspecies safety factor to the NOEL of 0.033 mg/kg bw for the inhibition of RBC ChE activity 24 hours after the first dose.

Poisons Schedule

At present, dichlorvos is listed in Schedule 7 of the SUSDP. There are two lower schedule entries to accommodate dilute preparations: a Schedule 6 entry for preparations containing 50% or less of dichlorvos; and a Schedule 5 entry for dichlorvos impregnated in plastic resin strip material containing 20% or less of dichlorvos, in sustained release pellets containing 20% or less of dichlorvos for the treatment of animals and in pressurised spray packs containing 10 g or less of dichlorvos.

Drinking Water Quality Guidelines

The Australian Drinking Water Guidelines (ADWG) are a joint publication of the National NHMRC and the Agricultural and Resource Management Council of Australia and New Zealand (see Australian Drinking Water Guidelines - 1996; ISBN 0 642 24462 6 or http://www.nhmrc.gov.au/publications/pdf/eh19.pdf). The ADGW are not legally enforceable but rather provide a standard for water authorities and State health authorities to ensure the quality and safety of Australia's drinking water.

The Guideline Value (mg/L) is analogous to an MRL in food and is generally based on the analytical limit of determination. It is set at a level consistent with good water management practice and that would not result in any significant risk to the consumer over a lifetime of consumption. If a pesticide is detected at or above this value then the source should be identified and action taken to prevent further contamination. The current Guideline Value for dichlorvos is 0.001 mg/L.

The Health Value (also expressed as mg/L) is intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The health values are derived so as to limit intake from water alone to approximately 10% of the ADI, on the assumption that

(based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg having a daily water consumption of 2 L over a lifetime. The current Health Value for dichlorvos is 0.001 mg/L

1.2 International Toxicology Assessments

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR)

Dichlorvos has been reviewed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1965, 1966, 1967, 1970, 1977 and 1993. An ADI of 0.004 mg/kg bw/d was established in 1966 and was reaffirmed at each subsequent meeting. The ADI was calculated by applying a 10-fold safety factor to the NOAEL of 0.04 mg/kg bw/d, based on the absence of the inhibition of RBC ChE activity at this dose in a 21-day human study. As of March 2004, the JMPR has neither considered nor set an ARfD for dichlorvos.

The United States Environmental Protection Agency (US EPA)

The US EPA commenced a special review of dichlorvos products in 1988 due to concerns over carcinogenicity and possible inadequate margins of safety for ChE inhibition and liver effects. In 1995, it was concluded that: dichlorvos posed carcinogenic risks of concern to the general population from dietary exposure; and posed risks of concern for ChE inhibition to residents and workers involved with mixing loading and applying dichlorvos, and also to persons re-entering treated areas. In order to reduce these risks, certain uses were cancelled and labels were modified.

In light of the Food Quality protection Act (1996), the US EPA re-evaluated the toxicology of dichlorvos and examined new information relating to dietary risk. A revised human health risk assessment was released in August 2000. No dietary cancer risks for dichlorvos were estimated. The carcinogenic potential of dichlorvos was classified as “suggestive” under the 1999 draft US EPA cancer guidelines. An ARfD was set at 0.002 mg/kg bw, based on the NOAEL of 0.5 mg/kg bw for alterations in FOB, decreased motor activity, catalepsy and reduction in body temperature at 35 mg/kg bw in an acute neurotoxicity study in rats and using a 300-fold uncertainty factor. The 300-fold uncertainty factor included a 10-fold factor for intraspecies variation, a 10-fold factor for interspecies variation and an additional 3-fold factor due to the lack of ChE measurement. The chronic reference dose (analogous to an ADI) was set at 0.0005 mg/kg bw/d based on the NOAEL of 0.05 mg/kg bw/d for inhibition of plasma and RBC ChE inhibition in males and females, and inhibition of brain ChE activity in males at 0.1 mg/kg bw/d in a 1-year dog study and using a 100-fold uncertainty factor (10-fold factors for intra- and interspecies variation).

International Agency for Research on Cancer (IARC)

IARC has classified dichlorvos in Group 2B (possibly carcinogenic to humans) based on inadequate evidence in humans and sufficient evidence in experimental animals (IARC 1991).

International Program on Chemical Safety (IPCS)

The International Program on Chemical Safety (IPCS) of the WHO published an Environmental Health Criteria (EHC) monograph in 1989 (EHC 79) covering the human health and safety aspects of dichlorvos.

Regulatory status in the UK

In 2002, the UK Department for Environment, Food and Rural Affairs suspended the sale of a range of agricultural, professional and domestic insecticide products containing dichlorvos. This decision was taken following the advice of the Advisory Committee on Pesticides (ACP), an independent expert scientific advisory Committee. The ACP was concerned that it could not satisfactorily rule out the possibility that dichlorvos causes cancer by directly acting on the genetic material. Therefore, a precautionary approach was adopted until acceptable data regarding the mutagenic and carcinogenic potential of dichlorvos can be presented to the ACP.

1.3 Chemistry - Active Constituent

Approved common name: dichlorvos (ISO)

Alternative names: Vapona, DDVP

Chemical name: 2,2-dichloroethenyl dimethyl phosphate (CAS)

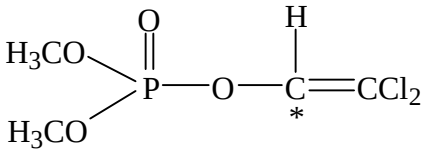
2,2-dichlorovinyl dimethyl phosphate (IUPAC)

CAS Registry number: 62-73-7

Empirical formula: $C_4H_7Cl_2O_4P$

Molecular weight: 221.0

Chemical structure:



*denotes position of the radiolabel in [^{14}C-vinyl]dichlorvos

Chemical class: Organophosphate

Structural analogues: None

Chemical and physical properties

Colour:	Colourless to amber
Odour:	Aromatic
Physical state:	Liquid
Boiling point:	234.1°C
Density (20^0C):	1.425
n-Octanol/water partition coefficient (log K_{ow}):	1.16
Vapour pressure at 25°C:	2.1 Pa
Solubility:	
in water:	8 g/L at 25°C
in organic solvents:	Completely miscible with aromatic hydrocarbons, chlorinated hydrocarbons and alcohols; moderately soluble in diesel oil, kerosene, isoparaffinic hydrocarbons, and mineral oils.
Stability:	Stable to heat. Slowly hydrolysed in water and in acidic media, and rapidly hydrolysed by alkalis to dimethyl hydrogen phosphate and dichloroacetaldehyde; DT50 (estimated) 31.9 d (pH 4), 2.9d (pH 7), 2.0 d (pH 9) (22°C)

Active constituent - Declaration of Composition and Batch Analysis

Impurities of Toxicological Concern

Dichlorvos active constituent contains no impurities of toxicological concern.

1.4 Products

In Australia, there are currently 14 registrants for 18 dichlorvos products.

2. METABOLISM AND TOXICOKINETICS

Casida J, McBride L & Niedermeier RP (1962) Metabolism of 2,2-dichlorovinyl dimethyl phosphate in relation to residues in milk and mammalian tissues. J Agric Food Chem 10: 370-377

The metabolism of radiolabelled dichlorvos in rats, cows and a goat was investigated in a series of experiments (see summary Table below).

Experimental summary

Species	Dose	Observations
Female rats	1.1 mg/kg bw [^{14}C-vinyl]dichlorvos, dichloroacetaldehyde or dichloroethanol	Rats placed in metabolism chambers for 24 hours for analysis of exhaled metabolites
Atropinised female rats	4 mg/kg bw [^{14}C-vinyl]dichlorvos ip or po	Maintained for 7 days. Urine and faecal samples collected. Tissues collected and extracted.
Male and female rats	10 mg/kg bw [^{32}P-methyl]dichlorvos, po	Individual rats sacrificed after 15 min, 1, 4 and 14 h, and 1, 4 and 7 days for tissue extraction and analysis.
Rats (n=1)	500 mg/kg bw [^{32}P]desmethyl dichlorvos or [^{32}P]dimethyl phosphate, po	Sacrificed after 90 h later for assessment of tissue distribution.
Guernsey cows (n=4)	1 or 20 mg/kg [^{32}P-methyl]dichlorvos, po (gelatine capsule); 1 mg/kg bw [^{32}P-methyl]dichlorvos, iv or sc	Radioactivity measured in milk, faeces and urine
Goat (n=1)	1.52 .g/kg bw [^{32}P-methyl]dichlorvos, sc	

^{32}P-dichlorvos given orally to male and female rats at 10 mg/kg BW was rapidly absorbed and distributed to various tissues. Highest blood (6-7 ppm) and stomach (123-130 ppm) levels of ^{32}P (expressed as radioactive equivalents) were evident in rats sacrificed after 15 min and 1 h. Peak small intestine (88 ppm), liver (60 ppm) and kidney (26 ppm) levels occurred 1 h after dosing. Other organs and tissues contained less ^{32}P, with the highest levels detected in bone (6 ppm after 1 h), brain (5 ppm after 1 h) and heart (6 ppm after 4 h). The highest levels after 7 days were found in bone (11 ppm), kidney (2.5 ppm) and liver (2.7 ppm). This tissue distribution reflected rapid metabolism (hydrolysis) to inorganic ^{32}P, which was incorporated into tissues.

Almost all the radioactive dose was eliminated in 90 h in the rat given [^{32}P]dimethyl phosphate orally. Urine contained only unchanged dimethyl phosphate (approximately half the dose) and no metabolites were present. The rat given [^{32}P]desmethyl dichlorvos excreted approximately 14% of the dose via the urine within 90 h; 86% of this as phosphoric acid and 14% as unchanged parent compound. Tissue distribution was similar to that of [^{32}P-methyl]dichlorvos.

Recovery of radioactivity within 7 days in faeces and urine was extensive in all animals given [^{32}P]dichlorvos; 100% from the goat (sc administration), 68–91% in cows (oral, subcutaenous and intravenous), and 71–81% in rats (oral). Urine was the principal route of excretion accounting for >60% of the dose in rats (both sexes), with 11-17% recovered from faeces. ^{32}P-containing urinary metabolites were predominantly mono- and dimethyl-phosphates (>80%), with desmethyl dichlorvos constituting <20%. The level of these urinary metabolites decreased over time. In contrast, the proportion (of radioactivity) attributable to inorganic phosphate increased in later urine samples.

The levels of [^{32}P]-metabolites in milk were measured in cows and goats in samples collected at intervals from 15 min to 144 h after dosing. Low levels of radioactivity were present in samples taken before 1-2 h. Peak radioactivity was observed between 4-12 h after subcutaneous and intravenous dosing, and at 12-24 h after oral dosing. The highest levels in cows treated at 1 mg/kg bw intravenously and subcutaneously were 2.51 and 2.15 ppm respectively; the cows treated orally at 1 and 20 mg/kg bw showed milk levels of 0.6 and 11.1 ppm after 24 and 12 h, respectively. Organosoluble ^{32}P was present in very small amounts only during the first hour after dosing.

In female rats administered 4 mg/kg bw [^{14}C-vinyl]dichlorvos orally or intraperitonealy, the major elimination routes were expired air and urine; 16% of the dose was eliminated as $^{14}CO_2$ in 24 h and 30% in the urine within 7 days (3% was excreted in faeces in 7 days). Substantial radioactivity remained in the tissues of rats sacrificed 7 days after dosing at 4 mg/kg (po or ip), with the highest levels found in the liver

(approximately 3 ppm), blood (1 ppm), kidney (0.5 ppm), small and large intestines (0.4 ppm) and heart (0.3 ppm). Lower levels were evident in skeletal muscle, fat and brain.

More than 90% of administered radioactivity appeared as a conjugate of dichloroethanol in the urine of rats given [^{14}C-vinyl]dichlorvos. This was split by acid reflux and β-glucuronidase, and was thus probably dichloroethyl glucuronide. A small amount of labelled dichloroacetaldehyde and/or desmethyl dichlorvos (<2% of the total) was evident. Little if any unconjugated dichloroethanol was present and there was no unchanged dichlorvos found in urine.

Hutson DH, Hoadley EC & Pickering BA (1971) The metabolic fate of [vinyl-I-14C] dichlorvos in the rat after oral and inhalation exposure. Xenobiotica 1: 503-611

Inhalation exposure: Three male rats were exposed to [^{14}C-vinyl]dichlorvos vapour (19 μCi/mg, Shell Chemical Co.) in which only their heads were exposed at a rate of 200 mL/min for 1 h. Summation of recovered label indicated that the rats received 1.07, 0.81 and 0.71 mg of dichlorvos. Following exposure, rats were placed in metabolism cages for 4 days. Respired air accounted for the greatest proportion of eliminated label and most label was recovered within 24 h. Compared to the total amount of $^{14}CO_2$ accumulated in 4 days, urine accounted for 38.4% and faeces 10.1%. Liver, gut, skin and carcass contained 10.3, 3.7, 25.6 and 34.6% of the total amount of $^{14}CO_2$, respectively.

Oral and intraperitoneal administration: Five female rats were orally dosed with 3.13 mg (approximately 12.5 mg/kg bw) [^{14}C-vinyl]dichlorvos in arachis oil and were maintained for 4 days in metabolism cages for collection of excreta. In addition, 2 female rats were given 37.5 mg (approximately 150 mg/kg bw) of sodium 2,2-dichloro-[1-^{14}C]vinyl methyl phosphate orally (in water), and 1 female was given 18.8 mg of [^{36}Cl]-dichlorvos by injection into the right groin (this animal also received 4.3 mg atropine and 22.5 mg obidoxime to protect it from the acute toxicity of dichlorvos).

Paper chromatograms of urine from the rats given [^{14}C-vinyl]-labelled or [^{36}Cl]-labelled dichlorvos orally showed free ^{36}Cl ion and at least 7 metabolites containing ^{14}C (4 of which also contained ^{36}Cl). The parent compound was not identified. Metabolite A constituted 8.3% of urinary radioactivity (approximately 2% of the dose) and was identified as hippuric acid. This was also found in the urine of rats given desmethyl dichlorvos orally. Metabolite B (10.9% of urinary radioactivity) was identical to desmethyl dichlorvos. The probable identity of metabolite C was dichloroethanol glucuronide (2,2-dichloroethyl-β-D-glucopyranosiduronic acid), determined by paper chromatography and evaporation following incubation with β-glucuronidase. Radio-scanning of paper chromatograms indicated this constituted 27% of urinary radioactivity.

The structures of other metabolites were not identified. Metabolite D, making up 25% of urinary radioactivity was separated by prolonged paper chromatography into 3 components. Metabolite E was 4% of urinary radioactivity (ie. 1% of the dose) and metabolites F and G made up 7 and 5% of urinary radioactivity, respectively. The presence of dichloroacetaldehyde and dichloroacetic acid could not be demonstrated: ^{14}C-urea made up 3.1% of total urinary metabolism.

The livers from 5 female rats treated with [^{14}C-vinyl]dichlorvos (0.72 mg po, approximately 3 mg/kg bw) were obtained 4 days after dosing, then homogenised and separated into soluble, lipid, nucleic acid and protein fractions. These fractions contained 10.9, 11.8, 2.7 and 74.6% of the total radioactivity respectively. Most of the protein fraction was made up of ^{14}C-label incorporated into glycine and serine.

Analysis of urine after ip administration of 0.67 mg [^{14}C-vinyl]dichlorvos in 0.5 mL water-ethanol indicated the presence of small amounts of hippuric acid and desmethyl dichlorvos (2-5%), and dichloroethanol glucuronide present at a high level (76% of total urinary radioactivity). No unchanged dichlorvos was identified.

Metabolites present in 0-24 h urine of rats following inhalation exposure were hippuric acid (9.3% of total urine radioactivity), desmethyl-dichlorvos (4.3%), and urea (5.3%). Unchanged dichlorvos was not found.

Hutson DH & Hoadley EC (1972a) The comparative metabolism of [^{14}C-vinyl] dichlorvos in animals and man. Arch Toxikol 30: 9-18

Hutson DH & Hoadley EC (1972b) The metabolism of [^{14}C-methyl]dichlorvos in the rat and mouse . Xenobiotica 2:107-116

In a series of experiments, [^{14}C-vinyl]dichlorvos (19 μCi/mg, Shell Chemical Co.) was given by gavage to male and female mice (0.2 mg, approximately 8 mg/kg), and Syrian hamsters (0.22–0.56 mg,

approximately 4 mg/kg bw), and to one man orally (5 mg in 100 mL orange juice, approximately 70 μg/kg bw) and another by inhalation (38 mg/m^3 unlabelled dichlorvos for 105 min). Elimination rates and urinary excretion were compared with results obtained from a second experiment using rats (Hutson and Hoadley 1972b).

Approximately 90% of the administered radioactivity was recovered in 24 h in mice; approximately 30% in each of the urine, respired air and carcass, and 3% in faeces. In hamsters during the same period, approximately 20% was recovered in urine, over 30-40% in expired CO_2, and approximately 3% in faeces. In the man given 5 mg labelled dichlorvos, approximately 27% was eliminated in CO_2 in 0-8 h, compared to only 9% in 0-48 h urine. Data given for rats indicated that excretion via the urine constituted approximately 10%, faeces 1.5% and expired CO_2 approximately 30% in 0-24 h. Overall, the data indicated that the rates and routes of elimination were largely similar (predominantly air and urine) in all species and there was no marked sex difference in the laboratory animals.

Paper chromatography and isotope dilution analysis were used to identify metabolites following oral administration of [^{14}C-methoxy]dichlorvos in rats and mice given 3.3 and 25 mg/kg bw orally, respectively. The major urinary metabolite in both species was dimethyl phosphate, constituting approximately 70% of urinary radioactivity (approximately 40% of the dose). Desmethyl dichlorvos constituted 4% of total urinary radioactivity in rats and 28% in mice. Minor metabolites present at less than 2% of total urinary radioactivity were S-methyl-L-cysteine (both species), S-methyl-L-cysteine oxide and methylmercapturic acid (mouse only), and methylmercapturic acid S-oxide (rat only). Two other minor chromatographic peaks were not identified.

Similar techniques were used to identify metabolites of [^{14}C-vinyl]dichlorvos in hamsters (given 4 mg/kg bw, po), mice (8 mg/kg bw, po) and a man (5 mg po). Hippuric acid, desmethyl dichlorvos and urea radiolabelled metabolites were found in the urine of mice and the man, with only hippuric acid measured in hamsters. A dichloroethanol conjugate in the urine of a man exposed to dichlorvos by inhalation (38 μg/L for 105 min) was detectable by GLC but quantification was not possible because of interference from endogenous peaks.

Blair D, Hoadley EC & Hutson DH (1975) The distribution of dichlorvos in the tissues of mammals after inhalation or intravenous administration. Toxicol Appl Pharmacol 31: 243-253

Groups of male CFE rats were exposed to atmospheres containing different concentrations of dichlorvos vapour: 0.05 or 0.5 mg/m^3 (both in open chambers for 14 days), 50 mg/m^3 for 2-4 h, and 10 and 90 mg/m^3 for 4 h in glass tubes allowing head-only exposure. Female rats and male and female mice were exposed only at the highest concentration. Three groups of 3 male rats exposed to 50 mg/m^3 for 4 h were subsequently maintained in clean laboratory air for 15, 30 and 60 min after exposure before they were sacrificed and their kidneys analysed for dichlorvos content. Other animals were sacrificed immediately after exposure for dissection and tissue analysis.

In addition to the animal experiments, 2 male human subjects were exposed in a 20 m^3 chamber to atmospheres containing 0.25 mg/m^3 for 10 h or 0.7 mg/m^3 for 20 h. Blood was taken for analysis within 1 min of the men leaving the chambers but dichlorvos could not be detected. In both animal and human experiments, blood and/or tissue were analysed for dichlorvos using GLC with a quantification limit of 10 ng/g tissue.

At low concentrations (<1.0 μg/g) recovery of dichlorvos from blood and tissues was difficult because the compound was rapidly degraded. Thus, at low concentrations, results were reported in terms of extractable dichlorvos rather than real values representing accurate tissue concentrations.

Low tissue concentrations of dichlorvos were found in blood and tissues of rats and mice sacrificed immediately after exposure to atmospheres of 90 mg/m^3 for 4 h. The highest levels were in the kidneys (0.54 μg/g in female mice to 2.39 μg/g in male rats). Concentrations of approximately 1 μg/g were found in the fat of male mice but values were lower in female mice and in rats. Low concentrations (generally <0.2 μg/g or less) were found in blood, liver, testes, lung and brain of rats and mice. No dichlorvos was found in tissues of rats exposed to 10 mg/m^3 atmospheres for 4 h, or in blood or tissues exposed to 0.05 or 0.5 mg/m^3 for 14 days. Mean kidney concentrations of dichlorvos from 5 male rats/group ranged from 1.63 ± 0.22 μg/g in a group sacrificed immediately after 4 h exposure, to 0.10 ± 0.04 μg/g in a group sacrificed 60 min later.

The distribution of dichlorvos in rats following intravenous injection was examined to provide a comparison with inhalation exposure. Male rats were given dichlorvos dissolved in infonutrol at a dose level of 0.83 mg/kg bw injected into the tail vein. Three animals were sacrificed after 10 min and a further 3 sacrificed 30

min after the injection for tissue analysis. Little or no dichlorvos (detection limit 0.01 μg/g) was found in liver, fat, testes or brain at either time. Dichlorvos was detected in blood in only 1/3 animals at either time interval, with levels of 0.16-0.15 recorded respectively (detection limit 0.10 μg/mL). Kidneys contained 1.44-2.25 μg/g after 10 min, declining to 0.15-0.80 μg/g after 30 min, indicating rapid biotransformation of dichlorvos.

Overall, these data indicated that dichlorvos was rapidly metabolised *in vivo*.

Cheng T (1989) Metabolism of ^{14}C-DDVP in rats (preliminary and definitive phases). HLA study No. 6274-105. Lab: Hazleton Laboratories America Inc., Madison, Wisconsin, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 18th October 1988 to 24th January 1989. Report date: 30th August 1989.

Cheng T (1991) Supplement to: Metabolism of ^{14}C-DDVP in rats (preliminary and definitive phases) (MRID 41228701). HLA study No. 6274-105-1. Lab: Hazleton Laboratories America Inc., Madison, Wisconsin, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 21st August 1989 to 27th November 1990. Report date: 28th March 1991.

GLP compliant (US EPA; 40 CFR Part 160) and QA study.

Materials and Methods

In a preliminary study, [vinyl-1-^{14}C] dimethyl dichlorovinylphosphate (^{14}C-dichlorvos) (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 2534-039; 100% purity; 12.9 mCi/mmol specific activity) was administered to Crl:CD®(SD)BR rats (2/sex; age unspecified; Charles River Laboratories Inc., Portage, Michigan, USA) as a single gavage dose at 1.2 mg/kg bw in deionised water. The bodyweights of the two males were 221 and 236 g, while the bodyweights of the two females were 193 and 212 g.

In the main study, 5 rats/sex/group were administered ^{14}C-dichlorvos as a single intravenous dose of 1.0 mg/kg bw, a single gavage dose of 0.8 or 21.0 mg/kg bw, or 15 daily gavage doses of 0.8 mg/kg bw unlabelled dichlorvos (lot No. KB-40-10-4) followed by a single radiolabelled dose of 0.8 mg/kg bw on the 16th day. A control group consisted of 2 untreated rats. The bodyweights of males and females ranged from 213-330 and 189-246 g, respectively. The radiochemical purity and stability of ^{14}C-dichlorvos was analysed by Thin Layer Chromatography (TLC) and Gas Chromatography (GC). Dose solutions of ^{14}C-dichlorvos and dichlorvos were prepared from methylene chloride stock solutions on the day of dosing.

Rats were acclimatised for an unspecified period and housed individually under standard conditions. Rats were randomly assigned to treatment groups based bw. Prior to dosing rats were housed overnight in metabolism cages and fasted until 4 hours postdose, after which time they remained in the metabolism cages for the collection of expired air, urine and faeces. Purina Certified Rodent Chow® #5002 (Ralston Purina Company, St Louis, Missouri, USA) and water (unspecified source) were available *ad libitum*.

Rats were observed at least twice daily for mortalities and clinical signs. Bodyweights were recorded at the commencement of dosing and thereafter on a weekly basis. Expired CO_2 was trapped in a solution of ethanolamine:ethoxyethanol (1:3), with activated charcoal used to trap expired organic volatiles (preliminary study only). During the preliminary study, CO_2, organic volatiles, urine and faeces were collected at 0-12 and 12-24 hours postdose, then daily for 7 days. During the main study, CO_2, urine and faeces were collected at 0-6, 6-12 and 12-24 hours postdose, then daily for 7 days. Rats were sacrificed at 7 days postdose by halothane anaesthesia and exsanguinated. Blood was collected during the main study. The following tissues were collected and analysed for total radioactivity: bone (femur), brain, fat, heart, kidneys, liver, lungs, muscle (thigh), ovaries, pancreas, spleen, testes, uterus and the residual carcass. Cages were washed with a sodium phosphate solution, which was then analysed for radioactivity.

Radioactivity was analysed by liquid scintillation counting (LSC). Samples of CO_2, urine and the cage washes were analysed directly. Tissue and faecal samples were homogenised prior to combustion and then LSC. Blood samples were combusted prior to LSC. Data were expressed as means and standard deviations.

For the analysis of dichlorvos metabolites, faecal and urine samples collected at 0-24 hours postdose were pooled by dose group and sex. Faecal samples were extracted with methanol, dried then combusted. The total radioactivity in each pooled urine and faecal sample was determined by LSC. ^{14}C residues in urine and faecal samples were characterised by TLC and subsequently quantified by LSC. Hippuric acid and urea were used as analytical standards. Selected urine samples were treated with β-glucuronidase, subjected to ethyl acetate or methanol extraction then analysed by GC or TLC. Control samples were not

treated with β-glucuronidase. Metabolites were also isolated using a flash chromatographic method, characterised by preparative TLC, then quantified by LSC. Metabolites were also analysed by MS.

Results

Preliminary study: Approximately 95% of radioactivity was recovered. The highest level (expressed as the mean % recovery ± 1 SD) was detected in expired CO_2 (35.6±2.60%), followed by the carcass (25.9±2.42%), urine (16.4±2.29%), faeces (13.1±2.57%), the tubing used to connect the trap system (3.4±1.0%), cage wash (0.22±0.114%) and volatiles (0.06±0.039%). There was no apparent difference in the level of radioactivity between males and females. Given the low proportion of volatile organics trapped by the charcoal filter, it was not used in the main study.

Main study: ^{14}C-dichlorvos and dichlorvos were reportedly stable in methylene chloride for the duration of dosing. One female from the 21.0 mg/kg bw group died at 2.5 hours postdose, while all other rats in this group displayed cholinergic signs (tremors and salivation). Dark urine samples were noted on a number of occasions in 3 males and 3 females given the single intravenous dose of 1.0 mg/kg bw, and in two males from the 0.8 mg/kg bw repeat-dose group.

There was almost complete recovery of radioactivity (89-98%), with the maximum level detected in expired CO_2 (40-58%), followed by the carcass (13-26%), urine (10-15%) and faeces (4-7%) (see Table below). The liver contained 3.5-4.8%, with all remaining tissues containing 1-1.5%. The cage wash contained 0.06-0.36%. There was no apparent difference in the distribution of radioactivity following oral or intravenous dosing and there did not appear to be any difference between the low and high oral doses, or the repeat oral dose. There was no sex differences.

Recovery of radioactivity (%) 7 days after the administration of ^{14}C-dichlorvos

Group	Urine	Cage wash[1]	Faeces	Carcass	Liver	Tissues[2]	CO_2	TOTAL
1.0 mg/kg bw, single iv dose								
Males	15±3.0	0.18±0.087	4.7±2.6	26±3	4.8±0.65	1.5±0.27	40±2	92±1.1
Females	13±2.4	0.20±0.079	5.8±2.3	17±1.3	4.6±0.32	1.4±0.07	50±1.5	92±1.8
0.8 mg/kg bw, single po dose								
Males	14±1.1	0.06±0.057	4.2±0.9	24±0.89	3.5±0.20	1.4+0.14	41±3.0	89±4.3
Females	10±2.9	0.13±0.13	7.1±2.6	13±1.2	3.9±0.33	1.3+0.11	54±0.68	93±1.1
0.8 mg/kg bw, multiple po dose								
Males	13±4.39	0.21±0.16	4.8±3.9	20±1.43	3.4±0.67	1.4±0.20	55±3.74	97±2.80
Females	14±1.41	0.07±0.10	4.9±1.3	16±0.95	3.7±0.31	1.3±0.18	58±1.92	98±1.52
21.0 mg/kg bw, single po dose								
Males	15±3.41	0.17±0.14	5.9±1.7	20±3.20	4.4±0.70	1.2±0.17	45±2.1	91±5.0
Females	17±1.87	0.36±0.60	6.5±2.7	13±0.70	4.5±0.43	1.0±0.12	52±3.70	94±4.5

Results expressed as the mean % recovery ± 1 SD; 1 = includes cage wipe; 2 = excludes carcass and liver

Results of the kinetic analysis for the excretion of radioactivity via exhaled CO_2 are summarised in the Table below. The highest levels of radioactivity were excreted over the first 0-6 hours and ranged from approximately 20-30%. Thereafter, radioactivity steadily declined over 7 days. It should be noted that a second smaller peak of radioactivity was detected at 24-48 hours in all groups, except in males receiving multiple oral doses of 0.8 mg/kg bw, and in females receiving a single 21 mg/kg bw oral dose.

Kinetics of ^{14}C-dichlorvos excretion via exhaled CO_2

Time (hours)	1 mg/kg bw, single iv dose		0.8 mg/kg bw, single po dose		0.8 mg/kg bw, multiple po dose		21.0 mg/kg bw, single po dose	
	♂	♀	♂	♀	♂	♀	♂	♀
0-6	18.88	27.14	21.70	29.17	29.13	30.19	25.38	28.72
6-12	3.90	5.04	4.39	5.59	5.41	6.22	4.84	5.96
12-24	3.43	4.09	3.41	4.41	4.72	4.80	3.00	4.08
24-48	4.03	4.29	3.50	4.62	4.01	4.81	3.66	3.98
48-72	2.86	2.94	2.87	3.35	3.29	3.37	2.35	2.79
72-96	1.92	2.14	1.83	2.32	2.36	2.60	1.76	2.36
96-120	1.47	1.82	1.37	1.96	1.88	2.04	1.34	1.81
120-144	1.38	1.32	1.12	1.39	1.49	1.59	1.10	1.30
144-168	1.15	1.17	0.92	1.14	1.20	1.26	0.93	1.02

Results expressed as the mean % recovery; bolded values indicate peaks of radioactivity

Results of the kinetic analysis for the excretion of radioactivity via the urine are summarised in the Table below. The majority of urinary excretion of radioactivity occurred within the first 24 hours. Following a single intravenous dose of 1 mg/kg bw, males showed two peaks of radioactivity, the major one at 0-6 hours and a second smaller peak at 12-24 hours. In females from this same group, a single peak was evident at 6-12 hours. Following a single oral dose of 0.8 mg/kg bw, males showed a single peak of radioactivity at 0-6 hours. Females from this same group showed two peaks of radioactivity, the major peak at 0-6 hours and a smaller peak at 12-24 hours. Following multiple oral doses of 0.8 mg/kg bw, rats showed a single peak of radioactivity (0-6 hours in males and 6-12 hours in females). Rats receiving a single oral dose of 20 mg/kg bw showed a single peak of radioactivity at 0-6 hours.

Kinetics of ^{14}C-dichlorvos excretion via the urine

Time (hours)	1 mg/kg bw, single iv dose		0.8 mg/kg bw, single po dose		0.8 mg/kg bw, multiple po dose		21.0 mg/kg bw, single po dose	
	♂	♀	♂	♀	♂	♀	♂	♀
0-6	8.72	2.48	7.94	4.11	6.75	4.59	8.73	9.27
6-12	1.78	5.13	2.58	1.98	2.40	5.17	2.25	3.02
12-24	1.80	2.11	1.34	2.04	1.24	1.57	1.06	1.58
24-48	0.97	1.18	0.71	0.88	0.78	0.95	0.81	1.05
48-72	0.72	0.63	0.53	0.51	0.73	0.74	0.51	0.61
72-96	0.49	0.45	0.39	0.32	0.45	0.49	0.42	0.45
96-120	0.37	0.31	0.31	0.28	0.36	0.35	0.30	0.30
120-144	0.29	0.23	0.23	0.17	0.23	0.24	0.22	0.23
144-168	0.24	0.19	0.17	0.14	0.19	0.19	0.18	0.16

Results expressed as the mean % recovery; bolded values indicate peaks of radioactivity

Results of the kinetic analysis for the excretion of radioactivity via the faeces are summarised in the Table below. Two peaks of radioactivity were detected in all groups, the major one at 12-24 hours and a second one at 144-168 hours. In multiple-dosed rats, a third peak was detected in males at 0-6 hours, and in females at 96-120 hours.

Kinetics of ^{14}C-dichlorvos excretion via the faeces

Time (hours)	1 mg/kg bw, single iv dose		0.8 mg/kg bw, single po dose		0.8 mg/kg bw, multiple po dose		21.0 mg/kg bw, single po dose	
	♂	♀	♂	♀	♂	♀	♂	♀
0-6	<0.01	<0.01	0.13	NS	0.74	<0.01	0.14	0.06
6-12	0.04	0.98	0.28	0.03	0.04	0.26	0.50	0.66
12-24	1.95	1.64	1.39	3.28	1.83	1.72	2.59	3.33
24-48	0.77	0.67	0.73	1.21	0.84	0.69	0.92	0.85
48-72	0.38	0.47	0.50	0.93	0.21	0.27	0.48	0.48
72-96	0.25	0.25	0.19	0.47	0.14	0.19	0.25	0.28
96-120	0.28	0.27	0.14	0.33	0.09	0.30	0.22	0.16
120-144	0.15	0.41	0.12	0.42	0.14	0.18	0.13	0.12
144-168	0.85	1.06	0.74	0.44	0.73	1.25	0.64	0.54

Results expressed as the mean % recovery; bolded values indicate peaks of radioactivity

In terms of the tissue distribution of ^{14}C-dichlorvos after 7 days, most radioactivity (13-26% of the total dose) was detected in the carcass (see Table above) followed by the liver (3.5-4.8% of the total dose). Of the remaining 1-1.5% detected in other tissues, most was found in the kidneys (0.23-0.45%) and blood (0.32-0.49%). There was no apparent difference in the tissue distribution of ^{14}C-dichlorvos following intravenous or oral dosing, or between single or multiple oral doses.

Metabolite analysis: The concentrations of ^{14}C (expressed as ^{14}C-dichlorvos equivalents) in pooled urine and faecal samples are summarised in the Table below. Radioactivity was approximately 5-10 times higher in urine than in faeces and approximately 20-30 times higher at 20 mg/kg bw than at 0.8 or 1 mg/kg bw.

^{14}C-dichlorvos equivalents in pooled urine and faecal samples (ppm)

Group	Urine (M/F)	Faeces (M/F)
1.0 mg/kg bw, single iv dose	1.84/1.18	0.241/0.218
0.8 mg/kg bw, single po dose	1.23/0.917	0.106/0.269
0.8 mg/kg bw, multiple po dose	1.23/1.25	0.162/0.167
21.0 mg/kg bw, single po dose	26.9/31.3	3.80/5.82

M = male; F = female

In pooled faecal samples, 52.8-78% of radioactivity was extractable while 22.5-56.7% was non-extractable. Several radioactive metabolites were detected in urine, including urea (19-33%) and hippuric acid (3.78-19.5%). In faeces, numerous uncharacterised minor metabolites were detected in addition to hippuric acid (<6%) and urea (2.7-29.7%). Treatment of urine samples with β-glucuronidase increased the level of radioactivity in the organic fraction by 5.5-13.4%. Analysis of these organic fractions by TLC was reportedly unsuccessful due to the low concentrations of metabolites and/or the volatility of the radioactive

components. It was reported that the urinary profile changed when the thin-layer chromatogram was stored for 3 months at -20°C.

Given the reported low recoveries from the urine thin layer chromatogram 7 days after analysis, and from the faecal chromatogram after 24 hours, the authors concluded that dichlorvos metabolites or their breakdown products were volatile. Mass spectrometric analysis confirmed the formation of urea and hippuric acid, but was unable to identify any other metabolites. The authors suggested that dichlorvos metabolites were dehalogenated due to the lack of a characteristic chlorine cluster in the mass spectra. Based on the relatively large amount of radioactivity excreted via expired CO_2 and the formation of urea and hippuric acid, the authors proposed that the metabolism of dichlorvos involves a one carbon pool biosynthesis pathway

Conclusions: The major route of excretion of ^{14}C-dichlorvos in rats was via CO_2 exhalation (40-58%) followed by the urine (10-15%) and faeces (4-7%). Excretion was rapid, occurring within the first 24 hours after dosing. Marked levels of ^{14}C (13-26%) remained in the carcass seven days after dosing. Relatively low levels of ^{14}C were found in other tissues such as the liver (3.5-4.8%), blood (0.3-0.5%) and kidneys (0.2-0.5%). Excretion and tissue distribution appeared to be independent of douse route and sex. Urea and hippuric acid were identified as urinary and faecal metabolites of ^{14}C-dichlorvos. A number of other urinary and faecal components were detected but were unable to be identified due to their low concentrations and/or volatility.

2.1 Metabolic pathway for dichlorvos

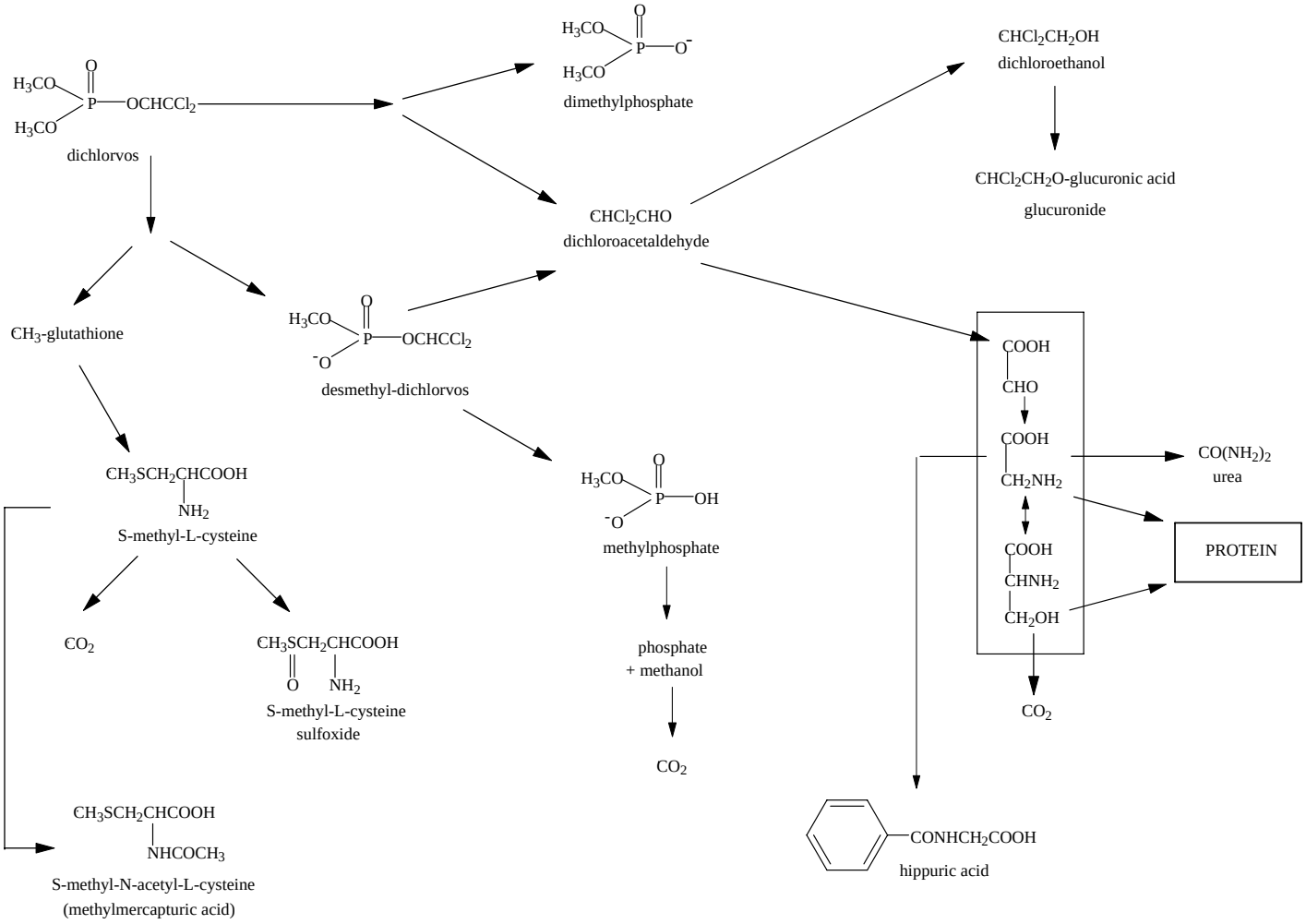
Metabolic dispositional studies using radiolabelled dichlorvos were reported in the rat, mouse, hamster, pig and man. In some studies, labelling at different sites (eg ^{14}C-methyl; ^{14}C-vinyl; ^{36}C1-chlorovinyl; ^{32}P-phosphate) enabled specific pathways to be traced (Hutson et al 1971, 1972a & b). The influence of the dose form was assessed using various routes of administration.

Analysis of urinary metabolites showed that there was very little species or route variation in metabolic pathways. Hydrolysis generally predominated over the O-demethylation, although the latter was slightly more important in the mouse. Metabolic cleavage was rapid to the extent that the half-life for unchanged dichlorvos *in vivo* was not determined with any accuracy.

Unchanged dichlorvos was only detectable in blood or tissues after administration at high dose levels. This was explained by rapid metabolism rather than poor absorption since the radiolabel was extensively incorporated into various metabolites and distributed widely, and persistently, into tissues. Expired $^{14}CO_2$ accounted for a major proportion of the dose, irrespective of whether the label was on the C-methyl or C-vinyl group, confirming rapid and extensive metabolism. The pattern of distribution of ^{32}P-label reflected biotransformation to, and deposition of, inorganic phosphate in the tissues.

The major metabolic pathways for dichlorvos are summarised in the figure overleaf.

Metabolic pathway of dichlorvos in rats



2.2 Percutaneous absorption

Jeffcoat R (1990) Dermal absorption of dichlorvos® in rats. RTI Study No. 4615-1. Lab: Research Triangle Institute, Research Triangle Park, North Carolina, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 15th November 1989 to 18th December 1989. Report date: 23rd March 1990.

GLP compliant (US EPA; 40 CFR Part 160) and QA study.

Materials and Methods

Male Crl:CD®(SD)BR rats (unspecified age) were purchased from Charles River Laboratories Inc. (Raleigh, North Carolina, USA) and quarantined for at least 7 days. Rats were housed under standard conditions, with Purina Certified Rodent Chow® #5002 and tap water available *ad libitum*. Rats were randomly assigned to treatment groups based on bodyweight (220-254 g). Approximately 24 hours before dosing, rats were sedated with ketamine:xylazine (7:1; 60 mg/kg bw, im), their backs clipped free of hair and wiped with acetone. Rats were then acclimatised in individual glass metabolism cages. After clipping and immediately prior to sample application, the application site was examined for signs of damage and any rats omitted from the study if necessary.

^{14}C-dichlorvos, labelled at the vinyl carbon (NEN Research Products; Lot No. 2675-018; >95% purity; 20.31 mCi/mmol specific activity), was applied to 12 rats/dose at 3.6, 36 or 360 μg/rat to 12 cm^2 of skin (equal to 0.5, 3 or 30 μg/cm^2, respectively) in a total volume of 100 μL water. A control group of four rats was treated with water. The rationale for the dose selection was not specified, although three *in vitro* and two *in vivo* pilot studies were performed using these same dose levels. It should be noted that treatment of the control and high-dose groups commenced approximately 4 weeks after the low- and mid-dose groups.

During sample application, a sorbent charcoal tube attached to a glass funnel was used to trap any volatiles (3.6 and 36 μg groups only). After dosing, the needle was wiped, with the swab subsequently analysed by liquid scintillation spectrometry (LSS). The application site was covered with a non-occluding dressing, which contained a charcoal-impregnated covering to absorb any ^{14}C-dichlorvos evaporating from the skin. Following dosing, the radiochemical purity of each dose solution was analysed using HPLC. The concentration of ^{14}C in each dose solution was analysed by LSS at the time of formulation, then at regular unspecified intervals during the exposure period. It should be noted that it was necessary to stabilise the ^{14}C-dichlorvos stock solution (formulated in hexane) with benzene, due to evidence of degradation (ie. loss of purity).

Urine, faeces and $^{14}CO_2$ were collected at 10 and 24 hours, then every 24 hours until sacrifice. After 10 hours of exposure, all rats were anaesthetised with ketamine:xylazine (60 mg/kg bw, im), the protective dressing removed, and the application site washed with soapy water and gauze sponges. Blood samples were taken from 4 rats/dose by cardiac puncture, which were then sacrificed by injecting T-61 euthanasia solution into the heart. Bladder contents were removed and pooled with the final urine samples. The skin at the application site was excised and the remaining carcass kept for analysis. Cage rinses were also collected for analysis. New protective dressings were fitted to the remaining rats and the above procedures repeated on 4 rats/dose at 24 and 102 (360 μg group) or 120 hours (36 and 3.6 μg groups). The high-dose group was sacrificed at 102 rather than 120 hours due to an impending ice storm near the performing laboratory.

All samples were analysed by LSS. Urine, cage and skin washes, and aliquots of the dose formulations were analysed directly, while all other samples were subjected to a solubilisation and extraction process. Each non-occlusive dressing was solubilised in methanol and scintillation fluor for at least 4 hours. Blood was solubilised with soluene-350. Faecal samples were homogenised then solubilised with soluene-350. Carcasses and skin samples were homogenised for at least 26 and 24 hours, respectively, in ethanolic NaOH. Charcoal filters were extracted with methanol for at least two weeks and then completely burned in a sample oxidiser; both the methanol and combusted filters were analysed. Charcoal tubes used during sample application were extracted with methanol, which was analysed separately from the solid charcoal. No statistical analysis was performed.

Results

The three dose formulations were within ±10% of the nominal concentrations, with an approximately 1-2% variation in the concentration of ^{14}C-dichlorvos in 10 aliquots of each formulation. The time at which these aliquots were collected was unclear. Pilot studies found that ^{14}C-dichlorvos was stable for 6 hours at room temperature, however data from these studies were not provided. The actual doses received by rats in the 3.6, 36 and 360 μg groups were determined to be 3.36-3.51, 33.0-36.3 and 319-334 μg/rat, respectively.

The level of recovery of radioactivity in the various samples is summarised in the Table below. Recovery was consistent across all groups and time points and totalled approximately 90%. The highest level of radioactivity was detected in the charcoal filter attached to the dressing (38-55%), followed by the skin at the application site (12-20%), the first skin wash (8-12%), the non-occluding dressing at 10 hours post-application (6-12%) and carcass (3-6%). The level of recovery in exhaled air, urine, faeces and blood ranged from 2.2-4.9%, 1-2%, 0.1-1.6% and 0.09-0.2%, respectively. There was an apparent time-related increase in the levels of radioactivity in exhaled CO_2, urine and faeces.

Radioactivity (% of applied dose) in various samples following dermal application of ^{14}C-dichlorvos to rats

Sample	3.6 μg/rat			36 μg/rat			360 μg/rat		
	10 h	24 h	120 h	10 h	24 h	120 h	10 h	24 h	102 h
Carcass	2.91	4.04	1.51	5.12	4.10	3.31	6.27	4.75	3.93
Skin (dose site)	18.19	12.44	17.14	20.02	13.58	16.27	19.27	15.14	12.10
Blood	0.19	0.20	0.09	0.20	0.19	0.05	0.16	0.12	0.09
Exhaled CO_2	2.20	3.46	3.89	2.71	3.29	4.62	3.10	3.47	4.89
Urine	0.99	1.49	1.84	1.29	1.56	2.15	1.19	1.49	2.03
Faeces	0.07	0.30	1.61	0.09	0.26	0.96	0.08	0.16	0.50
Wash 1 (10 h)	10.28	11.09	7.81	11.27	11.27	9.65	12.64	11.00	12.42
Dressing 1 (10 h)	5.60	11.84	7.85	9.08	8.77	9.27	7.53	6.82	7.51
Charcoal filter 1	51.55	43.97	55.49	41.68	44.01	41.48	37.67	46.63	47.10
Wash 2	-	1.67	1.05	-	1.24	0.95	-	1.70	1.09
Dressing 2	-	0.39	0.56	-	0.34	0.55	-	0.38	0.43
Charcoal filter 2	-	0.53	1.19	-	0.18	0.98	-	-	-
Cage rinse	0.19	0.19	0.14	0.20	0.11	0.04	0.59	0.11	0.05
Dosing trap	0.11	0.04	0.06	0.10	0.08	0.05	-	-	-
TOTAL	92	92	96	92	89	90	89	92	92

Results expressed as the mean % recovery (n=4); - not recorded

The total level of dermal absorption (calculated as the sum of the % radioactivity in the carcass, skin, urine, faeces, blood and expired air) was consistent across all doses and times (see Table below) and was approximately 22-30%. This indicated that absorption occurred within the first 10 hours of sample application, with the actual amount of ^{14}C-dichlorvos absorbed proportional to the dose. The level of dermal penetration (calculated as the sum of the % recovery in the carcass, urine, faeces and expired air) was also consistent across all doses and times and was approximately 6-11%.

Total absorption of ^{14}C-dichlorvos (% of dose)

Time (h)	Dose rate (μg/cm^2)		
	0.5	3	30
10	24.5±3.1	29.4±2.8	30.1±7.1
24	21.9±3.3	23.0±9.6	25.1±3.6
102	-	-	23.6±1.9
120	26.1±11.4	27.4±1.5	-

Results expressed as the average % of the dose ± 1 SD; - not recorded

There was a dose-related increase and a time-related decrease in the concentration of ^{14}C-dichlorvos in blood (see Table below). The highest blood levels were detected 10 hours after sample application indicating that absorption occurred within the first 10 hours.

Concentrations of ^{14}C-dichlorvos in blood (ng dichlorvos equivalents/g blood)

Time (h)	Dose rate (μg/cm^2)		
	0.5	3	30
10	1.3±0.2	15.7±2.8	101.5±16.6
24	1.2±0.1	13.4±2.5	87.0±9.0
102	-	-	49.6±10.7
120	0.7±0.1	6.0±0.9	-

Results expressed as the mean ± 1 SD; - not analysed

Conclusions: A substantial proportion of ^{14}C-dichlorvos (38-55%) evaporated from the skin surface following application to rats. The total level of dermal absorption was 22-30% and was consistent over time and dose rate (ie. 0.5 to 30 μg/cm^2). Absorption occurred within the first 10 hours of sample application, with the actual amount of ^{14}C-dichlorvos absorbed increasing with dose. The excretion routes of ^{14}C-dichlorvos following dermal absorption were via exhaled air (CO_2) (2.2-4.9%), urine (1-2%) and faeces (0.1-0.6%). Blood levels decreased over time. The volatility and stability of dichlorvos are important issues for consideration and these appeared to be addressed by the study authors.

3. ACUTE STUDIES

3.1 Active Constituent

3.1.1 Acute median lethal dose studies

Summaries of submitted and published findings of acute median lethal dose studies with technical dichlorvos are shown in the Tables below. In general, the signs of acute dichlorvos intoxication are consistent with that of the OPs as a class.

Acute oral toxicity of dichlorvos

Species (strain)	Sex	Vehicle	LD_{50} mg/kg bw	95% CI mg/kg bw	Comments	Reference
Mouse	NS	NS	124	Not given	No details	Hazleton Laboratories (1960)
Rat (Sherman)	M F	peanut oil	80 56	62-104 48-65	Deaths in 1 h; recovery from other effects after 1 d	Durham et al (1957)
Rat (Sherman)	M F	peanut oil	80 56	NS	No details	Gaines (1969)
Rat (NS)	M F	PEG	108 79.5	92.3-127 45.7-138	Deaths in 1 h; clinical signs at all doses	Tierfarm (1969a)
Rat (Tif.RAI)	M&F	CMC	46.4	40.1-53.7	Deaths in 2 h; signs at all doses	Ciba-Geigy (1973)
Rabbit (NS)	M&F	PEG	74	51-107	No details	Tierfarm (date unspecified)
Dog (mongrel)	NS	gelatine capsule	100-316	Not given	No details	Hazleton Labs (1960)

CI = confidence interval; M = male; F = female; NS = not specified; PEG = polyethylene glycol; CMC = carboxymethyl cellulose

Acute intraperitoneal toxicity of dichlorvos

Species (strain)	Sex	Vehicle	LD_{50} mg/kg bw	95% CI mg/kg bw	Comments	Reference
Mouse (MF-2)	M&F	CMC	24.0	16.9-33.9	signs at all doses: recovery in 4 days	Tierfarm (date unspecified)

M = male; F = female; CMC = carboxymethyl cellulose

Acute inhalational toxicity of dichlorvos

Species (strain)	Sex	Vehicle	LC_{50} mg/kg bw	95% CI mg/kg bw	Comments	Reference
Mouse (CF1)	M&F	vapour 4h (head only)	>218	-	one dose level only; no deaths	MacDonald (1982)
Rat (wistar)	M	Aerosol 1 h Aerosol 4 h	455 340	209-1244 63-544	No details	Kimmerle (1966)
Rat (Wistar)	M&F	vapour 4h (head only)	>206	85-250#	10/10 dead at 210, 3/10 dead at 250 mg/m^3	MacDonald (1982)
Rat (Wistar)	M&F	vapour 4h (head only)	>116*	-	one dose level only; no deaths	Pauluhn (1984)
Rat	M	aerosol 4 h	523	435-632	recovery from	Pauluhn

Species (strain)	Sex	Vehicle	LC_{50} mg/kg bw	95% CI mg/kg bw	Comments	Reference
(Wistar)	F	(head only)	447	379-529	signs in 7 days.	(1984)

M = male; F = female; NS = not specified; # = dose range tested; * = reported to be the maximum achievable vapour concentration.

Acute dermal toxicity of dichlorvos

Species (strain)	Sex	Vehicle	LD_{50} mg/kg	95% CI mg/kg	Comments	Reference
Rat (Sherman)	M F	xylene	107 75	NS	No details	Gaines (1969)
Rat (RAC)	M&F	concentrate or PEG	210	80-200#	occluded for 24 h; deaths in 1-7 days.	Tierfarm (1969b)

M = male; F = female; NS = not specified; PEG = polyethylene glycol; # = dose range tested

3.1.1.1 Oral

Rats

Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson AM & Hayes WJ jr (1957) Studies on the toxicity of 0, 0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). Arch Ind Health 15: 340-349

This published paper reported on a series of experiments that investigated a number of endpoints as well as acute oral toxicity in rats. Dichlorvos was prepared in-house at the Technical Development Laboratories of the Communicable Disease Centre, Public Health Service, US Department of Health, Education and Welfare. Information on the purity of the test material was not provided.

Dichlorvos was given in peanut oil by gavage with a dose volume of 5 mL/kg bw to groups of white Sherman rats (group size not reported). LD_{50} values were 80 mg/kg bw (95% CI 62-104 mg/kg bw) in males and 56 mg/kg (48-65 mg/kg bw) in females respectively. All animals that died did so within 1 h of dosing and complete recovery was observed in survivors within 24 h. Adverse signs included bulging eyes, lachrymation, sialorrhoea, generalised muscle fasciculations and tremors. Convulsions were observed in some animals prior to death. There was no apparent weight loss amongst survivors. Necropsy and pathology were not reported.

Gaines TB (1969) Acute toxicity of pesticides. Toxicology and Applied Pharmacology 14: 515-53.

This study reported on LD_{50} values for a large number of pesticides studied by the author since 1960 using Sherman rats. No details about the source or supply of dichlorvos were provided. The LD_{50} was reported to be 80 (males) and 56 (females) mg/kg bw following gavage administration in peanut oil. The original data may have been reported by Durham et al (1957) but this was not credited in the present paper.

Tierfarm, Sissel, Switzerland (1969a) Report on the determination of the oral LD_{50} to the rat of DDVP – technical. No study/report No. Lab/Sponsor: unspecified. Report date: unspecified.

Dichlorvos, as a 0.5-1% v/v solution in PEG, was given by gavage to groups of 5 rats per sex (bred at Tierfarm AG laboratories; strain not given) at doses of 10-100 mL/kg bw. No information was provided regarding the purity, source or batch of dichlorvos. Adverse signs were evident at all dose levels, increasing in severity with dose. Initial signs included slight tonic-clonic spasms of limb muscles, cramps of cheek muscles (trismus) and tachypnoea. Exophthalmia, prostration, lachrymation, dyspnoea and secretion from the Hardersche (Harderian) gland were observed at doses where lethality occurred. LD_{50} values were 108 mg/kg bw in males and 79.5 mg/kg bw in females (95% CI = 92.3-127 and 45.7-138 mg/kg bw, respectively). Deaths occurred within 1 h of administration, and surviving animals recovered after 4-5 days. Organs were examined macroscopically, showing liver

congestion and GIT bloating in animals dying after treatment, with no changes observed in animals surviving to 7 days after dosing.

Ciba-Geigy Ltd, Basle (1973) Acute oral LD_{50} of technical dichlorvos (G177) in the rat. Toxicology/pathology PH 2.635. Report number Siss 3361 Report date: 30th August 1973

Tif. RAI rats (5/sex/group; 6-7 weeks old) were given technical dichlorvos (purity and source not provided) dissolved in 2% carboxymethyl cellulose (CMC) by gavage at doses ranging from 31.7 to 100 mg/kg. Observations continued for 7 days. The mean LD_{50} was 46.4 mg/kg bw (40.1-53.7 mg/kg bw), calculated by probit analysis. Adverse signs included dyspnoea, exophthalmus, curved position, trismus, tonic-clonic muscle spasms and ruffled fur,with these signs occurring at all dose levels within 2 h of administration. These signs increased in severity with dose. All survivors recovered within 4 days. Gross examination, carried out on all dead animals and on the survivors sacrificed at the end of 7 days, revealed no treatment-related changes.

Lamb IC (1992) A range-finding acute study of dichlorvos in rats. WIL study No. WIL-188002. Lab: WIL Research Laboratories Inc, Ashland, Ohio, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 3rd March 1992 to 14th September 1992. Report date: 14th September 1992.

This GLP compliant study (US EPA; 40 CFR Part 160) was commensurate with a dose-range-finding study. The aim was to establish dose levels for an acute neurotoxicity study and to estimate the time of peak effect in rats. A single dose of dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 97.87% purity) was administered orally to SD Crl:CD®BR rats (one/sex/group; Charles River Breeding Laboratories Inc, Portage, Michigan, USA) by gavage at 0.1, 0.5, 1.0, 10.0, 20.0, 30.0, 40.0, 60.0, 70.0 or 80.0 mg/kg bw. The vehicle was deionised water and the dose volume was 10 mL/kg bw. [An initial study had tested doses of 0.1, 1, 5, 10, 20, 40, 50, 60, 75 or 100 mg/kg bw/d using a dose volume of 5 mL kg/bw. However, due to problems associated with poor solubility, the dose volume was increased to 10 mL/kg bw for the current study.]

Rats were approximately 7 weeks old at the time of dosing and had been acclimatised for at least 12 days. On receipt, rats were housed 3/sex/cage for approximately three days and thereafter, individually. Rats were arbitrarily assigned to treatment groups based on bodyweight, which was 167-313 and 139-201 g for males and females, respectively. Rats were housed under standard conditions. Purina® Certified Rodent Chow® #5002 and tap water were available *ad libitum*.

Rats were observed twice daily for mortalities and clinical signs. Detailed clinical examinations were performed the day prior to, and of, dosing, then at approximately 15, 30, 45, 60 and 90 minutes, and 2, 4 and 24 hours, after dosing. These detailed clinical observations consisted of the following: pupillary reflex, general appearance and condition, open field observations (arousal, surface righting reflex and gait, forelimb and hindlimb grasp reflexes). Bodyweights were recorded at days -1, 0 and 1. Any dead rats were necropsied, which included an examination of the external surfaces, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including the vicera.

The 80 mg/kg bw male was found dead approximately 15 minutes after dosing. There were no other deaths. There was no effect on bw. A range of clinical signs were observed at and above 0.5 mg/kg bw (see Table below), with the estimated time to peak effect ranging from 15-45 minutes after dosing. The predominant clinical signs included gait alterations, whole body tremors, reduced limb grasp, constricted pupils and exophthalmus. All rats had recovered by 24 hours.

Clinical signs, NOELs and estimated time to peak effect in rats

Clinical Sign	NOEL (mg/kg bw)	Estimated time to peak effect (min)
Rocking, lurching or swaying	1.0	15
Prostration	60.0	15
Tremors	20.0	15-30
Reduced forelimb grasp	0.5	15-30
Reduced hindlimb grasp	40.0	15-30
Absent hindlimb grasp	0.5	15-30
Constricted pupils	40.0	15-45

Exophthalmus	0.5	15-30
Labored respiration	40.0	15-30
Salivation	40.0	15-45

Rabbits

Tierfarm, Sissel, Switzerland (undated) Report on the determination of the acute oral LD_{50} to the rabbit of DDVP - technical. No Study No. Lab/Sponsor: unspecified.

Dichlorvos (purity and source unspecified) was given by gavage to male and female 'yellow silver strain' rabbits, as a 5% solution in PEG, at dose levels in the range 46.4-100 mg/kg bw. The LD_{50} was given as 74 mg/kg bw (95% CI 51-107 mg/kg bw) with no differentiation made between sexes. No details of adverse clinical signs or time of death were provided.

Dogs

Hazleton Laboratories, Falls Church, Virginia (1960) Acute oral administration – Dogs. No study/report No. Lab/Sponsor: unspecified.

This was a summary statement of results without reference to materials and methods. The oral LD_{50} of technical dichlorvos (capsule) in dogs was reported to be 100-316 mg/kg bw. No experimental details were provided. LD_{50} values for the mouse and rat were also given as 124 mg/kg bw (sex not stated), and 80 (males) and 56 (females) mg/kg bw, respectively.

Snow DH & Watson ADJ (1973) The acute toxicity of dichlorvos in the dog: 1. Clinical observations and clinical pathology. Australian Veterinary Journal 49: 113-119

Snow DH (1973) The acute toxicity of dichlorvos in the dog: 2. Pathology. Australian Veterinary Journal 49: 120-125

In this unconventional study, a 96% preparation of dichlorvos (source not specified) was given to 21 dogs (15 greyhounds, 6 cross-bred) as follows: three greyhounds received 11 mg/kg and 6 received 22 mg/kg bw in gelatine capsules; three cross-breeds/sex were given 22 mg/kg orally; and one or 2 greyhounds at each dose level received 2.2, 4.4, 8.8 or 11 mg/kg bw intravenously (undiluted or in a saline emulsion). A number of parameters were monitored before and after administration, including plasma and RBC ChE activity, serum ALT, AST and AP activities, haematology, and clinical signs.

Adverse clinical signs were observed in all dogs, with wide variability. Following oral administration, signs were similar at both dose levels and included apprehension and restlessness, fine muscle fasciculations, ataxia and generalised tremors. Some dogs became hypersensitive to sound and touch. Miosis, salivation, vomiting, diarrhoea and tenesmus were also observed. Four dogs most severely affected had an increase in body temperature of 0.5-2.8°C. Signs generally disappeared within 4 h but persisted for 30 h in one dog. Two dogs showed persistent weight loss in the weeks following administration. Hct increased at 1-3 h after dosing, >10% in those animals showing severe signs of poisoning. Total plasma protein also rose in correlation with the severity of symptoms. Three of 12 dogs given 22 mg/kg bw died. Death occurred within 16-20 min in 2 dogs and after 155 min in the third, and followed pronounced hyperpnoea, dyspnoea, tonic-clonic convulsions, and in one case, coma, prior to respiratory failure. Serum AST and CPK, and to a lesser extent ALT, were elevated in 3 surviving dogs, indicating muscle damage rather than hepatobiliary effects in these animals. Wide variability in plasma and RBC ChE activity was observed pre- and post-treatment. Blood samples taken 1 h after administration showed depression of plasma ChE by 59-88% and RBC ChE by 69-97% independent of the dose. Plasma ChE levels returned to pre-treatment values within 96-144 h but RBC ChE generally remained low through to 144 h, the last time point sampled. Brain ChE activity was measured in the three dogs that died and this was inhibited by 33-36% of normal level of activity.

Five of the 6 dogs given dichlorvos intravenously died within 7 min to 2 h following adverse signs similar to those reported for oral administration. The dog given the lowest dose of 2.2 mg/kg bw in emulsion survived and showed only mild signs of intoxication. Following intravenous administration, almost complete inhibition of plasma ChE activity and 95% inhibition of RBC ChE activity was

observed at 4.4 and 8.8 mg/kg bw. Brain ChE activity was reduced to 87% in the female given 4.4 mg/kg bw and to 44-59% in the male and female given 8.8 mg/kg bw.

The pathology of dichlorvos poisoning in the same dogs was described in an accompanying publication (Snow 1973). This described generalised congestion and hyperaemia in dogs that died rapidly, and cardiovascular abnormalities, including extensive haemorrhages, when death was delayed. Muscle fibre degeneration and necrosis were also observed, confirming the pattern of serum enzymes elevation observed in clinical chemistry analysis.

The present study provided detailed information on the effects of acute dichlorvos poisoning in dogs. However, its use for regulatory purposes was limited as adverse effects were observed at all dose levels.

3.1.1.2 Intraperitoneal

Tierfarm AG, Sissel, Switzerland (undated) Report on the determination of the acute Intra-peritoneal LD50 to the mouse of DDVP – technical. No study No. Lab/Sponsor: unspecified.

Groups of 5 mice/sex (MF-2 strain, bred at Tierfarm AG) received 12.9-77.5 mg/kg bw dichlorvos dissolved at 0.2-1% in 2% sodium CMC by ip injection. No information on the purity or source of dichlorvos was provided. All animals showed tonic-clonic muscle spasms, exophthalmia and dyspnoea within 5 minutes of administration, with lateral position appearing later. These signs increased dose-dependently. The LD_{50} was 24.0 (95% CI 16.9-33.9) mg/kg bw for both sexes (individual sex data was not presented). The time to death was not given but surviving animals recovered within 3-4 days. Congested livers were reported in dead animals but no macroscopic abnormalities were observed in those surviving the 14-day observation period.

3.1.1.3 Inhalation

Rats and mice

Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson AM & Hayes WJ Jr (1957) Studies on the toxicity of 0, 0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). Arch Ind Health 15: 340-349.

Weanling Sherman rats (10/sex/group) were maintained for two weeks in Peet-Grady chambers that had previously been sprayed once with an emulsion containing 2.5% dichlorvos at a rate of approximately 100 or 195 mg dichlorvos per square foot (ie. equivalent to 9.29 or 18.12 mg/m^3). Air levels of dichlorvos in the chamber sprayed at the lower rate ranged from 6 mg/m^3 at the start and 0.1 mg/m^3 at the end of the two-week period. No adverse signs or body weight changes were observed. Plasma and RBC ChE activities were slightly (<20%) but significantly lower than controls in males after 1 week but not after 2 weeks in the chambers. No other effects of exposure were reported.

In a series of experiments, Sherman rats (6/sex/group) were placed in 'Hazeldene-type' chambers in which the air supply was passed through a gas saturation bottle containing 250 mL dichlorvos. The vapour concentration was not measured and it was not possible to determine the inhaled dose from the data presented. Animals were supplied with food but not water and were removed from the chambers for 1 h/day when water was supplied. Incoming and exhaust air was monitored for dichlorvos by measuring total phosphorus. All animals in all trials exposed to dichlorvos in the air died, with symptoms including slow laboured respiration, sialorrhoea and paleness of ears and feet. Under the most severe exposure, these signs appeared within 2 h, with average survival time 6.9 and 10.1 h in males and females respectively.

Kimmerle G (1966) Letter re. DDVP (Lo-No. 271) / inhalation study. Bayer AG Institute of Toxicology, 56 Wuppertal-Elberfeld. Ref. Dr.Ki/Sp dated 7th December 1966.

A single-page note reported on the LC_{50} of dichlorvos in male rats (the source and purity of dichlorvos and strain of rat were not provided). Groups of 20 animals were exposed to analysed dichlorvos aerosol concentrations of 0.209-1.244 mg/L for 1 h, or 0.063-0.544 g/L for 4 h. In addition, 2 groups of rats received (theoretical) 0.049 or 0.109 mg/L for 4h/d for 5 consecutive days. The 1-h LC_{50} was

0.455 mg/L (455 mg/m^3), and the 4-h LC_{50} was 0.340 mg/L (340 mg/m^3). Adverse signs were observed in 3/20 rats at 0.319 mg/L and in most animals exposed to 0.479 mg/L or more for 1 h, and in all animals exposed to 0.082 mg/L or more for 4 h. There were no clinical signs and no animals died in the repeat-dose section of the study.

Macdonald R (1982) Toxicology of consumer products: the acute (4 h) inhalation toxicology of dichlorvos in rats and mice. Document No SBGR.82.145. Lab: Shell Research Ltd, Sittingbourne, UK. Sponsor: Temana. Report date: March 1982

QA study.

Groups of 10 animals (5/sex, CF1 mice and Wistar rats, both from Shell's Tunstall Laboratory) were subjected to head-only exposure to vapour containing dichlorvos (Windmill Plastics Ltd; batch No. ST82/016; 97.8% purity) for 4 h. Observations on health and behaviour were made during and for 14 days after exposure. All mice exposed to 218 mg/m^3 (the only dose tested) survived. Adverse signs were body tremors and lethargy, with 3 mice displaying hind limb paresis and all showing splayed gaits. The clinical signs reversed by the second day. In male rats, 3/10 animals exposed to 250 mg/m^3 (saturated air) died while all 10 animals exposed to 210 mg/m^3 died. Subsequent exposure to 142 and 85 mg/m^3 resulted in one male death at the lowest concentration. Two further experiments were performed, using concentrations of 206 and 198 mg/m^3, with no deaths observed. Adverse signs in rats were observed at the highest concentrations, and included ataxia and hypersensitivity to noise, both clearing rapidly after exposure. Lethargy persisted for up to 3 days in survivors. Dead rats showed signs of respiratory failure but no treatment-related effects were seen in survivors sacrificed after 14 days.

In conclusion, the LC_{50} (4h) for dichlorvos in mice was >218 mg/m^3 and in rats was >206 mg/m^3. Considerable variability was evident, with all animals exposed to 210 mg/m^3 dying but no deaths observed at 206 mg/m^3.

Pauluhn J (1984) Dichlorvos (L15/20, DDVP) study for acute inhalation toxicity. Report No. 13124 Lab/Sponsor: Bayer AG Institute of Toxicology, Wuppertal-Eberfeld. Report date: 12th December 1984

This study was undertaken to assess the acute inhalational toxicity of dichlorvos aerosol and vapour and was stated to be in conformance with OECD guidelines and GLP. Young adult Wistar (Bor:WISW) rats were exposed to dichlorvos (98.7 % pure, batch no. 809 436 388, lot no. 3379) vapour in Battelle chambers (preventing exhaled air mixing with fresh supply of test atmosphere), or aerosol (sprayed undiluted) in 40 L inhalation chambers, with rats confined to tubes to allow only head and nose exposure.

For the vapour experiment, 5 animals/sex were exposed for 4 h to a nominal concentration of 342 mg/m^3 (the analysed concentration was 116 mg/m^3, the maximum achievable as vapour). All animals showed adverse signs including bristling and ungroomed coat, reduced motility and high gait but there were no deaths. No effects on weight gain were observed during the 14-day observation period and autopsy revealed no treatment-related gross effects.

In the aerosol experiment, 10 animals/sex were exposed to nominal air concentrations of 1500 - 15000 µL/m^3 (230-1926 mg/m^3) for 4 h, with the particle mass median diameter ranging from 2.8 to 6.4 µm. The LC_{50} was 523 (95% CI 435-632) mg/m^3 for males and 447 (379-529) mg/m^3 in females. Adverse signs, evident immediately after exposure (ie when rats were removed from tubes) were severe muscle tremors and weakness, convulsions, recumbence on side, ataxia, apathy and dyspnoea. More persistent signs included ungroomed coat, bristling fur and high gait. All surviving animals appeared to behave normally during the second week of observation. Body weight gain was normal. Animals that died during exposure showed a number of changes at autopsy: distended lung; oedematous and pale, patchy liver with lobulation; pale spleen and kidney; hyperaemia of the glandular stomach and serosa of the small intestine; and blood and mucus in the gut. No treatment-related gross effects were observed in those animals surviving the 14-day observation period.

This study was of sufficient quality to provide confidence in the measured concentrations of atmospheres to which the rats were exposed and in the LC_{50} values derived. A lethal vapour

concentration could not be generated (maximum concentration was 116 mg/m^3) but the LC_{50} values for aerosol exposure were 523 and 447 mg/m^3 in males and females, respectively.

Monkeys

Durham WF, Gaines TB, McCauley RH, Sedlak VA, Mattson AM & Hayes WJ jr (1957) Studies on the toxicity of 0, 0-dimethyl-2,2-dichlorovinyl phosphate (DDVP). Arch Ind Health 15: 340-349

Rhesus monkeys were exposed to dichlorvos in Peet-Grady chambers (1 or 2) that had been sprayed with dichlorvos emulsion at 9.29 or 18.12 mg/m^3 in the same experiment previously reported for rats. There were no deaths, no adverse signs and no effect on bw. Plasma and RBC ChE activities were measured in samples collected weekly for 10 weeks (including the initial 2-week exposure period) from monkeys exposed to the higher concentration. Graphical representation showed that ChE activities had declined by approximately 60-70% in samples taken after one week of exposure at 18.12 mg/m^3. ChE activity recovered over the next 3-4 weeks. The study was not amenable to statistical analysis.

3.1.1.4 Dermal

Gaines TB (1969) Acute toxicity of pesticides. Toxicol Appl Pharmacol 14: 515-53

The oral and dermal LD_{50} values of a large number of pesticides studied by the author since 1960 were reported in summary. The dermal LD_{50} of dichlorvos in Sherman rats was reported to be 107 (males) and 75 (females) mg/kg bw following administration to the skin in xylene.

Tierfarm, Sissel, Switzerland (1969b) Report on the determination of the acute dermal LD_{50} to the rat of DDVP technical. No Study/Report No. Lab/Sponsor: uspecified. Report date 10th July 1969.

Dichlorvos (purity and source not given) was applied as a concentrate or dissolved in PEG (10% v/v) to a 10 cm^2 area of the shaved back of RAC rats (6/sex/group) at doses in the range 80-200 mg/kg bw. The skin was occluded with aluminium foil and sticking plaster for 24 h before washing with warm water, and animals were observed for 7 days after administration. The LD_{50} (males plus females) was 210 mg/kg bw, with most deaths occurring after 24 h. No adverse signs were observed at 80 or 100 mg/kg bw (in PEG). At 150 or 200 mg/kg bw (concentrate), adverse signs were observed within 15-30 minutes of application and included dose-dependent trismus, tonic-clonic spasms of limb muscles, prostration, exophthalmus, dyspnoea, and lachrymation and secretion from the Hardersche (Harderian) glands. Symptoms persisted for up to 3 days but surviving animals recovered fully after 5-6 days. Necropsy of animals dying during the observation period revealed acute liver, spleen and kidney congestion, bloated intestines and inflamed peritoneum. Animals sacrificed after 7 days showed enlarged livers, and bloated or slack intestines. No other macroscopic effects were reported. No skin irritation was evident.

3.1.2 Skin and Eye Irritation Studies

Pauluhn J (1985) L 15/20 (DDVP) technical active ingredient (c.n. dichlorvos): Study for irritant/corrosive effect on skin and eye (rabbit). Report No. 13500. Lab/Sponsor: Bayer AG Institute of Toxicology, Wuppertal-Elberfeld. Report date: 22nd May 1985

These studies conformed with OECD guidelines 404 and 405. To assess dermal irritation, groups of 3 HC:NZW rabbits (from Hacking and Churchill, Huntingdon, UK) were shorn over areas of their flanks and exposed to 2.5 x 2.5 cm cellulose squares to which water (control) or 500 µL dichlorvos (batch no. 809 436 455, 97.3% pure) had been applied. After 4 h the dressings were removed and areas washed with water; skin reactions where scored after 1, 24, 48 and 72 h, and 7 and 14 days. Easily perceptible redness (erythema grade 2) and slight swelling (oedema grade 1) were identified in all three rabbits during the first 48 h after exposure. This declined in 2 rabbits to erythema grade 1 after 7 days but increased to grade 3 (moderate) in the third. After 14 days this rabbit showed slight erythema (grade 1) with no other effects evident in other animals. Overall, dichlorvos was slightly irritating to rabbit skin.

In the ocular irritation test, 100 µL of dichlorvos was applied to the conjunctival sac of one eyelid of 6 rabbits, the eyelid was held shut for one second and washed with saline 24 h later. Cornea and iris

findings were obtained optically, and the corneal area affected was determined using a drop of 1% fluorescein applied to the eye after 24 h. Adverse signs were observed in all rabbits immediately after administration. These included miosis, muscular fasciculations, tremor, staggering gait and recumbence on stomach, cyanosis, hyper salivation, tachypnoea and bronchosecretion. One male rabbit died 25 min after administration. Corneal opacity was evident, graded as light and affecting up to 3/4 of the cornea, in all rabbits during the first 72 h. Conjunctival redness and swelling, and increased tear flow was also observed in all animals, generally graded as slight to pronounced (grade 1-3). Little effect on the iris reaction to light was observed. All surviving animals had recovered within 14-21 days after administration. Under the conditions of this study, dichlorvos was a severe eye irritant in rabbits.

3.1.3 Skin Sensitisation Studies

Ueda A, Aoyama K, Manda F, Ueda T & Kawahara Y (1994) Delayed-type allergenicity of triforine (Saprol®). Contact Dermititis 31:140-145.

Humans: The delayed-type allerginicity of seven pesticides (triforine, dichlorvos, chlorothalonil, quinomethionate, cyhexatin, methomyl and benomyl) and chrysanthemum extracts (known to induce contact dermatitis due to their sesquiterpene lactones) were studied in 59 male and 48 female chrysanthemum growers following the spring harvest season. The average age of males was 38.6±11.8 years and the average age of females was 42.2±9.6 years. Subjects had worked in flower growing for an average of 14 years and had reportedly been exposed to a range of pesticides. Health information was obtained using questionaires (work-related allergies, family history of allergies and general health information). Patch testing of the 7 pesticides and extracts was performed on the inner side of the forearm and reactions read after 2 days. Subjects were also analysed for serum immunoglobulins (IgE, IgA, IgG and IgM).

Approximately 60% of subjects were reported to have one or more work-related allergic symptoms (immediate-type or delayed-type), 25% had a personal history of allergic diseases and 26% a family history of allergic disease. Of the 27% of subjects who reported experiencing work-related skin conditions, approximately half attributed this to pesticides. Pesticides thought to be causative factors included dichlorvos, chlorothalonil, methomyl, maneb and macozeb. Twenty percent of males and 44% of females patch-tested positive to at least one or more of the compounds. When subjects were patched tested with 0.02% dichlorvos, 10% of males (6/59) and 19% of females (9/48) exhibited a positive reaction (average of 14%). On this basis, dichlorvos is concluded to be a skin sensitiser in humans.

Guinea pigs: A guinea pig maximisation test was performed to determine the allergenicity of dichlorvos or triforine, and their potential cross reactivity (only the allergenicity of dichlorvos is considered here). Nine Dunkin-Hartley female guinea pigs (300-400 g bw) were induced topically with 25% dichlorvos under occluded conditions. Animals were then challenged with 0.005, 0.05 or 0.5% dichlorvos and any skin reactions scored at 24 and 48 hours according to the procedure of Kligman (1966). At 24 hours post-challenge, 0, 56 and 100% of guinea pigs tested positive at 0.005, 0.05 or 0.5% dichlorvos, respectively, decreasing to 0, 22 and 67%, respectively, at 48 hours. There was a dose-related increase in the grade of the reaction. On this basis, dichlorvos is concluded to be a skin sensitiser in guinea pigs.

3.2 Metabolites/Degradation Products

There were no acute toxicity studies on dichlorvos metabolites/degradation products submitted for evaluation.

3.3 Products/Formulations

There were no acute toxicity studies performed on any of the currently registered Australian dichlorvos products.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1. Oral Administration

4.1.1 Rats

Kleeman JM (1988a) One-week gavage toxicity study with DDVP in rats. HLA Study No. 6274-101. Lab: Hazelton Laboratories America Inc, Madison, Wisconsin, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 6th June 1988 to 15th June 1988. Report date: 24th August 1988.

This study was commensurate with a range-finding study and was aimed at determining the effect of dichlorvos on plasma and RBC ChE activities in rats. dichlorvos (presumably sourced from AMVAC Chemical Corporation, Los Angeles, CA, USA; unspecified lot No. & purity) was administered orally by gavage to 5 female Crl:CD®(SD)BR rats per group (Charles River Laboratories Inc., Portage, Michigan, USA) at 0, 0.1, 10 or 20/40 mg/kg bw/d for up to 7 days. The vehicle was deionised water and the dose volume was 10 mL/kg bw. Food and water (unspecified) were available *ad libitum* except for 4 hours prior to blood collection when it was withheld. Bodyweights were recorded on the first day of treatment. Observations for mortality and clinical signs were performed twice daily. Blood samples were collected before the first and on the 7th day of treatment for analysis of plasma and RBC ChE activities. All animals were sacrificed by an unspecified means and discarded on the last day of dosing.

At 40 mg/kg bw/d, one rat died on the first day of dosing, with the 4 remaining rats appearing hypoactive and displaying tremors. Consequently, this dose was reduced to 20 mg/kg bw/d following a 2-day washout period (the dosing period at 20 mg/kg bw/d was therefore 4 days). Five additional rats were dosed with 10 mg/kg bw/d dichlorvos for 6 days, however no pretreatment blood samples were collected from this group. Rats from the control and 0.1 mg/kg bw/d groups appeared normal. Plasma and RBC ChE activities were significantly lower ($p<0.05$; unspecified statistical test) than the control group at and above 10 mg/kg bw/d. The level of inhibition was approximately 73% for plasma ChE activity and 30% for RBC ChE activity.

4.2. Inhalational Administration

4.2.1 Dogs, cats and rabbits

Walker AIT, Blair D, Stevenson DE and Chambers PL (1972) An inhalational toxicity study with dichlorvos. Arch Toxikol 30:1-7.

Dogs (6 male and 4 female beagles), cats (6 male, 2 female) and rabbits (8 male, 2 female NZW) were housed in cages in a kennel and exposed to dichlorvos in the air continuously for 8 weeks. The dichlorvos was generated from two commercial resin strip formulations containing 20% dichlorvos (AC 6750 and AC 6741, differing in plasticiser incorporated into the PVC, supplied by Shell Chemical Co.), placed in the kennel at the same time. Air levels of dichlorvos were determined using a ChE inhibition assay (Michel 1949). This was done initially daily and later at weekly intervals throughout the 8-week exposure period.

The mean level of dichlorvos in air ranged from 0.05 to 0.3 µg/L (mg/m^3) after equilibrium was reached within 3 days. No effects were observed on the general health of animals or on EEG recordings obtained from selected animals of each species. There was no treatment-related effect on RBC or plasma ChE activities.

5. SUBCHRONIC STUDIES

5.1 Rats

Kleeman JM (1988b) 13-week gavage toxicity study with DDVP in rats. HLA study No. 6274-102. Lab: Hazleton Laboratories America Inc., Madison, Wisconsin, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 22nd June 1988 to 23rd September 1989. Report date: 28th December 1988.

GLP compliant (US EPA; 40 CFR Part 160) and QA study.

Materials and Methods

Dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 98.3% purity) was administered orally by gavage to Crl:CD®(SD)BR rats (10/sex/group; Charles River Laboratories Inc., Portage, Michigan, USA) over 5 days/week for 13 weeks at 0, 0.1, 1.5 or 15 mg/kg bw/d. The vehicle was deionised water and the dose volume was 10 mL/kg bw. The dose selection was based on the results of a previous one-week range-finding study (Kleeman 1988a), where 0.1 mg/kg bw/d dichlorvos did not produce clinical signs or inhibit plasma or RBC ChE activity. Dosing solutions were prepared daily for the first 4 weeks of the study and thereafter weekly, with all solutions stored refrigerated. The homogeneity of all dose levels was analysed. The stability of dichlorvos in deionised water was analysed following refrigeration for 0.25, 1, 2 and 5 days or when stored frozen for 1, 2, 5 and 9 days and 7 weeks. dichlorvos concentrations were verified by HPLC in duplicate samples taken during the first 4 weeks, then at week 8 and 12.

Upon receipt, rats were approximately 28-days old and were acclimatised for 9 days prior to the initiation of treatment. The mean bodyweights of males and females were approximately 164 and 143 g, respectively. Rats were housed individually under standard conditions. Purina Certified Rodent Chow® #5002 and water (unspecified source) were available *ad libitum*. Rats were randomly assigned to each group based on bw.

Rats were observed for mortalities and moribundity twice daily, and once daily for clinical signs. Rats were also observed for clinical signs approximately 30 minutes after dosing. Bodyweights were recorded weekly from day 0 and then at termination. Food consumption was recorded weekly. Water consumption was not recorded. Ophthalmic examinations were performed during the acclimatisation period and prior to termination. Plasma and RBC ChE activities were measured during week 7 and at termination (week 14) along with brain ChE activity. No pretreatment ChE measurements were taken. After 13 weeks of treatment, all surviving rats were fasted overnight and blood collected following ketamine anaesthesia. Standard haematology and clinical chemistry parameters were analysed (refer Appendix II), with the exception of γ–glutamyl transpeptidase, CPK and triglycerides. No pretreatment haematology or clinical chemistry parameters were analysed. Urinalysis was not performed.
Rats were sacrificed at week 14 by methoxyflurane anaesthesia, exsanguinated and necropsied. Necropsies involved an examination of the following: external surface of the body; all orifices; cranial cavity; carcass; external surfaces of the brain and spinal cord; nasal cavity and paranasal sinuses; thoracic, abdominal and pelvic cavities and their vicera. The following organ weights were recorded: brain, kidneys, liver, ovaries and testes. The standard range of tissues were preserved (see Appendix III), with the exception of a blood smear, the gall bladder, harderian glands, vagina and Zymbal's gland. A histopathological examination was performed on these tissues in the control and 15 mg/kg bw/d groups. Additionally, macroscopic lesions, the lungs, liver and kidneys were histopathologically examined in the 0.1 and 1.5 mg/kg bw/d groups.

The following statistical analyses were performed: Levene's test (determination of the homogeneity of the variance); log, square, square root, reciprocal, angular and rank transformations (to convert heterogenous to homogenous data); ANOVA followed by a Dunnet's t-test (pairwise comparisons of homogenous or ranked data); Kruskal-Wallis H-test ANOVA followed by the Nemenyi-Kruskal-Wallis test for multiple comparison of the Wilcoxon-Mann-Whitney two-sample rank test (non-transformable data).

Results

Analysis of dosing solutions: The concentrations of dichlorvos in the top, middle and bottom of the dosing solutions were consistent (~5% variation) indicating that the solutions were homogenous. dichlorvos was stable in water when refrigerated for 5 days and when stored frozen for up to 9 days. However, there was an approximately 15% reduction in dichlorvos when the dosing solutions were stored frozen for 7 weeks. The concentrations of dichlorvos in the dosing solutions were generally within ±10% of the nominal concentrations, with slight exceedances (1-2%) of this figure measured on 1-2 occasions in the 0.01 and 0.15 mg/mL solutions (used to formulate the 0.1 and 1.5 mg/kg bw/d doses).

Mortalities, clinical signs and ophthalmoscopy: An accidental death occurred in the female control group. There were no other mortalities. Treatment-related clinical signs were confined to the 15 mg/kg bw/d group and included salivation (7 males and 4 females) and urine stains (7 males and 5 females), both reportedly occurring 30-60 minutes postdose. There were no treatment-related ophthalmoscopic abnormalities observed at termination.

Bodyweights and food consumption: There was no treatment-related effect on bodyweight, bodyweight gain and average weekly food consumption.

ChE activity: Results of the analysis of plasma, RBC and brain ChE activities at weeks 7 and 14 are summarised in the Table below. At week 7, significant inhibition ($p<0.05$) of plasma and RBC ChE activities occurred in males at and above 1.5 mg/kg bw/d, with the level of inhibition greater than 20% (relative to the control) and therefore considered toxicologically significant. In females, plasma ChE activity was significantly inhibited at 15 mg/kg bw/d (58%; $p<0.05$), while RBC ChE activity was inhibited at 1.5 and 15 mg/kg bw/d (25 and 42%, respectively; $p<0.05$). At termination (week 14), inhibition of plasma and brain ChE activities was confined to the 15 mg/kg bw/d group (~25%, $p<0.05$), while RBC ChE activity was inhibited at and above 1.5 mg/kg bw/d. Females from the 0.1 mg/kg bw/d group had significantly lower (8%; $p<0.05$) RBC ChE activity than the control, but as the level of inhibition was below 20%, this finding was not considered to be toxicologically significant.

Plasma and RBC ChE activities in rats treated with dichlorvos

ChE	Dose (mg/kg bw/d)							
	0		0.1		1.5		15	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 7								
Plasma	318±67 (0%)	813+326 (0%)	285±32 (10%)	933±382 (-14%)	226±49 (29%)*	692±90 (15%)	112±24 (65%)*	338±79 (58%)*
RBC	1195±163 (0%)	1269±245 (0%)	1166±244 (2%)	1148±124 (10%)	903±138 (24%)*	956±146 (25%)*	629±109 (47%)*	740±95 (42%)*
Week 14 (termination)								
Plasma	314±57 (0%)	1091±462 (0%)	282±60 (10%)	1150±485 (-5%)	259±70 (18%)	1020±257 (6%)	204±45 (35%)*	575±142 (47%)*
RBC	1358±146 (0%)	1321±82 (0%)	1247±114 (8%)	1212±81 (8%)*	1014±63 (25%)*	1002±82 (24%)*	787±104 (42%)*	874±87 (34%)*
Brain	1105±377 (0%)	1338+490 (0%)	1213±656 (-10%)	1290+376 (4%)	1060±183 (4%)	1290+337 (4%)	791±290 (28%)	680+217 (49%)*

Results expressed as the mean MU/mL ± 1 SD (% inhibition relative to the control); *$p<0.05$

Haematology and clinical chemistry: Possible treatment-related effects on haematology and clinical chemistry parameters are summarised in the Table below. At 15 mg/kg bw/d in both sexes, RBC, Hb and Hct were significantly lower than the control group ($p<0.05$). In males, Hb and Hct were also significantly lower than the control at 1.5 mg/kg bw/d. MCV was significantly elevated ($p<0.05$) in females at 15 mg/kg bw/d, while cholesterol was significantly elevated ($p<0.05$) in males at this same dose.

Haematology and clinical chemistry findings in rats treated with dichlorvos

Parameter	Dose (mg/kg bw/d)							
	0		0.1		1.5		15	
	♂	♀	♂	♀	♂	♀	♂	♀
RBC(10^6/μL)	9.68± 0.443	8.86± 0.201	9.85± 0.658	8.76± 0.471	8.95± 0.981	8.55± 0.496	8.78± 0.451*	7.69± 0.522*
Hb (g/dL)	16.2± 0.95	15.8± 0.56	16.2± 1.17	15.4± 0.68	14.6± 1.48*	15.4± 0.74	14.8± 0.79*	13.8± 0.97*
Hct (%)	53.2± 2.62	52.1± 1.01	53.8± 3.56	51.4± 2.72	49.0± 4.27*	50.9± 1.79	49.4± 2.14*	46.9± 2.48*
MCV (fL)	55±1.4	59±1.4	55±2.7	59±1.3	55±2.0	60±2.0	56±1.4	61±2.0 *
Cholesterol (mg/dL)	75±13	91±16	78±11	89±18	80±21	92±16	102±30*	103±16

Results expressed as the mean ± 1 SD; *p<0.05

An examination of individual animal data for the haematology findings indicated that there were no obvious outliers. Across all groups, RBC was within the historical control range of 7.0-9.8 10^6/μL (males) and 6.5-9.2 10^6/μL (females) for aged-matched (18-20 weeks) CD® rats (Charles River Laboratories 1993). With regard to Hb, only the high-dose female group was slightly below the historical control range of 14-17 g/dL. Hct was generally within the historical control range of 36-52%, with the exception of control and 0.1 mg/kg bw/d males, which had slightly higher values. MCV was within the historical control range of 50-60 fL across all groups, except for high-dose females, where it was slightly higher (61%). Given that the magnitude of the difference between the statistically-significant findings at 1.5 or 15 mg/kg bw/d and the control was modest, and that most parameters fell within their respective historical control range, the slight anaemia observed here is of limited toxicological significance. However, as the anaemia occurred in both sexes, was statistically significant and was dose-related it is likely to be treatment-related.
The significant elevation in cholesterol in males at the top dose was probably attributable to a single outlying rat with high cholesterol (166 mg/dL). Furthermore, the average cholesterol concentration of 103 mg/dL was only slightly above the historical control range for age-matched CD® rats (50-100 mg/dL; Charles River Laboratories 1993). Therefore, this finding was not considered treatment-related.

Pathology: Terminal bodyweights, absolute and relative organ weights and organ:brain weights were unaffected by treatment. No treatment-related macroscopic abnormalities were detected at necropsy. Histopathological examination revealed kidney tubular mineralisation in females at 1.5 and 15 mg/kg bw/d (1/10 and 3/10 rats, respectively), which was not detected in the control or 0.1 mg/kg bw/d groups. The historical control incidence of this finding in 3-month old female Crl:CD®(SD)BR rats is 10% (Charles River Laboratories 1992) and therefore the effect at 15 mg/kg bw/d could have been treatment-related. At 15 mg/kg bw/d, 2/10 males exhibited pancreatic islet cell hyperplasia and pigmentation of the islet cells, which were not detected in any other groups. Historical control data for these finding in age and sex matched rats of the same strain were

Conclusions: The NOEL following gavage dosing of rats 5 days/week for 13 weeks was 0.1 mg/kg bw/d, based on the inhibition of plasma ChE activity in males at week 7 and the inhibition of RBC ChE activity in both sexes at week 7 and at termination (week 14) at 1.5 mg/kg bw/d. At the highest dose of 15 mg/kg bw/d, inhibition of plasma, RBC and brain ChE activities, and clinical signs (salivation, presence of urine stains), occurred. A slight anaemia was seen in males at and above 1.5 mg/kg bw/d and in females at 15 mg/kg bw/d. Kidney tubular mineralisation in females and an equivocal increased occurrence of hyperplasia and pigmentation of the pancreatic islet cells was seen in males at 15 mg/kg bw/d.

5.2 Dogs

Hine CH (1962) 90-day chronic toxicity studies of Vapona insecticide for dogs. Report No. 1. Lab: The Hine Laboratories, San Francisco, USA. Sponsor: Shell Chemical Company, Agricultural Chemical Division, New York, New York, USA. Study duration: unspecified. Report date: 1st September 1962.

Methods

Groups of 3 male and 3 female beagle dogs (Lunor Dog Farms, Kalamaroo, Michigan) were given dichlorvos (Shell Chemical Co.; Batch No. 7362; 93% purity) in gelatine capsules for 90 days. The doses used were 0, 0.3, 0.9 or 1.4 mg/kg bw/d. These doses were selected because they were calculated to be equivalent to dietary concentrations of 0, 5, 15 and 25 ppm, based on a standard kennel diet of approximately 1 lb (0.45 kg) food/dog/d. As no physiological signs of treatment were evident at any dose level, the dose in the group receiving 5 ppm was increased to 50 ppm (3.0 mg/kg bw/d) on day 21, and a fifth group (3/sex) was added subsequently and dosed at 5 ppm. All animals were treated for 90 days.

Behavioural observations were made 3 times/week and body weight was measured at 2-weekly intervals. Clinical chemistry (BUN, bilirubin) and haematological assessment (white blood cell count, haemoglobin measurements, differential cell counts) were performed before treatment and at termination. RBC and plasma ChE activities were determined prior to treatment and at 2-weekly intervals, and brain ChE was measured in frontal lobe samples collected at termination. The animals were sacrificed for necropsy and organ weights were obtained for heart, lungs, liver, kidney and spleen. Tissues removed for histology included brain, parotid and submaxillary glands, lymph nodes, trachea, thymus, stomach, lungs, heart, aorta, vena cava, thyroid, oesophagus, gall bladder, adrenal gland, kidney, liver, bladder, pancreas, bone marrow, rib, sternum, seminal vesicles, prostate, testicles, ovary, oviduct and uterus.

Results

Mortalities and clinical signs: All animals receiving 3.0 mg/kg bw/d and 2/6 receiving 1.4 mg/kg bw/d showed signs of increased excitation and aggression. Urinary output was also increased in dogs from these groups. However, no effects on pupil size or response to light, muscle tone or muscle fasciculations were noted. There were no deaths and no treatment-related effects on body weight gain or food consumption.

ChE activity: Considerable variability in plasma and RBC ChE activities was recorded. However, a dose-dependent suppression in mean ChE activity was evident. At 3.0 mg/kg bw/d, inhibition of plasma, RBC and whole-blood ChE was 54, 41 and 48% respectively, compared to the controls, after 54 days. The greatest level of inhibition at 0.9 and 1.4 mg/kg bw/d was observed after 60 days; at 0.9 mg/kg bw/d, plasma, RBC and whole blood ChE activities were reduced to 66, 54 and 60%, respectively, while at 1.4 mg/kg bw/d, plasma, RBC and whole blood ChE activities were reduced to 61, 41 and 51%, respectively. At termination, mean plasma, RBC and whole blood ChE activities were within 80% of controls for the 0.9 and 1.4 mg/kg bw/d groups, whilst in the 3.0 mg/kg bw/d group, mean plasma and whole blood ChE activities were similar to controls but RBC activity was reduced to 68%. At the lowest dose of 0.3 mg/kg/d, no inhibition of RBC or whole blood ChE activity was observed, with mean values generally greater than 100% compared to controls. However, plasma ChE activity was commonly suppressed throughout the study, ranging from 62% after 54 days to 79% after 74 days. Individual animal data indicated reduced plasma, RBC and whole blood ChE activities compared to pre-treatment levels at 0.9, 1.4 and 3.0 mg/kg bw/d, particularly after 54-74 days. However, no effects were evident at 0.3 mg/kg bw/d.

Given the inherent variability in the data, the observed relative increase in RBC and total ChE activities compared to controls, and the lack of a change compared to pre-treatment levels, 0.3 mg/kg bw/d appeared to be the NOEL for plasma and RBC ChE inhibition.

Brain ChE activity was inhibited at 3.0 mg/kg bw/d, with the mean activity reduced to 33% of the controls. No significant inhibition was observed at or below 1.4 mg/kg bw/d.

Necropsy: There was no treatment-related effect on any clinical chemistry or haematology parameter or on gross pathology or organ weights. A number of histopathological lesions were observed but were randomly distributed and gave no indication of a treatment-related effect.

Conclusion: The study revealed some behavioural effects indicating autonomic stimulation in dogs given dichlorvos in gelatine capsules at doses of 1.4 or 3.0 mg/kg bw/d for 90 days. The NOEL was 0.3 mg/kg bw/d based on the inhibition of RBC and plasma ChE activities at and above 0.9 mg/kg bw/d, whilst brain ChE activity was only inhibited at 3.0 mg/kg/d.

6. CHRONIC STUDIES

6.1 Oral Administration

6.1.1 Mice

NCI (National Cancer Institute) (1977) Bioassay of dichlorvos for possible carcinogenicity. National Institutes of Health, Bethesda, Maryland, USA.

Materials and Methods

Dichlorvos (Shell Chemical Company; Batch No. unspecified; 94% purity) was dissolved in a small amount of acetone and admixed in the diet at 1000 or 2000 ppm and fed to groups of 50/sex B6C3F1 mice (Charles River, Massachusetts). Control groups of 10 mice/sex were given untreated feed. Corn oil was added to the feed of control and treated groups at 2%. All mice were 35 days old at the start of the treatment period. As well as the concurrent matched controls, analyses employed pooled controls of 100 male and 80 female mice from concurrently run experiments in the performing laboratory. As the initial concentrations were poorly tolerated, they were decreased to levels of 300 and 600 ppm after two weeks. Treatment continued for a total of 80 weeks and all surviving animals were sacrificed for necropsy after an additional 12-14 weeks without treatment. Controls were sacrificed after 92 weeks. As stability studies showed that concentrations of dichlorvos in the diet remained within 10% of the initial value over 7 days at 4°C, the diet was formulated weekly, stored at 0°C and changed daily.

Mice were observed twice daily for clinical signs, and weighed and palpated for masses periodically. Those appearing moribund at the time of the clinical examination were sacrificed and necropsied. Gross and microscopic examination was undertaken for all major tissues, organs or gross lesions taken from sacrificed or dead mice during the study, and from animals sacrificed at term. Routine tissues examined included brain, pituitary, adrenal, thyroid, parathyroid, trachea, oesophagus, thymus, salivary gland, lymph nodes, heart, lung, spleen, liver, kidney, stomach, pancreas, small intestine, large intestine, urinary bladder, prostate or uterus, testis or ovary, mammary gland, skin, bone and marrow.

Statistical analysis of tumour incidence was made using the Fisher exact test for intergroup comparison, and the Armitage and Cochran test for linear trend in proportions (with continuity correction). In addition, the exact 95% confidence interval for the odds ratio was calculated.

Results

Bodyweights and observations: Time-weighted average dietary concentration of dichlorvos were 318 ppm in the low-dose group and 635 ppm in the high-dose group (equivalent to 47.7 and 95.3 mg/kg bw/d, respectively). Average body weights of high-dose mice tended to be slightly lower than low-dose or controls after the initial growth phase although the difference was slight. Symptoms of severe toxicity (tremors, rough coat, diarrhoea and poor general appearance) were observed in low-dose and high-dose groups during the first 2 weeks, but mice recovered when the doses were reduced. The only adverse clinical signs noted after the first 2 weeks were alopecia and rough coats, particularly in treated males, which developed from approximately week 20. Survival rates were unaffected by treatment, with group survival ranging from 74% (low-dose females) to 94% (high-dose males) after 90 weeks.

Necropsy: Non-neoplastic proliferative or inflammatory lesions, most commonly adrenal cortical hyperplasia (both sexes) and cystic endometrial hyperplasia (females), occurred with approximately equal frequency in control and treated mice. The only noteworthy neoplastic lesions were squamous cell carcinoma of the oesophagus in 1/47 low-dose males and 1/41 high-dose females, and a single oesophageal papilloma observed in 1/41 high-dose females. Epithelial hyperplasia of the oesophagus was evident in 3/47 low-dose males. These lesions were not observed in any other groups (although samples from matched and pooled control groups were fewer: 8 female and 10 male matched control samples, and 16 female and 27 pooled control samples). No other uncommon tumours appeared only in treated groups. Overall, there were no statistically significant differences in tumour incidence between treated and untreated mice.

The Charles River Laboratories historical control incidence of oesophageal squamous papillomas in male B6C3F1 mice is 1/1112, while in females it is 1/1111. The NTP historical control incidence of squamous cell carcinoma of the forestomach in males is 2/1159 and in females it is 1/1158. The NTP historical control incidence of squamous cell papilloma is 13/459 in males and 18/1158 in females. Based these historical control data and given the lack of dose-response relationships and statistical significance, the low incidence of forestomach lesions seen in this study is considered to show an equivocal relationship with treatment.

Conclusion: The LOEL following dietary administration for 80 weeks was approximately 48 mg/kg bw/d, based on the occurrence of clinical signs (alopecia and rough hair coats) and oesophageal hyperplasia (males). The occurrence of a squamous cell carcinoma in a single male and female at 48 and 96 mg/kg bw/d, respectively, and the presence an oesophageal papilloma in a single female at 96 mg/kg bw/d, were considered to show an equivocal relationship with treatment.

Konishi Y, Denda A & Kitaoka R (1981) Studies on carcinogenicity of dichlorvos in B6C3F1 mice. Ministry of Health and Welfare, Tokyo, Japan (Cooperative studies on carcinogenicity test on mutagens). Unpublished.

This study has not been independently evaluated by the OCS. The following text is based on the 1989 IPCS monograph on dichlorvos (EHC 79).

Groups of 50 B6C3F1 mice/sex were given freshly-prepared drinking water *ad libitum* containing 0, 400 or 800 mg/L dichlorvos for 102 weeks (estimated oral doses of 56 and 112 mg/kg bw/d, respectively, based on an average bodyweight of approximately 50 g and a daily water intake of 7 mL). Drinking water solutions were renewed daily. Surviving mice were sacrificed on cessation of treatment for autopsy. There was a dose-dependent decrease in bodyweight gain in both sexes at both concentrations of dichlorvos. There was no treatment-related effect on mortality (survival rates of control, low dose and high dose groups after 102 weeks were 62, 66 and 84% in males, and 66, 50 and 80% in females, respectively). The proportions of mice with tumours in these groups were 22.4, 39.1 and 23.4% in males, and 29.3, 16.2 and 9.1% in females, respectively. The main tumour types were lung adenomas and tumours of the liver, spleen, thymus and salivary gland, which occurred in all three groups. There was no statistically significant difference in tumour incidences at any site in any group.

Horn KH, Teichmann B & Schram T (1987) Investigations with dichlorvos (DDVP) I. Testing dichlorvos for carcinogenic activity in mice. Archiv für Geschwulstforschung 57: (5): 353-360 (Translated from German)

Materials and Methods: Dichlorvos (VEB Chemiekombinat Bitterfeld; Batch No. unspecified; 97% purity) was freshly dissolved in water and administered by oral gavage to groups of male and female inbred C57B1/6/Bln mice (5-6 weeks of age) at 0.2 mg/mouse (~10 mg/kg bw) either 2- or 3-times per week for 50 weeks (designated as DDVP-1 and DDVP-II groups, respectively). These doses are equivalent to 2.9 and 4.3 mg/kg bw/d. Additional groups of mice served as controls and were either untreated (UC) or gavaged with water 3-times per week (WC). Group sizes were not given but appeared to be $\geq$79/sex in the treated groups and $\geq$35/sex in the control groups. Mice were maintained for up to 60 weeks after the cessation of dosing.

Mice were inspected daily and weighed periodically. Caudal vein blood samples were collected from 15 animals/sex/group at 6-monthly intervals for evaluation of differential cell counts. All animals dying or killed during the study were necropsied. Few animals (unpsecified numbers) reportedly survived to 110 weeks, with all survivors necropsied. Organs showing macroscopic variations were examined histopathologically. Major organs (lung, liver, stomach, kidneys, adrenals and urinary bladder) were also examined histopathologically. The method of scoring lesions and tumours was unusual in that the number of mice evaluated was determined separately for each lesion and tumour type. Thus, the numbers of mice evaluated varied widely between groups (eg. the incidence of histological foci in liver cells was evaluated in 8 males and 63 females in the group dosed twice weekly with dichlorvos). Results were reportedly analysed using χ^2 and t-tests but no details were provided.

Results: Survival times did not appear to be affected by treatment. Average survival times in males were given as 74, 62, 62 and 90 weeks in DDVP-1, DDVP-II, WC and UC groups, respectively. In females, the survival times in the corresponding groups were 70, 55, 64 and 93 weeks, respectively. Survival curves and numbers, and body weights and clinical signs were not reported. There was a marginal increase in the incidence of focal hyperplasia of the bladder in treated animals compared to controls (5-12% *versus* 0-9%), which was not considered treatment-related. There were no other non-neoplastic lesion, and no neoplastic lesions, which could be attributed to treatment. Of particular note was a lack of effect on the incidence of forestomach lesions (cf other rodent studies): hyperplasia was noted in animals from all groups, ranging from 10/119 (8.4%) in DDVP II females to 12/35 (34.2%) in untreated control females; and papillomas in 2/96 (2%) DDVP II males and 1/66 (1.5%) WC males.

Conclusion: This study was considered to have limited regulatory value due to the poor study design and lack of reporting detail. However, it did indicate that chronic oral administration of dichlorvos to mice was not carcinogenic.

Chan PC (1989) NTP Technical Report on the Toxicology and Carcinogenesis Studies of Dichlorvos in F344/N rats and B6C3F1 mice (Gavage Studies). Lab: Southern Research Institute for the National Toxicology Program, Research Triangle Park. Technical Report Series NTP TR No.342.

Materials and Methods

Dichlorvos (Shell Development Company, Houston, Texas, USA; Batch No. SDC 092179; 99% purity) was administered orally in corn oil by gavage to B6C3F1 mice (50/sex/group; 8 weeks old; Charles River, Michigan, USA) at dose levels of 0, 10 or 20 mg/kg bw/d (males) or 0, 20 or 40 mg/kg bw/d (females) 5 days/week for 103 weeks. The doses were based on the results of a preliminary 13-week study using groups of 10/sex in which 160 mg/kg bw/d was generally fatal for both sexes and 80 mg/kg bw/d caused 50% mortality in males.

Mice were housed 5/cage, with food and water available *ad libitum*. Clinical signs were assessed twice daily. Mice were weighed weekly during the first 12 weeks and monthly thereafter. Necropsy examinations were performed on animals dying or sacrificed *in extremis* during treatment, and on all animals surviving to termination. Routine histological examination was made of all major tissues and organs including central nervous system (CNS), bone and marrow, gut, urinary tract, liver, spleen, gall bladder, reproductive organs, lymph glands, thymus, thyroid and parathyroid, salivary gland, lung and trachea, pituitary, mammary gland, skin, and any tissue masses observed macroscopically. The effect of dichlorvos on plasma and RBC ChE activities was analysed in a separate experiment where 10 mice/sex/dose were dosed orally by gavage at 0, 5, 10, 20 or 40 mg/kg bw/d 5 times per week for 37 days. Plasma and RBC ChE activities were measured on days 11, 25 and 33.

Survival rates were statistically analysed using Kaplan and Meier curves, and tumour incidence was analysed by Fisher exact and Cochran-Armitage trend tests.

Results

Mortalities and Clinical Observations: Food and water intake were not assessed but bodyweight gain was similar in all groups. Survival was unaffected by treatment, with rates of 70, 54 and 58% recorded in control, low-dose and high-dose males, respectively, and 50, 58 and 68% in the corresponding female groups, respectively.

ChE: In the satellite study, plasma ChE activity was significantly and dose-dependently reduced in both sexes by 50% at 5 mg/kg/d to 85% at 40 mg/kg bw/d. This was consistent throughout the one month treatment period (first measurement after 10-11 days). No marked effects on RBC ChE activity were evident at any dose level in either sex.

Necropsy: Non-neoplastic lesions occurred with similar frequency in all groups and no effects of treatment on incidences were observed.

In female mice, there were statistically significant negative trends for lymphomas and pituitary *pars distalis* adenomas in female mice. Pair-wise comparisons between treated and control groups were not statistically significantly different. The only treatment-related increase in tumour incidence was of the forestomach squamous epithelium. Data are summarised in the Table below.

Incidence forestomach lesions in mice

Lesion	Dose (mg/kg bw/d; 5 days/week)							
	0		10		20		40	
	M	F	M	F	M	F	M	F
Hyperplasia	11(22)	12(24)	5(10)	-	9(18)	7(14)	-	5(10)
Papilloma	1(2)	5(10)	1(2)	-	5(10)	6(12)	-	18(36)
- day 1st	729	669	714	-	729	442	-	520
Carcinoma	0	0	0	-	0	0	-	2(4)

Results expressed as the number of mice exhibiting the lesion (% incidence; n=49 or 50)

The incidence of forestomach squamous cell papillomas was elevated at 20 and 40 mg/kg bw/d, in males and females, respectively. This was statistically significant on trend analysis in both sexes, but only in females using a pair-wise comparison between the high dose and control groups. The historical control incidence of squamous cell papillomas from 8 studies from the performing laboratory, using corn oil vehicle, was 3/396 (0.75%; range 0/50-3/49) in males and 4/396 (1%; range 0/50-2/50) in females. These values were similar to those obtained from NTP's overall pooled historical controls of 34 studies using corn oil gavage. The historical control incidence of sqamous cell papillomas from the current NTP database is 13/1159 (1%) in males and 18/1158 (1.5%) in females. Therefore, the occurrence of squamous cell papillomas in males at 20 mg/kg bw/d and in females at 40 mg/kg bw/d are considered to be treatment-related. Forestomach carcinomas were evident in 2 females (4%) at 40 mg/kg bw/d but not in any other group. While this finding was higher than the NTP historical control incidence of 1/1158 (0.1%) in females (and 0.2% in males), the occurrence of forestomach carcinomas in females at 40 ppm was concluded to show an equivocal relationship with treatment. There were no statistically significant intergroup differences in forestomach hyperplasia, a lower grade lesion on the morphological, proliferative continuum leading to papilloma.

Conclusions: The LOEL following dietary administration of dichlorvos to mice for one month was 5 mg/kg bw/d, based on the inhibition of plasma ChE activity in the satellite study. The NOEL for forestomach lesions (specifically papillomas) following chronic dietary administration was 10 mg/kg bw/d for males and 20 mg/kg bw/d for females. The occurrence of forestomach carcinomas in females at 40 mg/kg bw/d showed an equivocal relationship with treatment. Mechanistic studies discussed in Section 12, demonstrate that dichlorvos is irritating to the mouse GIT, as it is to rabbit eyes and skin. Given its irritancy and consistently negative results in *in vivo* genotoxicity studies, the increase in forestomach papillomas seen in this study are concluded to be irrelevant to human dietary risk assessment.

Horn KH, Teichmann B & Schramm T (1990) Studies on dichlorvos. III. Testing of dichlorvos for co-carcinogenic activity in mice. Archiv für Geschwulstforschung 60:117-124.

This study has not been independently evaluated by the OCS. The following text is based on the 1993 JMPR evaluation report on dichlorvos.

There was no evidence that dichlorvos was co-carcinogenic in C57Bl/6/Bln mice when administered by oral gavage 3 times/week at 0.2 mg/mouse (~4.3 mg/kg bw/d), with mice also subcutaneously

injected with 50 µg N-nitrosodiethylamine. Mice were treated for 50 weeks and then observed for up to 110 weeks.

6.1.2 Rats

Witherup S, Stemmer KL & Pfitzer EA (1967) The effects exerted upon rats during a period of two years by the introduction of Vapona® insecticide into their daily diets. No study or Report No. Lab/Sponsor: The Kettering Laboratory, Department of Environmental Health, College of Medicine, University of Cincinnati, Ohio, USA. Report date: 14th February 1967.

Materials and Methods

Dichlorvos (Shell Chemical Co; batch No. unspecified; 93% purity) was diluted in ethanol, admixed in the diet (Purine Laboratory Chow) and fed to CD weanling rats (40/sex/group; unspecified strain; Charles River, Massachusetts, USA) at 0, 0.1, 1, 10, 100 or 500 ppm for 2 years (equivalent to 0, 0.005, 0.05, 0.5, 5 and 25 mg/kg bw/d, respectively, using a dietary conversion factor of 20). Diets were prepared weekly. Composite samples collected at 2, 4 and 7 days after preparation were stored frozen prior to analysis off-site by an unspecified method (Residue analysis Laboratory, Agricultural Research Division, Shell Development Company, Modesto, California, USA). Rats had been acclimatised for two weeks prior to the initiation of treatment and were randomly assigned to each group. Rats were housed 3/cage in a room maintained at 76±2°F.

Rats were observed daily for clinical signs. Bodyweights were recorded weekly for the first year and then twice weekly for the second year. Food consumption was not measured. Five rats/sex/group were sacrificed by exsanguination after 6, 12 or 18 months, with all remaining survivors sacrificed at the end of the treatment period (24 months). All rats were necropsied and examined for gross visceral abnormalities. The following organs were weighed: liver, heart, lungs, kidneys, spleen, brain, gonads, pituitary, adrenals, and thyroid. These same organs were histopathologically examined in addition to sections of the CNS.

Blood and urine were sampled from 10 rats/group at 6, 12, 18 and 24 months for analysis. The following haematology parameters were analysed: Hb, Hct, total and differential leukocyte count. The following clinical chemistry parameters were analysed: protein, albumin/globulin (A/G) ratio, and plasma and RBC ChE activities. Plasma and RBC ChE activities were also measured at regular intervals during the study (13 times). Brain ChE activity was measured at each of the 4 sacrifice times. The following urinalysis parameters were analysed: albumin, glucose, acetone, pH and microscopic analysis for cellular components, crystals of uric acid, calcium oxide and triphosphates, and for amorphous material.

Results

Dietary analysis: There was considerable loss of dichlorvos associated with a gradual increase in DCA. At the 5 dose levels, analytical dichlorvos concentrations were 0.08, 0.80, 8.0, 80 and 400 ppm after 12 h, declining to 0.022, 0.224, 2.24, 22 and 112 ppm after 156 h, respectively. Average concentrations of dichlorvos were 0.047, 0.467, 4.67, 46.7 and 234 ppm. The average DCA content present was 0.014, 0.114, 0.887, 6.86 and 28.6 ppm in these groups, respectively.

Mortalities, clinical signs and bodyweight effects: There was no treatment-related effect on mortalities, clinical signs or bodyweight gain.

Haematology, clinical chemistry and urinalysis: There was no treatment-related effect on any heamatology, clinical chemistry or urinary parameter.

ChE activity: Plasma and RBC ChE activities were measured 13 times during the study, with no significant effects observed in the three lowest dose groups. In the 100 ppm group, plasma and RBC ChE activities were reduced to 60-90% and to 50-90% of controls in males and females, respectively. In the 500 ppm group, activities were reduced to 20-70% and 20-60%, respectively. Activities tended to increase as the study progressed. At termination, brain ChE activity was significantly decreased

only in the 500 ppm group, by 45-47% in rats sacrificed after 6 months, declining to 5-15% in those sacrificed after 24 months.

Pathology: There was no treatment-related effect on organ weights of rats sacrificed at scheduled intervals or at termination, and there were no treatment-related macroscopic lesions. Histology revealed hepatocellular fatty vacuolisation in all 500 ppm rats, and in approximately 80% of females and 62% of males at 100 ppm. This was only evident in animals sacrificed after 18 or 24 months. All neoplastic lesions were benign, with most occurring in the mammary and pituitary glands of both sexes without dose-dependency. There were no tumours that occurred only at the high-dose. Overall, no treatment-related effects were observed on the incidence or timing of tumours.

Conclusion: The NOEL following 2-years of dietary exposure to dichlorvos was 10 ppm (mean analytical concentration of 4.67 ppm; equivalent to 0.23 mg/kg bw/d) based on the inhibition of plasma and RBC ChE activity at and above 100 ppm (mean analytical concentration 46.7 ppm; equivalent to 2.3 mg/kg bw/d). Hepatocellular abnormalities were evident at 100 and 500 ppm, but these were insufficient to indicate impairment of normal liver function.

NCI (National Cancer Institute) (1977) Bioassay of dichlorvos for possible carcinogenicity. Bethesda, Maryland, USA.

Materials and Methods

This study was undertaken concurrently with the NCI mouse study reported above and used dichlorvos from the same source and the same assessment and analysis protocol. Osborne-Mendel rats (50/sex/group; Charles River, Massachusetts, USA) were fed diet containing dichlorvos at 500 and 1000 ppm (equivalent to 25 and 50 mg/kg bw/d, using a dietary conversion factor of 20). Control groups of 5 rats/sex were matched with each dose group and sex (ie. 10 control rats/sex in total) and given untreated feed. Corn oil was added to the feed of control and treated groups at 2%. Results were analysed using comparisons with pooled data from control rats (60/sex), used in other pesticide studies performed at the same time, as well as with matched controls. The initial dose was poorly tolerated in the high-dose group and was reduced to 300 ppm after 3 weeks (equivalent to 15 mg/kg bw/d). The low-dose group dose was reduced to 150 ppm to maintain the protocol (low dose required to be half the high dose) (equivalent to 7.5 mg/kg bw/d). The low-dose group and 5 of the controls/sex were started on the new lower dose level 4 weeks after the high-dose groups because of a shortage of rats. Treatment continued for 80 weeks and all surviving rats were sacrificed for necropsy after an additional 30 weeks without treatment (ie. at 110 weeks). Controls were also sacrificed after 110 weeks. The diet was formulated weekly, stored at 0°C and changed daily. Rats were observed twice daily for clinical signs and weighed and palpated for masses periodically during the study. Most rats dying before term and all those sacrificed after 110 weeks were necropsied.

Results

Mortalities and Clinical Signs: No adverse effects of treatment were observed on mortality; 64 and 76% of males and 80 and 84% of females survived in the low dose and high dose groups respectively. Four/10 control males and 1/10 females died in the post-treatment period before scheduled termination. Marked adverse clinical signs were observed in high dose rats during the first 3 weeks (tremors, rough coat, diarrhoea and poor general appearance). Rats recovered when the dose was reduced to 300 ppm. Alopecia, epistaxis, haematuria, dark urine, palpable masses, and abdominal distension developed in control and treated groups as the study progressed, increasing in incidence in the treated groups and predominating in high dose females. Average body weights of high dose males and females were consistently lower than controls during the 80-week treatment phase, recovering during the post-treatment period.

Necropsy: There was no treatment-related effect on the occurrence of non-neoplastic proliferative or inflammatory lesions. Common findings included: focal hepatocytomegaly; chronic nephritis, renal tubular dilatation and regeneration, and hyperplasia of the renal pelvis epithelium (and urinary bladder epithelium); and thyroid C-cell and parathyroid hyperplasia. There were no treatment-related effects on the total number or time of onset of neoplastic lesions. Thyroid, pituitary and mammary gland tumours were the most common. The only tumour that exhibited a positive trend with dose was malignant fibrous histiocytoma of the subcutis in males (see Table below). The trend was significant

(p=0.018) when compared to the pooled controls but not in matched controls. Given that the occurrence of malignant fibrous histiocytoma was dose-related and statistically significant, the possibility that is was due to dichlorvos can not be discounted, despite the absence of this tumour type in other rat studies.

Occurrence of malignant fibrous histiocytoma in rats

Males				Females			
Pooled control	Matched control	Low Dose	High Dose	Pooled control	Matched control	Low Dose	High Dose
2/58 (3%)	1/10 (10%)	4/48 (8%)	8/50 (16%)	1/60 (2%)	0/10 (0%)	5/48 (10%)	1/50 (2%)

Results expressed as the number of rats affected/total number of rats (% incidence)

Conclusion: Chronic dietary administration of dichlorvos to Osborne-Mendel rats resulted in a significant dose-related increase in malignant fibrous histiocytoma in males at 7.5 and 15 mg/kg bw/d. The relevance of this neoplasm to the hazard assessment of dichlorvos is unclear as other studies using different rat strains and higher doses failed to cause a similar effect.

Enomoto MF, Nakadate M, Ninomia K, Hayakawa Y, Ito H, Igarashi S, Uwanuma Y, Nakasato R & Hatanaka J (1981) Studies on carcinogenicity of DDVP (2,2-dichlorovinyl dimethyl phosphate) mixed in drinking-water in rats. Ministry of Health and Welfare, Tokyo, Japan (Cooperative studies on carcinogenicity test on mutagens). Unpublishe).

This study has not been independently evaluated by the OCS. The following text is based on the 1989 IPCS monograph on dichlorvos (EHC 79).

Groups of F344 rats (50/sex; 6 weeks of age) were given freshly prepared drinking water *ad libitum* containing 0, 140 or 280 mg/L dichlorvos for 104 weeks. Estimated oral doses were 14 or 29 mg/kg bw/d, respectively, assuming a daily water intake of 10 mL/100 g bodyweight and bodyweights of 400 g for males and 250 g for females. Drinking water solutions were renewed daily. All surviving animals were sacrificed for necropsy after a further 4-week recovery period. Slight inhibition of body weight gain was observed in high-dose males, but there was no effect on mortality. Survival rates in week 108 were 82, 75 and 75% in males, and 86, 71 and 82% in females in control, low dose and high dose groups, respectively. The organs and tissues of all animals dying during the study and those surviving to term were examined microscopically. The overall tumour incidences were 100, 96 and 98% in males and 37, 31 and 33% in females in the control, low dose and high dose groups, respectively. High incidences of interstitial cell tumours of the testes were seen in males across all groups without dose-dependency (49/51, 41/48 and 47/48 in control, low- and high-dose groups). Mononuclear cell leukaemia was found in all groups at 4-12%. There was no statistically significance difference in tumour incidence at any site in any group.

Horn KH, Teichmann B, Schramm T & Nischan P (1988) Studies on dichlorvos. II. Testing of dichlorvos for carcinogenic activity in rats. Archiv für Geschwulstforschung, 58: 1-10. (Translated from German)

Materials and Methods: Groups of inbred BD IX/Bln rats (6-8 weeks of age) received 0.1 mg dichlorvos (VEB Chemiekombinat Bitterfeld; batch no. unspecified; 97% purity) freshly dissolved in 0.2 mL water by oral gavage twice (low-dose; 70/sex) or 3 times (high-dose; 99/sex) per week for 60 weeks (doses were approximately equivalent to 0.07 and 0.11 mg/kg bw/d, respectively). Thereafter, rats were observed for another 51 weeks before they were sacrificed for necropsy. Control groups of 59 males and 60 females received 0.2 mL water 3 times per week. Caudal vein blood samples were collected from 15-20 animals/sex/group at 7-monthly intervals for evaluation of differential cell counts. All animals dying or sacrificed during the study were necropsied and organs showing macroscopic variations were examined histologically. Major organs (lung, liver, stomach, kidneys, adrenals and urinary bladder) were also examined histologically. The method of scoring lesions and tumours was similar to that employed by Horn et al (1987), with the number of rats evaluated determined separately for each lesion and tumour type.

Results: Mortality did not appear to be affected by treatment, with average survival times in males given as 62, 63 and 67 weeks in control, low- and high-dose groups, respectively. In females, survival times in the corresponding groups were 74, 75 and 77 weeks, respectively. Survival curves and numbers, and body weights and clinical signs were not reported.

There was a marginal increase in focal hyperplasia of the forestomach at both doses and in both sexes, with forestomach papillomas also detected in a small number of rats at the high dose (see Table below). These findings were not statistically significant and did not follow a dose-response relationship (recognising that the latter was possibly the result of the closeness of the dose selection). While the occurrence of hyperplasia and papillomas are consistent with studies conducted in mice, in the absence of suitable historical control data for this particular rat strain, the current findings are considered equivocal. There was an apparent dose-related increase in focal oval cell or bile duct proliferation males, which was statistically significant at the high dose (no p value given) (see Table below). However, the interpretation of this finding was complicated by the lack of suitable historical control data. The incidence of focal bladder hyperplasia was increased in males (15, 28 and 26% in control, low- and high-dose groups, respectively), but was not statistically significance. No neoplastic lesions were detected that could be attributed to treatment.

Incidence of forestomach and liver lesions in rats

Lesion	Control		0.1 mg, 2x week		0.1 mg, 3x week	
	♂	♀	♂	♀	♂	♀
Forestomach hyperplasia	4/54 (7%)	8/56 (14%)	10/66 (15%)	15/66 (23%)	13/97 (13%)	20/97 (21%)
Forestomach papillomas	0/10	0/22	0/13	0/20	2/26 (8%)	1/38 (3%)
Oval cell/ bile duct prolif.	12/40 (30%)	8/42 (19%)	23/48 (48%)	27/54 (50%)	41/77* (53%)	53/79 (67%)

* Significantly different to the control

Conclusion: While the current study had a number of deficiencies, which limited its regulatory value, such as the low and poor dose selection and lack of reporting detail, it indicated that dichlorvos is unliklely to be carcinogenic in this particular rat strain. The occurrence of proliferative lesions of the forestomach and bile duct showed an equivocal relationship with treatment.

Chan PC (1989) Toxicology and carcinogenesis studies of dichlorvos (CAS No. 62-73-7) in F344/N rats and B6C3F1 mice (gavage studies). Performed at Southern Research Institute for the National Toxicology Program, Research Triangle Park. Technical Report Series NTP TR No.342.

Materials and Methods

Fischer 344/N rats (50/sex/group; 7 weeks old; Charles River, Michigan, USA) were administered dichlorvos (Shell Development Company, Houston, Texas, USA; batch No. SDC 092179; 99% purity) orally in corn oil by gavage at doses of 0, 4 or 8 mg/kg bw/d, 5 days per week for 103 weeks. The dose selection was based on the results of a preliminary 13-week study. [In this particular study, 1/10 male rats died at 32 or 64 mg/kg bw/d, and 4/10 females died at 16 mg/kg bw/d (an additional male died at 16 mg/kg bw/d but this was related to gavage injury). Terminal bodyweight was reduced by 5% in females at 8 or 16 mg/kg bw/d, but there were no adverse clinical signs and no treatment-related macroscopic or microscopic abnormalities.]

Rats were housed 5/cage, with food and water available *ad libitum*. Clinical signs were assessed twice daily. Rats were weighed weekly during the first 14 weeks and monthly thereafter. Necropsy examination was performed where possible on rats dying or sacrificed *in extremis* during treatment and on all rats surviving to termination. Routine histological examination was made of all major tissues and organs including CNS, bone and marrow, gut, urinary tract, liver, spleen, reproductive organs, lymph glands, thymus, thyroid and parathyroid, salivary gland, lung and trachea, pituitary, mammary gland, skin, and any tissue masses observed macroscopically.

The effect of dichlorvos on plasma and RBC ChE activities was analysed in a separate experiment where 10 rats/sex/dose were dosed orally by gavage at 0, 2, 4, 8 or 16 mg/kg bw/d 5 times per week for 37 days. Plasma and RBC ChE activities were measured on days 10, 24 and 32.

Survival rates were statistically analysed using Kaplan and Meier curves, and tumour incidence was analysed by Fisher exact and Cochran-Armitage trend tests.

Results

Mortalities and Clinical Signs: Mild diarrhoea was reportedly treatment-related but there were no other adverse clinical signs. No treatment-related effects on bodyweight gain or survival (48-64% in males, 52-62% in females) occurred.

ChE activity: ChE activity, determined in an adjunct study, showed that RBC ChE activity was slightly (<20%) but significantly reduced in males from 4 mg/kg bw/d but was unaffected in females. Plasma ChE was significantly reduced (>30%) in both sexes at all doses.

Necropsy: Cytoplasmic vacuolisation of the liver and adrenal cortex was observed at increased frequency in low-dose (26%) and high-dose (38%) males compared to controls (14%). Adrenal cortical cytoplasmic vacuolisation was also observed in high-dose males (26% compared to 6% in controls) and low-dose females (34% compared to 9% in controls).

Non-neoplastic and neoplastic lesions of the pancreas were observed in both sexes. Initial routine cross-sections indicated a higher incidence of focal atrophy in high-dose females. Acinar hyperplasia was evident in 18% of male rats from each of the control, low-dose and high-dose groups, and in 4%, 6% and 0% of control, low dose and high dose females, respectively. In a second analysis, horizontal sections of pancreas from each rat were examined, showing higher levels of hyperplasia: 66, 88 and 78% in control, low-dose and high-dose males, respectively, and 42, 44 and 60% in corresponding females. The incidence of hyperplasia in treated groups of either sex was not significantly different from the controls.

The incidence of exocrine proliferative lesions of the pancreas is summarised in the Table below, which shows that there was an increase in acinar adenomas in males at 4 and 8 mg/kg bw/d. In the initial cross-sectional sampling, acinar adenomas were significantly increased ($p<0.05$) at both doses, with the result following a relatively shallow dose-response relationship. When supplementary horizontal sections were prepared an increase in acinar adenomas was also evident at both doses, but the result was not statistically significant nor did it follow a dose-response relationship. When the cross-sectional and horizontal sectional data were combined, the total incidence of adenomas was significantly higher ($p<0.05$) at both doses compared to the concurrent controls. The overall interpretation of these findings is complicated by the high background incidence in the concurrent control (which was significantly greater than the mean historical control incidence) and the large historical control range (0-28%). A further complication is the fact that corn oil gavage is known to increase the incidence of acinar cell adenomas in male rats (Haseman 1985). Therefore, the occurrence of pancreatic acinar adenomas is considered to show an equivocal relationship with treatment.

Incidence of exocrine pancreas proliferative lesions in rats

Sex		Hist. Control[1] (SRI)	Hist. control* (NTP)	Concurrent control	4 mg/kg	8 mg/kg
♂	Cross-section adenomas	30/347 (8.6%) (Range 0-22%)	90/1624 (5.5%) (Range 0-28%)	16/50 (32%)	25/50* (51%)	30/50* (60%)
	Horizontal-section adenomas			15/50 (30%)	23/50 (46%)	17/50 (34%)
	Total adenomas			25/50 (50%)	30/50* (60%)	33/50* (66%)

Sex		Hist. Control[1] (SRI)	Hist. control* (NTP)	Concurrent control	4 mg/kg	8 mg/kg
♀	Cross-section adenomas	1/397 (0.3%) (Range 0-4%)	7/1679 (0.4%) (Range 0-4%))	1/50 (2%)	1/50 (2%)	4/50 (8%)
	Horizontal-section adenomas			1/50 (2%)	2/50 (4%)	2/50 (4%)
	Total adenomas			2/50 (4%)	3/50 (6%)	6/50 (12%)

Results expressed as the number of rats showing the lesion/group size (% incidence); 1 = Historical control data from 8 studies at Southern Research Institute (SRI), or from 34 NTP studies, both using corn oil gavage; *Significantly different from concurrent controls, $p<0.05$

The incidence of mononuclear cell leukaemia (MCL) in males was significantly increased [11/50 (22%), 20/50 (40%), 21/50 (42%) in control, low dose and high dose groups, respectively]. The historical control range for corn oil gavage was 2-18% for rats from SRI (8 studies), and 2-44% for rats from 34 NTP studies. Thus, the incidence of MCL in males in the present study was within the NTP historical control range and was therefore not biologically significant. The incidence of MCL in females was 17/50 (34%) in the controls, 21/50 (42%) at the low dose and 23/50 (46%) at the high-dose but the biological significance of the finding could not be determined due the absence of suitable historical control data.

Alveolar/bronchiolar adenomas were observed in 3/50 (6%) high dose-males but in no other group. This incidence was within the historical control range of 0-8%.

In females, the incidence of mammary gland fibroadenomas was significantly but not dose-dependently increased; 9/50 (18%) in controls, 19/50 (38%) in low-dose and 16/50 (32%) in high-dose groups. Multiple fibroadenomas were observed in 6 (12%) of the low dose and 3 (6%) of the high-dose females, and one adenoma (2%) was observed in a high-dose female. Two mammary gland carcinomas were observed in each of the control and low-dose groups (4%). Incidences of fibroadenomas in historical controls were 16-18% for RSI and 12-40% for NTP controls. Thus, the incidence of mammary tumours in the present study was within the NTP historical control range and therefore not biologically significant.

Conclusion: No NOEL was established in this study because plasma ChE activity was inhibited at every dose. There was a significant increase in exocrine proliferative lesions of the pancreas in males at 4 and 8 mg/kg bw/d, which were considered to show an equivocal relationship with treatment. The increased incidences of MCL in males and mammary tumours in females were not considered biologically significant due to the lack of a dose-response relationship and as the incidences fell within the NTP's respective historical control range. Overall, dichlorvos was considered to show equivocal evidence of carcinogenicity in rats.

6.1.3 Dogs

Jolley PW, Stemmer KL & Pfitzer EA (1967) The effects exerted upon beagle dogs during a period of two years by the introduction of Vapona® insecticide into their daily diets. Report/Study No. unspecified. Lab: Kettering Laboratory, University of Cincinnati, Ohio, USA. Report date: 19th January 1967.

Materials and Methods

Dichlorvos (Shell Chemical Company, unspecified location; unspecified batch No.; 93% purity) was dissolved in ethanol, admixed in the diet and fed to beagle dogs (3/sex/group; Lunar Farms Inc, Kalamanza, MI, USA) at 0, 0.1, 1.0, 10.0, 100 or 500 ppm for 2 years (equivalent to 0, 0.0025, 0.025, 0.25, 2.5 or 12.5 mg/kg bw/d, respectively, using a dietary conversion factor of 40). Dogs were 4-5 months at the commencement of treatment and had been acclimatised for 3 months. Food was prepared weekly.

Dogs were observed daily for adverse signs. Bodyweight and food consumption were measured weekly. The following haematology parameters were measured 1, 2, 6, 9, 18 and 24 months: Hct, Hb, leucocytes and differential leucocytes. The following urinalysis parameters were measured after 1, 2, 9, 8 and 24 months: albumin, sugar, acetone, pH and microscopy; The following clinical chemistry parameters were measured prior to treatment and at 24 months: SGOT, SGPT, AP, total protein and A/G. Plasma and RBC ChE activities were measured at 3 and 2 weeks prior to dosing and then after 1, 4 and 6 weeks, and 2, 3, 6, 9, 18 and 24 months of dosing. ChE activity was determined in brain tissue at necropsy. All dogs were sacrificed at the end of the treatment period and autopsied. Major organs (liver, heart, lungs, kidneys, spleen, brain, gonads, pituitary, adrenals, thyroid) were weighed and, with stomach, small and large intestine and pancreas, were examined histologically. Gross pathological abnormalities were also examined microscopically.
Results

Achieved doses: Volatilisation reduced the concentration of dichlorvos present in the feed. Pooled samples taken daily during each week indicated that average concentrations were 0.09, 0.32, 3.2, 32.0 and 256 ppm at 0.1, 1.0, 10.0, 100 or 500 ppm, respectively. DCA was also detected at 0.60, 6.4 and 20.0 ppm in the 10.0, 100 or 500 ppm diets, respectively.

Mortalities, clinical signs and effects on bw, food consumption, haematology, clinical chemistry and urinalysis: One male dog (500 ppm group) was sacrificed *in extremis* after 68 weeks with a diagnosis of bronchitis and pneumonia. There were no treatment-related clinical signs, effects on food consumption or on bodyweight. There was no treatment-related effect on any haematology, clinical chemistry or urinalysis parameter.

ChE activity: There was dose-dependent depression of RBC ChE activity in dogs, particularly during the earlier periods of treatment. For males this was evident at 10-100 ppm and in females at 100-500 ppm, with RBC activity reduced 50-90% in both sexes over these concentrations. Plasma ChE followed a similar trend but was only depressed in dogs from the 100 and 500 ppm groups. ChE activity had returned to pretreatment levels at the end of 2-year treatment period. No effects of treatment were observed on the ChE activity in brain cortex or brain stem at necropsy.

Necropsy: Absolute and relative liver weights in males of the 100 and 500 ppm group and females of the 500 ppm group were marginally higher than those of controls. This was reported as of 'borderline significance' but no details of statistical methodologies were provided. No other effects on organ weight were observed. Pathology revealed a number of spontaneous lesions, with chronic bronchitis, pulmonary granulomas and renal granulomas the most common. These were unrelated to treatment. Rarefaction of the cytoplasm of hepatic cells, with cellular enlargement and thickening of the cell wall, increased in incidence and severity with dose; graded as slight in 1/3 females at 10 ppm and 1/3 males and 3/3 females at 100 ppm, and as moderate in all 500 ppm dogs. There were no architectural changes to the liver. The hepatocellular effects were of such magnitude that liver function was not likely to have been affected, and serum liver enzymes revealed no hepatic damage. Two independent pathologists, blind to the experimental protocol, were reported to have not seen significant differences in hepatocytes between treated and control animals.

Conclusion: This study preceded codified QA or GLP protocols but was adequate for the assessment of the chronic toxicity of dichlorvos in dogs. Based on the inhibition of RBC ChE activity in males at and above 3.2 ppm or more (10 ppm group), the NOEL was 0.32 ppm, equivalent to approximately 0.008 mg/kg/d.

Markiewicz VR (1990) A 52-week chronic toxicity study on DDVP in dogs. HLA study No. 2534-102. Lab: Hazleton Laboratories America Inc. Vienna, Virginia USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 11th August 1988 to 14th November 1989. Report date: 6th August 1990.

GLP compliant (US EPA; 40 CFR Part 160) and QA study. Study performed according to US EPA Pesticide Assessment Guidelines (Subdivision F, series 158, 83-1).

Materials and Methods

Dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 100% purity) was administered orally to purebred beagle dogs (4/sex/group; Hazleton Research Products Inc, Cumerland, Virginia, USA) in gelatine capsules (1/4 oz; Torpac Ltd, Toronto, Canada) for 52 weeks at 0, 0.05 (0.1 for the first 3 weeks of the study), 1.0 or 3.0 mg/kg bw/d. The dose selection was based on the results of a previous range-finding study (HLA Project No. 2534-101), where 0.1 mg/kg bw/d dichlorvos did not produce clinical signs or inhibit plasma, RBC or brain ChE activity. Capsules were prepared weekly based on the most recent bodyweight of each dog, and each animal was dose daily with one capsule. The stability of the test material was analysed at the beginning of the previous range-finding and then every 4 months.

Dogs were approximately 6-7 months old and had been quarantined for at least 3 weeks prior to the initiation of treatment. The mean bodyweights of males and females were approximately 10 and 7 kg, respectively. Dogs were housed individually under standard conditions and fed Purina® Certified Canine Diet #5007. Tap water was available *ad libitum*. Dogs were randomly assigned to each group based on bw, with some rearrangements necessary to accommodate variations in pretreatment plasma ChE activity. During the dosing phase, dogs were fed daily over a 4-hour period, with dosing occurring approximately 2 hours after the presentation of food. Food was then removed approximately 2 hours after dosing.

All dogs were observed twice daily for clinical signs and mortality. Cageside observations were also made for approximately one hour after dosing. Physical examinations were performed weekly. Bodyweights and food consumption were recorded one day prior to the commencement of dosing, weekly from weeks 1-16, and then every 4 weeks until termination. Food consumption was not recorded during week 52 due to human error. Ophthalmoscopic examinations were performed at an unspecified time prior to the commencement of dosing and then at termination.

The standard range of haematology, clinical chemistry and urinalysis parameters were measured (see Appendix II) prior to the initiation of dosing (unspecified time) and during weeks 26 and 52. However, triglycerides, γ-glutamyl transpeptidase and lactate dehydrogenase (LDH) were not measured as part of the clinical chemistry analysis. Plasma and RBC ChE were measured in non-fasted dogs approximately 3 hours postdose during weeks -2, -1, 0, 2, 6, 13, 26, 39 and 52. Brain ChE activity was measured after 52 weeks (brain samples were obtained at necropsy and stored frozen until analysis).

After 52 weeks of treatment, all surviving animals were sacrificed by sodium thiamylal anaesthesia, and exsanguinated. Necropsies were performed on each dog, which involved an examination of the following: external surface of the body; all orifices; cranial cavity; carcass; external surface of the brain and spinal cord and the cut surfaces of the spinal cord and brain; nasal cavity and paranasal cavity; thoracic, abdominal and pelvic cavities and their vicera; and cervical tissues and organs. The following organ weights were recorded for each terminally sacrificed animal: adrenals, brain (including brainstem), heart, kidneys, liver with drained gall bladder, ovaries and testes. The standard range of tissues was histopathologically examined (see Appendix III).

Bodyweight, food consumption, haematology (except cell morphology), clinical chemistry (except ChE activity) and organ weight data were statistically compared to the control group of the same sex by ANOVA following the appropriate transformation of any heterogenous data sets. Significant differences were further analysed using a Dunnet's t-test. Plasma and RBC ChE data were analysed by factorial repeated measures ANOVA, with dose and sex used as "between factors" and time used as the "within factor". Statistical differences were considered to be significant when $p<0.05$.

Results

Chemical analysis: Single samples were periodically analysed for dichlorvos over approximately a 2-year period. These samples were determined to have relative purities of 97.3-99.5% and were therefore within acceptable stability limits.

Mortalities and clinical signs: No dogs died during the study. There was a range of incidental clinical signs observed in both the control and treatment groups, but none that were attributable to the test compound. One high-dose male was possibly given a "slight overdose" of dichlorvos during week 33,

which apparently led to ataxia, salivation and dyspnoea. However, this could not be confirmed by chemical analysis of the formulation or by examination of the dosing records. There was a clear treatment-related effect on the occurrence of emesis, which was more pronounced in males than females (see Table below).

Effect of dichlorvos on the incidence of emesis

Observation	0 mg/kg bw/d		0.05 mg/kg bw/d		1.0 mg/kg bw/d		3.0 mg/kg bw/d	
	♂	♀	♂	♀	♂	♀	♂	♀
Emesis								
Total incidences	1	1	1	6	10	5	38	13
No. dogs[1]	1	1	1	2	4	3	4	3

1 = No. dogs displaying the observation at least once during the study

Bodyweights and food consumption: There appeared to be a transient effect on bodyweight in high-dose males. The mean bodyweight of this group dropped to below the pretreatment weight by approximately 5% at week 2 and then gradually increased to re-establish the pretreatment weight by week 8. Mean bodyweight then continued to steadily increase, reaching an equivalent bodyweight as the control group by week 20, despite having a lower (~7%) pretreatment bodyweight than the control group. None of these findings appeared to be statistically significant. An examination of individual animal data revealed that the apparent effect on bodyweight in high-dose males was attributable to a single animal (#26237) who lost approximately 15% of its pretreatment bodyweight over the first 3 weeks of dosing, and never completely recovered. This animal also exhibited the highest incidence of emesis over the dosing period (29 of the 38 incidents). There was no treatment-related effect on food consumption.

Ophthalmoscopy: There were no ophthalmoscopic abnormalities that were attributable to the test compound.

Haematology, clinical chemistry and urinalysis: There was no treatment-related effect on any haematology, clinical chemistry or urinalysis parameter.

ChE inhibition: There was a clear dose-related inhibition of plasma and RBC ChE activities (see Table below). Toxicologically-significant inhibition of plasma and RBC (ie. >20% inhibition relative to pretreatment activity) occurred at and above 1.0 mg/kg bw/d at every sampling interval. Statistical analysis was only performed on data from week 13. The magnitude of inhibition was equivalent in males and females, ranging from 39-59% and 65-74% for plasma ChE inhibition at 1.0 and 3.0 mg/kg bw/d, respectively, and 33-65% and 68-94% for RBC ChE inhibition at 1.0 and 3.0 mg/kg bw/d, respectively. It was surprising that no cholinergic signs were evident at the highest dose given the magnitude of RBC ChE inhibition (up to 94%).

At the lowest dose (0.05 mg/kg bw/d), there was a transient inhibition of plasma and RBC ChE activities at week 2 and 6, respectively. It should be noted that this low-dose group was administered 0.1 mg/kg bw/d for 3 weeks, which was then decreased to 0.05 mg/kg bw/d from week 4 because of the fact that >20% inhibition of plasma ChE activity was detected after 12 doses. Therefore, the inhibition of plasma ChE activity at week 2 is attributable to the higher dose level of 0.1 mg/kg bw/d. The study authors attributed the inhibition of RBC ChE activity at week 6 in the low-dose group to the "residual effect on the erythrocytes of the higher dose of 0.1 mg/kg bw/d" despite the animals having received the lower dose of 0.05 mg/kg bw/d for 4 weeks. While this explanation is plausible, in the absence of sampling between weeks 2-6 and 6-13, there is no way of confirming this explanation. Examination of the individual animal data revealed that in males, only 2/4 dogs showed RBC ChE inhibition above 20% (35 and 38%), while in females, all animals showed RBC ChE inhibition above 20% (45-60%). The reviewing toxicologist considered that this effect was transient in nature and was therefore not attributable to the lower dose of 0.05 mg/kg bw/d.

Results of brain ChE activity measurements were not provided, although it was reported that statistical analysis revealed significant inhibition at and above 1.0 mg/kg bw/d in males ($p<0.05$-0.01) and at 3.0 mg/kg bw/d in females ($p<0.05$).

Gross Pathology and organ weights: There were no treatment-related macroscopic abnormalities observed in any dogs. Absolute and relative organ weights were unaffected by treatment.

Histopathology: There were no treatment-related histopathological abnormalities.

Effect of dichlorvos on the % inhibition[1] of plasma and RBC ChE activities

Dose	Week 2		Week 6		Week 13		Week 26		Week 39		Week 52	
mg/kg bw/d	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Plasma ChE												
0												
mean	7.0	-0.7	11.7	1.9	-4.3	-14	-0.5	-1.9	7.2	-8.1	2.0	-0.9
sd	8.72	3.33	8.33	5.27	15.57	12.9	7.39	5.65	7.66	19.2	9.69	6.66
0.05												
mean	21.1	25.7	11.0	10.3	-2.4	-4.8	-1.9	-8.6	6.0	-11	11.7	-9.3
sd	6.44	5.73	5.77	4.07	5.91	8.24	7.58	11.7	4.69	27.1	11.01	22.5
1.0												
mean	59.0	59.3	59.2	56.7	48.1	41.0	39.1	51.4	47.5	43.7	52.9	51.8
sd	6.3	1.68	3.88	4.35	3.8	6.69	18.73	11.2	7.12	9.17	8.54	2.88
3.0												
mean	66.6	65.2	74.3	73.9	68.1	61.1	71.2	74.2	65.1	67.6	71.5	64.6
sd	10.6	7.65	6.79	3.66	9.08	8.09	8.7	4.21	16.27	2.02	8.31	4.36
RBC ChE												
0												
mean	-0.8	-2.1	-2.4	0	-5.7	0.7	-8.6	-5.8	2.7	7.8	-8.7	-1.1
sd	3.02	9.53	15.55	11	7.9	3.31	8.16	10.4	16.62	8.04	19.39	10.3
0.05												
mean	1.6	-1.4	23.6	50.1	7.0	6.5	3.1	3.4	8.0	12.8	-2.6	2.3
sd	7.02	2.06	14.92	6.93	3.51	12.3	6.83	19.9	7.72	6.83	8.28	5.59
1.0												
mean	33.6	33.1	65.2	63.2	53.9	51.9	43.0	38.0	44.0	39.7	53.4	45.2
sd	6.02	6.68	3.89	9.18	5.88	7.42	3.81	5.71	10.6	9.56	9.41	9.98
3.0												
mean	75.3	67.5	94.0	90.2	86.9	82.4	84.7	82.5	81.2	79.2	85.1	81.1
sd	3.06	4.33	0.68	0.56	2.06	3.7	1.79	2.37	5.57	2.14	3.54	0.87

1 = compared to pretreatment values; Bolded values are significantly different to the controls (p<0.05)

Conclusions: The NOEL following 52-weeks of oral dosing in dogs was 0.05 mg/kg bw/d, based on the inhibition of plasma and RBC ChE activities at and above 1.0 mg/kg bw/d. There was an increase in the incidence of emesis in males at 3.0 mg/kg bw/d predominantly due to a single animal. Classic cholinergic signs were only observed when one of the high-dose males was inadvertently overdosed.

6.2 Inhalational exposure

6.2.1 Rats

Blair D, Dix KM & Hunt PF (1974) Two year inhalation exposure of rats to dichlorvos vapour. Report No. TLGR.0026.74. Lab: Tunstall Laboratory, Shell Research Ltd, Sittingbourne Research Center, UK. Report date: June 1974.

Blair D, Dix KM, Hunt PF, Thorpe E, Stevenson DE & Walker AIT (1976) Dichlorvos - a 2-year inhalation carcinogenesis study in rats. Arch. Toxicol. 35: 281-294.

Materials and Methods

Carworth Farm E (CFE) rats (50/sex/group) were exposed individually to air concentrations of dichlorvos (SNC Pernis, unspecified location; batch No. unspecified; >97% purity) at 0, 0.05, 0.5 or 5 mg/m^3 for 23 h/d for 2 years. Achieved mean concentrations were 0, 0.05, 0.48 and 4.70 mg/m^3, respectively. Trimethyl phosphate was also present at 0, 0.007 and 0.04 mg/m^3 in the low-, mid- and high-dose atmospheres, respectively, as was DCA at 0.007, 0.013 and 0.028 mg/m^3. The method of application meant that the rats were exposed dermally, orally (via contamination of food and water, cage interior and grooming fur), as well as by inhalation.

Rats were housed individually in metal wire cages and fed *ad libitum* with Diet 86 pellets (Charles River, UK). Body weights and feed intake were measured monthly and behaviour was monitored daily. Rats dying during the course of the study were necropsied where possible. Haematology (Hb, erythrocyte, total leucocyte and differential leucocyte counts, prothrombin time, and kaolin-cephalin coagulation time) and clinical chemistry (protein, urea, Na, K, glucose and Cl concentrations, and plasma AP, AST and ALT activities) were examined in blood samples collected at necropsy. RBC and plasma ChE activities were measured at the same time.

Necropsies were performed on all animals sacrificed at the end of the treatment period. Major organs (brain, heart, liver, spleen and kidneys) were weighed and these along with additional tissues (anterior pituitary, thyroid, adrenals, mammary gland, pancreas, skin, reticulo-endothelial system, tongue, nasal cavity, trachea, skeletal muscle, and eye and lachrymal gland) were examined microscopically. The left half of each brain was used for examination of ChE activity. Additional brain samples were provided to an independent laboratory (B. Holmstedt, Karolinska Intitutet, Stockholm) for assessment of acetylcholine and choline concentrations.

Statistical analysis consisted of analysis of variance with Student t-test or χ^2 test for comparison between treated and control groups.
Results

Mortalities, Clinical Signs and Effects on Bodyweight and Food Consumption: The average weekly concentrations of dichlorvos in test atmospheres were reasonably consistent and close to nominal throughout the treatment period. Mortality was highest in control groups and lowest in high-dose groups. Survival rates of 22, 42, 30 and 64% in males, and 50, 60, 58 and 76% in females recorded in control, low-, mid- and high-dose groups, respectively. The highest morbidity rate in controls occurred from approximately 75 weeks. The low survival in controls caused the early cessation of treatment in males during week 99; female treatment continued to week 104.

There were no adverse clinical signs indicative of OP poisoning but some rats, generally males, showed lameness and ulcerated hocks (probably related to the wire floor of the cage). The only lesion noted in treated but not control groups was a sore tail, often with necrosis of the tip, seen in 2 high-dose males and 12 high-dose females. Body weight gain was slightly though significantly reduced ($p<0.01$) in both sexes at the highest dose. This persisted to the end of the study, with terminal bodyweight depressed by 10-13% (only significant in males). Food consumption was unaffected by treatment.

Clinical chemistry: At termination there was a slight though significant increase ($p<0.05$) in plasma AST and ALT activities, and a decreased in Cl, in high-dose males. There was no treatment-related effect on any haematological parameters. Mean plasma and RBC ChE activities were dose-dependently and significantly reduced at the mid- (17-32%; $p<0.01$-0.05) and high-dose (78-96%; $p<0.01$) in both sexes. RBC ChE was also slightly though significantly reduced in low-dose females (12%; $p<0.05$). In brain tissue, ChE activity was reduced by approximately 10 and 80% at the mid- and high-dose, respectively ($p<0.01$). No treatment-related effect on acetylcholine or choline concentrations in brain tissue was observed.

Necropsy: Some of the rats found dead during the study could not be necropsied in detail because of advanced autolysis. Furthermore, survival in the male control group was low with only 11 surviving to

termination, potentially impacting on the analysis of the results of the study. However, the data were adequate, and the statistical analysis appropriate to show that there were no treatment-related effects on the type, distribution or incidence of non-neoplastic or neoplastic lesions. The most common lesions were chronic nephrosis, focal myocardial fibrosis, degenerative arterial disease especially of the mesenteric artery, lymphoid hyperplasia in the spleen, and testicular atrophy, occurring at all dose levels and commonly reported in aged CFE rats. Common tumours included chromophobe adenomas of the anterior pituitary, parafollicular cell adenomas, and carcinomas of the thyroid, phaeochromocytomas of the adrenal medulla, and mammary fibroadenomas. Chi-square analysis indicated that the odds ratios for males and females with at least one tumour, males with adrenal medulla tumours and females with mammary tumours, were significantly reduced ($p<0.05$) at the highest dose level.

Conclusion: The present studies showed no evidence for carcinogenicity at dichlorvos air levels up to 4.70 mg/m^3. The authors reported that test atmospheres of 5 mg/m^3 resulted in a retained dose of 0.5 mg/d in rats when only the head was exposed. The estimated daily ingestion of dichlorvos was 6.2 mg via food, 3.6 mg via grooming and less than 0.1 mg via drinking water (percutaneous absorption was unknown). Thus, the actual daily intake of dichlorvos was estimated at be approximately 10 mg/rat, or 25 mg/kg bw/d at the highest dose. The NOEC was 0.05 mg/m^3 (approximately equal to 0.25 mg/kg/ bw/d), based on the inhibition of plasma and RBC ChE activity at 0.5 mg/m^3.

7. REPRODUCTION STUDIES

7.1 Rats

Tyl RW, Myers CB & Marr MC (1992) Two-generation reproductive toxicity study of DDVP administered in the drinking water to CD® (Sprague-Dawley) rats. RTI ID No. 60C-4629-170. Lab: Reproductive and Developmental Toxicology Laboratory, Centre for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 9th January 1991 to 23rd June 1992. Report date: 31st August 1992.

Tyl RW, Myers CB & Marr MC (1993) Addendum to final report: Two-generation reproductive toxicity study of DDVP administered in the drinking water to CD® (Sprague-Dawley) rats. RTI ID No. 60C-4629-170. Lab: Reproductive and Developmental Toxicology Laboratory, Centre for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 9th January 1991 to 23rd June 1992. Addendum date: 5th May 1993.

GLP compliant (US EPA; 40 CFR Part 160) and QA study. Statement of compliance with US EPA and OECD test guidelines. Male reproductive toxicity assessed based on US EPA FIFRA Phase II Technical Guidance Document on Male Reproductive Assessment (Office of Pesticides, 24th December 1989).

Materials and Methods

Dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 98.3% purity) was administered to outbred virgin Crl:CD® (SD)BR weanling rats (30 sex/dose; 43-days old; 155 and 140 g bodyweight for males and females, respectively; Charles River Breeding Laboratories, Kingston, NY, USA) in their drinking water at 0, 5.0, 20.0 or 80.0 ppm for 10 weeks (approximately equal to 0, 0.5, 2 and 8 mg/kg bw/d, respectively, using a dietary conversion factor of 0.1). The dose selection was based on the results of a 2-week palatability study (RTI Project No. 60C-4629-50) and a reproductive toxicity range-finding study (RTI Project No. 60C-4629-60). Dosing solutions were prepared weekly and formulated in deionised/filtered tap water then stored refrigerated, as they were reportedly stable under these conditions. Furthermore, the dosing solutions were reportedly stable for 24 hours at room temperature under the conditions of administration (ie. in plastic containers exposed to light). Prior to experimentation, dosing solutions were analysed for stability and homogeneity. The concentration of dichlorvos was analysed weekly for the first 4 weeks and then on randomly selected samples on a monthly basis, using HPLC.

Rats had been quarantined for approximately two weeks prior to the initiation of dosing during which time they were housed 2/sex/cage. They were then randomly assigned to each treatment group based on bodyweight and housed individually. During mating, one male and one female from the same dose group were housed together. Following successful mating or at the end of the mating period, females were housed individually and separately from the males. All rats were housed under standard conditions. Purina® Certified Rodent Chow® #5002 and deionised/filtered tap water (containing dichlorvos) were available *ad libitum*, except during the rebreed of F1 females with naive males, when water was not available during the nights of the cohabitation period. At various times (see below), rats were sacrificed by CO_2 asphyxiation, while pups were sacrificed by CO_2 asphyxiation and/or decapitation.

The study design is summarised in the Table below. Following the 10-week dosing period, one randomly selected F0 male was mated with one F0 female from the same dose group for 21 days. If mating was unsuccessful during the first week then the male was replaced with a different male from the same dose group for the remaining 14 days. Females were examined daily during this mating period for the presence of sperm and/or a vaginal plug, both considered as evidence of successful mating. The day that sperm or a vaginal plug was observed was designated gestational day (gd) 0. In females showing no evidence of mating, the last scheduled day of mating was designated gd 0. Parental males were sacrificed after completion of the mating period. From gd 20, females were observed twice daily for evidence of littering and allowed to rear their F1 litters until weaning (day 21

post-partum). Four days after birth, the size of each litter was randomly adjusted to approximately 4 pups/sex, with the remaining pups sacrificed by decapitation.

At least one male and one female F1 weanling/litter were randomly chosen to produce the F2 generation. These F1 weanlings (30 sex/dose) were exposed to the same drinking water solution as their parents for at least 11 weeks and then mated when approximately 14-17 weeks of age. Ten F1 weanlings/sex/dose were also randomly assigned for necropsy. All remaining F1 weanlings were macroscopically examined, sacrificed and discarded. F2a litters were weaned at day 21 post-partum, with 10 weanlings/sex/dose randomly assigned for necropsy. All remaining F2a weanlings were macroscopically examined, sacrificed and discarded. Due to the low breeding performance of F1 rats, females were subjected to a 3-week prebreed vaginal cytology examination to examine oestrous cyclicity. F1 females were then remated with untreated age- and weight-matched males for 3 weeks to produce F2b litters. Parental females were sacrificed after F1 or F2b litters had been weaned.

Summary of study design

Event	Time	Observations
F0 premating exposure (30 rats/sex/dose)	10 weeks	Deaths, clin signs, bw, food & water consump
F0 mating (1:1)	21days	Deaths, clin signs, bw, evidence of mating
F0 males sacrificed (all)	-	Necropsy, histopathology (0 & 80 ppm groups only), brain & organ weights, ChE activity
F0 gestation	~22 days	Deaths, clin signs, bw, food & water consump
F0 lactation	21 days	Deaths, clin signs, bw, food & water consump; pups counted, sexed, macroscopically examined
Necropsy of F1 weanlings (10/sex/group)	-	Necropsy
Sacrifice F0 females (all)	-	Necropsy, histopathology (0 & 80 ppm groups only), brain & organ weights, ChE activity
F1 premating exposure (30 rats/sex/dose)	11-15 weeks	Deaths, clin signs, bw, food & water consump
F1 mating (1:1)	21 days	Deaths, clin signs, bw, evidence of mating
F1 males sacrificed (all)	-	Necropsy, histopathology (0 & 80 ppm groups only), brain & organ weights, ChE activity
F2a gestation	~22 days	Deaths, clin signs, bw, food & water consump
F2a lactation	21 days	Deaths, clin signs, bw, food & water consump; pups counted, sexed, macroscopically examined
Sacrifice F2a weanlings (10/sex/group)	-	Necropsy
F1 prebreed vaginal cytology	21 days	Histopathology
F1 remating with untreated males (1:1))	21 days	Deaths, clin signs, bw, evidence of mating
Sacrifice of untreated males (all)	-	Bw, necropsy, histopathology (0 & 80 ppm groups only), brain & organ weights, ChE activity
F2b gestation	~22 days	Deaths, clin signs, bw, food & water consump
F2b lactation	21 days	Deaths, clin signs, bw, food & water consump; pups counted, sexed, macroscopically examined
Necropsy F2b weanlings (10/sex/group)	-	Necropsy
Sacrifice F1 females (all)	-	Necropsy, histopathology (0 & 80 ppm groups only), brain & organ weights, ChE activity

Mortalities were recorded twice daily. Clinical signs were recorded daily. Bodyweights were recorded weekly throughout the premating and mating periods, with the bodyweights of females also recorded on gd 0, 7, 14 and 20. Dams producing litters were weighed on lactation days 0, 4, 7, 14 and 21.

Untreated males used for the rebreed with F1 females were weighed during the quarantine period and at sacrifice. Water consumption was recorded daily. Food consumption was recorded approximately every 3 weeks. Food consumption was recorded for pregnant F0 and F1 females at gd 0-7, 7-14 and 14-20. Maternal food consumption was also measured during lactational day 0-4, 7-14 and 14-21. Food and water consumption were not measured during the mating period.

At birth, all pups were counted, sexed and macroscopically examined for any abnormalities. This process was repeated at postnatal days 1, 4, 7, 14 and 21 (weaning). Survival (viability) indices were calculated for each litter at these same times (see Appendix II). Any deceased pups were necropsied.

At termination, plasma, RBC and brain ChE activities were measured in all F0 and F1 parents. Brain weights were recorded. A gross necropsy was performed on all parents, which included an examination of the external surfaces, all orifices, the carcass, external and cut surfaces of the brain and spinal cord, the cranial, thoracic, abdominal and pelvic cavities, including the vicera, and cervical tissues and organs. The following tissues from all adults from the control and 80 ppm groups were histopathologically examined: liver, pituitary, vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate and any treatment-related gross lesions. Any parental rat found dead or sacrificed in a moribund condition was subjected to a complete necropsy and histopathological examination.

Due to low reproductive performance, additional examinations were performed on F1 males and females. Males were subjected to a more extensive assessment of reproductive toxicity, with 10 rats/group at 5.0, 20 and 80 ppm, and 20 rats from the control group, randomly selected for the following: spermatid head counts; determination of sperm number, motility and morphology; examination of the epididymal fluid for debris and abnormal cells; histopathological examination of both testes. The following organs were weighed: right and left testis, right and left epididymis; prostate, seminal vesicles (with and without fluid) and pituitary. Both ovaries of F1 females were weighed. The untreated males that were used in the F1 rebreed were sacrificed, their testes weighed and stored for possible histopathological evaluation. Any females failing to produce a litter were stained with potassium ferricyanide and microscopically examined for pregnancy status.

Standard reproductive and lactation indices were calculated for all F0 and F1 parental rats and their litters (see Appendix IV). The following statistical tests were performed on parametric data: ANOVA (following arcsine-square root transformation of all litter-derived percentage data and Bartlett's test for homogeneity of variance) followed by a Dunnett's multiple comparison test to compare each treatment group with the control; Dunnet's t-test (adult bodyweight, maternal food and water consumption, pup bodyweight and % males/litter); χ^2-test for independence for differences among treatment groups followed by a one-tailed Fisher's Exact Probability test (for pairwise comparisons between each treatment group and the control). For non-parametric data, a Kruskal-Wallis one-way ANOVA by rank was used to test for differences among groups, with a Mann-Whitney U test used to compare individual treatment groups with the control.

Results

Analytical chemistry: Analysis of the 3 drinking water formulations determined that they were within ±10% of the nominal concentrations and therefore considered acceptable (range 92.3–110%). On separate occasions, low- and high-dose formulations were found to be 114 and 119% of the nominal concentrations and were therefore discarded and reformulated. However, they were used for one day while the new formulations were prepared and analysed. Results of a number of previous studies, which tested aqueous solutions of dichlorvos at various concentrations, found that solutions ranging from 5-480 ppm were homogenous (2-8% variability between the bottom, middle and top of the formulation). Five and 80 ppm solutions were stable for 9 days when stored refrigerated (2-3°C) in the dark. Five and 20 ppm solutions were stable for 24 hours when stored at ambient temperature in plastic drinking water bottles.

F0 parental rats

Mortalities and clinical signs: There was no treatment-related effect on mortality in both sexes and on clinical signs in males. Alopecia of the bilateral forelimbs increased over time in females at 80 ppm, reaching a maximum at premating day 49-62 (5/30 rats affected) and then slowly declining over the remainder of the dosing period (to day 91). Alopecia was also noted in up to 2/30 females at 20 ppm

during premating days 47-70. Alopecia of the bilateral forelimbs was not observed in the 5 ppm and control groups during the premating period. Alopecia of the bilateral forelimbs was also observed in maternal rats during gestation in the control, 20 and 80 ppm groups (1, 1-2, 1-3 rats, respectively), but due to the low incidence and lack of a dose-response effect, this finding was considered to be equivocal. Alopecia of the bilateral forelimbs was also observed during lactation and tended to be higher in the 80 ppm group (3-4 rats) compared to the control and 20 ppm groups (1-2 rats).

Effects on bodyweight: During the premating period there was no treatment-related effect on bodyweight. Maternal bodyweight gain during gestation was 9-15% lower at all doses of dichlorvos compared to the control but did not follow a dose-response relationship. Only the 20 ppm group was statistically different to the control ($p<0.05$).

Food and water consumption: Food consumption was comparable across all groups. At 80 ppm, weekly water consumption (g/kg bw/d) of males was significantly lower than the control ($p<0.05$) from prebreeding days 7 to 70. The magnitude of this difference was between 6-14% of the control. A similar effect was also seen in females at 80 ppm during the premating period [the difference compared to the control ranged from 8-14%, with statistical significance ($p<0.05$) reached at premating days 7-14, 21-28, 35-42 and 56-63]. At 80 ppm, maternal water consumption was significantly lower than the control ($p<0.05$) during gestation (13-20% of the control). Water consumption of maternal rats during lactation was also lower than the control group at 80 ppm (the difference ranged from 3-6% of the control) but was not statistically significant. Whether the lower water consumption at 80 ppm was due to compound-related toxicity or to the reduced palatability of the water is unclear (note that the previous 2-week palatability study found reduced water consumption at 240 and 480 ppm).

ChE activity: Results of ChE analyses are summarised in the Table below. There was a significant ($p<0.05$) dose-related decrease in plasma, RBC and brain ChE activities in males at and above 20 ppm. In females, RBC ChE activity was significantly inhibited at and above 5 ppm ($p<0.05$), while plasma and brain ChE activities were significantly inhibited at and above 20 ppm. The apparent affect on RBC ChE activity at 5 ppm was not considered to be toxicologically significant as inhibition of RBC ChE activity did not occur at this dose in F0 males or in F1 males and females (see below). Furthermore, neither plasma nor brain ChE activities were inhibited at 5 ppm.

ChE activities F0 rats treated with dichlorvos

Observation	Dose (ppm)							
	0		5		20		80	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma ChE	330± 24.9 (0%)	1822.6 ± 127.8 (0%)	318± 26.7 (4%)	1598± 95.6 (12%)	235.7± 13.5 (29%)*	819.6± 63.1 (55%)*	195.2± 19.0 (41%)*	303.7± 19.3 (83%)*
RBC ChE	951± 27.5 (0%)	881.9± 12.3 (0%)	884.6± 29.2 (7%)	675.9± 16.3 (23%)*	673.5± 29.1 (29%)*	534.8± 15.9 (39%)*	411.4± 13.7 (57%)*	348.4± 7.6 (60%)*
Brain ChE	6201± 147.3 (0%)	6265.1 ± 160.8 (0%)	6133± 89.6 (1%)	5862± 223.7 (6.4%)	5290.2± 133.8 (15%)*	4635.7 + 184.8 (26%)*	2890± 97.4 (53%)*	2545.2 + 181.7 (59%)*

Results expressed as the mean ± SEM mUnits/mL (% inhibition relative to the control); * $p<0.05$

Pathology: Necropsy was unremarkable in both males and females. There were some histopathological abnormalities detected in the testes of males (atrophy and degeneration of the seminiferous tubules), but due to the low incidence (maximum of 2/30 rats affected) and the lack of a dose-response effect, these findings were not considered to be treatment related. There were no treatment-related histopathological abnormalities in females.

Reproductive parameters: Reproductive and lactational indices were unremarkable.

Offspring (F1a litter)

At 80 ppm, the bodyweights of F1a male and female pups were lower than the control group from postnatal day 7, with statistical-significant reached at days 14 and 21 ($p<0.05$; ~8% lower than the control). A similar trend was evident in both F2a and F2b litters (see below), although the difference in bodyweight between the 80 ppm and control group was not statistically significant. No other treatment-related effects on any pup parameters were evident. There were no treatment-related clinical signs observed in F1a pups during lactation. Macroscopic examination of the 10 F1a pups/sex/dose designated for necropsy revealed no treatment-related abnormalities.

F1 parental rats

Mortalities and clinical signs: There were no treatment-related mortalities or clinical signs.

Effects on bodyweight: At 80 ppm, average male bodyweights were significantly lower than the control ($p<0.05$) during the premating period (days 0, 7, 14, 21 and 28; ~7-13% of the control b) but there was no difference in bodyweight gain. The bodyweights of females in the 80 ppm group were significantly lower than the control ($p<0.05$; ~7-13%) at premating day 0, 7, 14, 21 and 28, however, the bodyweight gain of this group was not significantly different to the control. Maternal bodyweight was unaffected by treatment during gestation and lactation of F2a litters. Females subjected to the 3-week prebreed vaginal cytology examination showed no treatment-related effects on bodyweight or bodyweight gain. There was no treatment-related effect on maternal bodyweight during F2b gestation and lactation.

Food and water consumption: Food consumption (g/kg bw/d) was significantly elevated ($p<0.05$) in males at 80 ppm relative to the control group at premating days 7-14, 14-21, 21-28, 35-42 and 42-49. The magnitude of this elevation (~6-11%) in females relative to the control, with statistical significance ($p<0.05$) reached at premating days 0-7, 7-14, 14-21, 21-28 and 28-35. Maternal food consumption was unaffected by treatment during gestation and lactation of F2a litters, however it should be noted that at 80 ppm the sample size at some observation points was very low (n=1-3) because results were inadvertently not recorded. Females subjected to the 3-week prebreed vaginal cytology examination showed no treatment-related effects on food consumption. There was no treatment-related effect on maternal food consumption during F2b gestation. During F2b lactation, maternal food consumption was significantly decreased ($p<0.05$) relative to the control (~18% reduction) at 80 ppm.

Water consumption (mg/kg bw/d) was significantly lower ($p<0.05$) in males at 80 ppm compared to the control (~10-14%) during premating days 28-35, 49-56, 56-63, 63-70 and 70-77. In females, water consumption was also significantly lower ($p<0.05$) at 80 ppm compared to the control (~10-19%) during premating days 28-35, 35-42, 42-49, 49-56, 56-63 and 63-70.
During gestation of F2a litters, maternal water consumption was significantly lower ($p<0.05$) than the control at 80 ppm (approximately 19% lower than the control). Maternal water consumption was unaffected by treatment during lactation of F2a litters, however it should be noted that at 80 ppm the sample size at some observation points was very low (n=1-3) because results were inadvertently not recorded. At 80 ppm, water consumption was significantly lower ($p<0.05$; ~20%) than the control in females subjected to the 3-week premating vaginal cytology examination. Maternal water consumption during F2b gestation was significantly lower ($p<0.05$) than the control at and above 20 ppm. Water consumption in the 20 ppm group was approximately 13% lower than the control over the entire gestation period (gd 0-20), while this figure reached 19% over gd 14-20. At 80 ppm, water consumption was approximately 17% lower than the control over gd 0-20 and as high as 28% at gd 0-7. Maternal water consumption during lactation of F2b litters was significantly lower ($p<0.05$) than the control at 80 ppm (~20% at days 4-7, 7-14 and 14-21).

ChE activity: Results of ChE analyses are summarised in the Table below. Significant ($p<0.05$) inhibition of plasma and RBC ChE activities occurred at and above 5 ppm in males, however the effect at this level was not considered to be toxicologically-significant as it was below 20%. Brain ChE activity was significantly inhibited ($p<0.05$) in males at and above 20 ppm, with only the effect at 80 ppm considered to be toxicologically-significant because it was >20% of the control. In females, significant inhibition ($p<0.05$) of plasma and brain ChE activities occurred at and above 20 ppm.

Significant (p<0.05) inhibition of RBC ChE activity occurred in females at all doses, but only the effect at 20 and 80 ppm was considered to be toxicologically significant as it was >20%.

ChE activities F1 rats treated with dichlorvos

Observation	Dose (ppm)							
	0		5		20		80	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma ChE	332.9± 16.2 (0%)	1443± 76 (0%)	282.2± 11.7 (15%)*	1308± 61 (9%)	244.9± 9.7 (26%)*	670.5± 33.3 (54%)*	141.1± 5.8 (58%)*	271.8± 26.1 (81%)*
RBC ChE	962.8± 15.4 (0%)	912.5± 25.9 (0%)	830± 23.3 (14%)*	754.6± 20.6 (17%)*	650± 13.2 (32%)*	528.6± 19.9 (42%)*	430.4± 14.2 (55%)*	384.3± 16.5 (58%)*
Brain ChE	6152.1± 86.5 (0%)	5941.8 ± 158.4 (0%)	6221.8± 126.1 (1%)	5820± 83.2 (2%)	5755.7± 124.4 (6.4%)*	4043.4 + 110.5 (32%)*	3668.8± 163.9 (40%)*	2356.3 ± 110.4 (60%)*

Results expressed as the mean ± SEM mUnits/mL (% inhibition relative to the control); * p<0.05

Pathology: The terminal bodyweight of males from the 80 ppm group was significantly lower (p<0.05) than the control (680.1±30.3 *versus* 755.2±11.4 g, respectively). Absolute organ weights were unaffected by treatment in males. The majority of relative organ weights were higher in males at 80 ppm compared to the control, with the effects significant (p<0.05) for the right epididymis and the seminal vesicles at 80 ppm. However, these findings can be attributed to the lower terminal bodyweight of the 80 ppm group. There were no treatment-related effects on any of the sperm parameters. Gross necropsy and histopathology of males was unremarkable. The bodyweight and combined testes weight of the untreated males used in the remating of F1 females was normal.

Results of the vaginal cytology examination for F1 females prior to remating with untreated males is summarised in the Table below. As shown, there was a significant decrease in cycling at 80 ppm, and a significant increase in females with abnormal cycles. There was no treatment-related effect on cycle length.

Vaginal cytology for F1 females prior to remating with untreated males

Parameter	Dose (ppm)			
	0	5	20	80
n	29	30	30	30
No. (%) cycling	25(86)	30(100)	26(87)	19(63)*
No. (%) abnormal cycles	4(16)	7(23)	2(8)	13(68)*

*p<0.05

Terminal bodyweights of F1 females were unaffected by treatment. There appeared to be a non-significant reduction in absolute and relative combined ovary weight at 80 ppm (absolute combined ovary weights of 0.2661±0.0146, 0.2598±0.0166, 0.2573±0.0121 and 0.2198±0.0165 g at 0, 5, 20 and 80 ppm, respectively; relative combined ovary weights of 0.0570±0.0034, 0.0547±0.0039, 0.0532±0.0027 and 0.0472±0.0039 g at 0, 5, 20 and 80 ppm, respectively). Gross necropsy and histopathology revealed no treatment-related abnormalities in F1 females at termination.

Reproductive parameters: Possible treatment-related effects on reproduction and lactation indices are summarised in the Table below. The interpretation of these findings was made difficult by the absence of dose-response relationships, lack of statistical significance and the absence of historical control data from the performing laboratory. At 80 ppm, both male and female fertility was approximately 20-25% lower than the control in both litters. An examination of historical control data from Charles River Laboratories (CRL) (1996) revealed that the female fertility indices of 55% (F2a) and 50% (F2b) were below the average historical control range of 60-100%. In both litters, the pregnancy index was lower at 80 ppm than in the control, suggesting that female fecundity was reduced with treatment. The number of females with live litters was also reduced at 80 ppm (both litters), with an apparent increase

in the stillbirth index in the F2b litter. The latter was outside of the historical control range of 0-10.2% (CRL 1996). Marginal reductions in pup survival (day 7) and the lactation index were noted in both litters but were not considered treatment-related. All other reproduction and lactation indices were unremarkable.

Reproduction and lactation indices for F2a and F2b litters

Indice	Dose (ppm)			
	0	5	20	80
F2a				
No. pregnant females/group (%)	17/30 (57%)	14/30 (47%)	16/30 (53%)	11/30 (37%)
Fertility Index ♀[1]	70.8	73.7	76.2	55.0
No males siring litters/group (%)	16/30 (53%)	13/30 (43%)	14/30 (47%)	11/30 (37%)
Fertility Index ♂[2]	76.2	68.4	73.7	55.0
Pregnancy index[3]	81.0	73.7	84.2	55.0
No. females with live litters/group (%)	16/30 (53%)	14/30 (47%)	16/30 (53%)	10/30 (33%)
Stillbirth index[4]	6.3±5.9	1.4±1.4	9.9±5.6	10.7±9.0
7 day survival index[5]	100.0	85.7	86.7	81.3
Lactation index[6]	100.0	83.9	86.7	80.0
F2b				
No. pregnant females/group (%)	19/29 (66%)	19/30 (65%)	17/30 (57%)	13/30 (43%)
Fertility Index ♀[1]	76.0	70.4	63.0	50.0
No males siring litters/group (%)	18/30 (60%)	18/31 (58%)	16/31 (52%)	13/31 (42%)
Fertility Index ♂[2]	75.0	69.2	61.5	50.0
Pregnancy index[3]	79.2	73.1	65.4	50.0
No. females with live litters/group (%)	19/29 (66%)	19/30 (63%)	17/30 (57%)	11/30 (37%)
Stillbirth index[4]	5.1±3.5	9.0±4.3	6.6±3.2	19.1±10.2
7 day survival index[5]	98.6±1.0	86.8±8.0	93.0±6.2	88.8±9.9
Lactation index[6]	98.6±1.0	84.6±8.0	92.2±6.2	86.3±9.9

1 = No. pregnant females/No. sperm positive females; 2 = No. males siring litters/No. males impregnating females; 3 = No. pregnant females/No. males impregnating females; 4 = No. dead postnatal day 0/total No. on postnatal day 0; 5 = No. surviving 7 days/No. live on postnatal days 4; 6 = No. surviving 21 days/No. live on postnatal day 4.

Offspring (F2a and F2b)

At 80 ppm, F2a pup weights (males and females) were 5-15% lower than the controls during the entire lactation period [postnatal days 1 (5%), 4 (15%), 7 (14%), 14 (12%) and 21 (9%)]. At 80 ppm, F2b pup weights were also lower than the controls during the lactation period, with a greater effect seen in males than females [at postnatal days 4 (11/14% M/F), 7 (18/7% M/F), 14 (16/3% M/F) and 21 (14/2% M/F)]. Although none of the findings in F2 pups were statistically significant, the consistent trend at 80 ppm in both litters and with the effects seen in F1 pups means that this effect on pup weight can not be discounted. There were no treatment-related clinical signs observed in pups during lactation. Macroscopic examination of the 10 pups/sex/dose designated for necropsy revealed no treatment-related abnormalities.

Conclusion: The NOEL for parental toxicity was 5 ppm (~0.5 mg/kg bw/d) based on the occurrence of toxicologically- and statistically-significant inhibition of plasma, RBC and brain ChE activities at 20 ppm (~2 mg/kg bw/d). The NOEL for pup toxicity was 20 ppm (~2 mg/kg bw/d) based on lower pup weights

in both generations at 80 ppm (~8 mg/kg bw/d). The NOEL for reproductive toxicity was 20 ppm (~2 mg/kg bw/d) based on reduced fertility and pregnancy indices, increased stillbirths in the F2 generation, and a reduction in cycling concomitant with an increase in abnormal cycling in F1 maternal rats, at 80 ppm (~8 mg/kg bw/d).

8. DEVELOPMENTAL STUDIES

8.1 Mice

Schwetz BA, Ioset HD, Leong BKJ & Staples RE (1979) Teratogenic potential of dichlorvos given by inhalation and gavage to mice and rabbits. Teratology 20:383-388.

CF-1 mice (Carworth Animals, Michigan, USA) were mated and exposed to dichlorvos (Shell Chemical Co.; 96% purity) by vapour inhalation (7 h/d in cages placed in a 3.7 m^3 chamber) or oral gavage (in corn oil), during gd 6-15 or 6-18, respectively. The mean inhalational exposure level, monitored daily, was 4.06 (±0.63) mg/m^3, and the oral dose was intended to be the maximum tolerated dose for the species (60 mg/kg/ bw/d). Mice were observed and weighed daily and sacrificed on gd 18-29. The number of live, dead and resorbed foetuses was recorded. All foetuses were weighed, measured and sexed, and examined for external alterations. One-third of each litter was dissected and examined for visceral anomalies using Staple's method; the heads were fixed in Bouin's fluid and sectioned; and all foetuses were preserved, cleared and stained (alizarin red-S) for skeletal investigation.

There were no mortalities or adverse clinical signs. Bodyweight was significantly depressed compared to controls on gd 16 (but not on gd 10 or 18) in mice given 60 mg/kg/d dichlorvos by gavage over gd 6-15. Twenty-eight control and 25 treated litters were delivered. There were no significant differences between treated and control groups in the number of live foetuses, resorptions, foetal weight and length, or sex ratio, or in the incidence and type of visceral or skeletal anomalies or malformations. Thus, oral administration of dichlorvos at a dose of 60 mg/kg/d was slightly toxic to pregnant mice (reduced weight gain) but there was no evidence of developmental toxicity.

In mice exposed to dichlorvos vapour during days 6-15 of gestation, there were no adverse clinical signs and no effects on bodyweight gain. Twenty control and 15 treated litters were delivered by caesarean section on gd 18. There were no significant differences between treated and control groups in the number of live foetuses, resorptions, foetal weight and length, or sex ratio, or in the incidence and type of visceral or skeletal anomalies or malformations. Thus, dichlorvos at 4.0 mg/m^3 (exposure was whole-body so mice would be exposed dermally and orally as well as through inhalation) was without maternal or developmental toxicity.

8.2 Rats

Thorpe E, Wilson AB, Dix KM & Blair DB (1971) Toxicity studies with dichlorvos: teratogenic studies in rats and rabbits given dichlorvos by inhalation. Project No. T507022/3. Lab & Sponsor: Pathology, Toxicology & Chemical Toxicology Divisions of Tunstall Laboratory and the Statistics Unit of Sittingbourne Laboratories, unspecified location. Study duration: unspecified. Report date: July 1971.

Materials and Methods

Female Carworth Farm E strain rats (unspecified age; 200-300 g bw; Tunstall Laboratory, unspecified location) were housed in groups of two with males of proven fertility and examined daily for the presence of sperm by vaginal smears. The day that sperm was observed was designated gd 0. Sperm-positive females were randomly assigned to treatment groups and housed individually within inhalation chambers for 20 days. During this time up to 15 rats/group were exposed to atmospheres of dichlorvos [unspecified source and batch/lot No.; technical grade (>97% purity)] at 0, 0.25, 1.25 or 6.25 μg/L (equivalent to 0, 0.25, 1.25 and 6.25 mg/m^3, respectively). Exposure was for 23 hours per day, 7 days per week. No rationale was provided for the dose selection. It was stated that atmospheres of dichlorvos were generated according to a previous unpublished method, however, no study citation or details were provided. Housing conditions were unspecified while food and water (unspecified) were available *ad libitum*.

Rats were observed daily for clinical signs and abnormal behaviour. Any rats dying during the study were necropsied. Maternal bodyweight and food consumption were not recorded. At the end of the 20-day exposure period, rats were sacrificed by an unspecified means and blood samples taken for

measurement of plasma, RBC and brain ChE activities. Maternal animals were not macroscopically examined for any structural abnormalities or pathological changes. Gravid uterine weights and the number of corpora lutea were not recorded. Numbers of live foetuses, stillbirths and resorptions were recorded. Foetuses were not sexed. Live foetuses were weighed and examined for external malformations. Approximately half of each litter was processed for examination of skeletal abnormalities, with the remainder examined for visceral abnormalities. Unspecified statistical tests were performed on ChE data.

Results

Graphically presented data illustrated that chamber dichlorvos concentrations were relatively stable at 0.25 and 1.25 mg/m^3, but were somewhat variable at 6.35 mg/m^3. However, insufficient data were provided to make an independent assessment of the stability and concentration of dichlorvos in the chambers.

No rats died during the study. It was reported that lethargy occurred in an unspecified number of rats at 6.35 mg/m^3, with no effects observed at 0.25 or 1.25 mg/m^3. Toxicologically- and statistically-significant inhibition ($p<0.01$) of plasma, RBC and brain ChE activities occurred at and above 1.25 mg/m^3 in maternal rats (see Table below).

Effect of dichlorvos on ChE activity in rats

ChE	Dichlorvos concentration (mg/m^3)			
	0 (n=16)	0.25 (n=9)	1.25 (n=10)	6.25 (n=9 or 10)
Plasma[1]	0.183±0.0050 (0%)	0.178±0.0066 (3%)	0.123±0.0063 (33%)**	0.050±0.0063 (73%)**
RBC[1]	0.148±0.0071 (0%)	0.156±0.0095 (0%)	0.090±0.0090 (39%)**	0.018±0.0090 (88%)**
Brain[2]	0.304±0.0042 (0%)	0.299±0.0055 (2%)	0.218±0.0053 (28%)**	0.052±0.0053 (83%)**

1 = results expressed as the mean ± SEM μmoles SH group/min/10 mL; 2 = results expressed as the mean ± SEM μmoles SH group/min/25 mg. The % inhibition relative to the control is shown in parentheses; *p<0.05 (unspecified test); *p<0.01 (unspecified test)

There were no treatment-related effects on pregnancy rates, the number of resorptions, foetal deaths and live litter sizes and foetal weights. There were no visceral or skeletal abnormalities that were attributable to dichlorvos.

Conclusions: The NOEL for maternal toxicity following 20 days of inhalational exposure to dichlorvos was 0.25 mg/m^3, based on the inhibition of plasma, RBC and brain ChE activities at and above 1.25 mg/m^3. The NOEL for foetal and developmental toxicity was 6.25 mg/m^3, the highest dose tested. Deficiencies noted in this study were the lack of methodological and reporting detail, and the absence of observational data for maternal animals.

Tyl RW, Marr MC & Myers CB (1990a) Developmental toxicity evaluation of DDVP administered by gavage to CD (Sprague-Dawley) rats. RTI ID No. 60C-4629-10/20. Lab: Reproductive and Developmental Toxicology Laboratory, Centre for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: Unspecified. Report date: 22nd February 1991.

GLP compliant (US EPA; 40 CFR Part 160) and QA study.

Materials and Methods

Dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 96.86% purity) was administered orally to timed-pregnant CD® SD rats (25/group; 10-weeks old; 206.48-268.92 g bw; Charles River Laboratories Inc, Raleigh, NC, USA) by gavage on gestational days (gd) 6 to 15 at 0, 0.1, 3.0 or 21.0 mg/kg bw/d in deionised or distilled water. The dose volume was 5.0 mL/kg bw. The dose selection was based on a previous dose rangefinding study in pregnant rats (RTI Project No. 60C-4629-10). Prior to experimentation, dosing solutions were analysed for stability and homogeneity, and the concentration of dichlorvos was verified using HPLC. Dosing solutions were prepared twice during the study and stored refrigerated.

On receipt, rats were quarantined for 7 days. One female was housed with one male and the following morning examined for the presence of sperm. The day that sperm was observed was designated gd 0. Sperm-positive females were randomly assigned to treatment groups based on bodyweight and housed individually under standard conditions. Food (#5002 Purina Certified Rodent Chow®) and tap water were available *ad libitum*.

Clinical observations were made once daily on gd 0-5 and 16-20, and twice daily at dosing, 1-2 hours after dosing and on gd 6-15. Maternal bodyweights were recorded on gd 0, 6, 9, 12, 15, 18 and 20. Food consumption was recorded on gd 0-6, 6-9, 9-12, 12-15, 15-18 and 18-20. Rats were sacrificed by CO_2 asphyxiation on gd 20 and body, liver and uterine weights recorded. Thoracic and abdominal cavities and organs of maternal rats were macroscopically examined. Pregnancy status was confirmed by uterine examination. Ovarian corpora lutea were counted and uterine contents recorded (implantation sites, resorptions, live and dead foetuses). All foetuses were dissected from the uterus then counted, weighed, sexed and examined for external abnormalities. Approximately half of each litter was examined for visceral malformations and variations. These same foetuses were decapitated, their heads fixed and sectioned then examined for soft tissue craniofacial malformations and variations. All foetuses were eviscerated, fixed, stained and examined for skeletal malformations and variations.

The following parametric statistical tests were performed: ANOVA (following arcsine-square root transformation of all litter-derived percentage data and Bartlett's test for homogeneity of variance) followed by a Dunnett's multiple comparison test to compare each treatment group with the control; General Linear Model analysis to determine the significance of dose-response relationships; one or two-tailed tailed Dunnet's t-test for all pairwise comparisons; χ^2-test for independence for differences among treatment groups (for nominal scale measures), and when significant differences were detected, a one-tailed Fisher's Exact Probability Test for pairwise comparisons was performed.

Results

Analytical chemistry: Analysis of the dosing solutions revealed that they were within $\pm$10% of the nominal concentrations. Results of a number of previous studies (RTI Project No. 60C-4629), which tested aqueous solutions of dichlorvos at various concentrations, found that solutions of 0.02 and 10 mg/mL were homogenous (3-4% variability between the bottom, middle and top of the formulation). These same solutions were stable for 7 days when stored refrigerated (2-3°C) in the dark or for 6 hours when stored at ambient temperature. The 0.02 mg/mL formulation was subsequently shown to be stable for 24 hours at ambient temperature in plastic water bottles.

Maternal rats: There were no deaths. Cholinergic signs were evident at 21.0 mg/kg bw/d during the dosing period and included tremors, prone positioning, excitability, leaning/swaying and lower jaw movement (see Table below). Tremors were reported to occur within 10-60 minutes of dosing. Rats

recovered following the cessation of dosing. Treatment-related clinical signs were not observed in any other group.

The average bodyweight of the 21.0 mg/kg bw/d group was significantly lower ($p<0.01$-0.05) than the control group (~5%) throughout the dosing period (gd 9, 12 and 15). Average bodyweight gain over the dosing period (gd 6-15) was 60.0±2.4, 56.8±3.6, 57.3±2.8 and 43.0±1.8 at 0, 0.1, 3.0 and 21.0 mg/kg bw/d, respectively, with the effect at 21.0 mg/kg bw/d statistically significant ($p<0.01$).

At 21 mg/kg bw/d, average food consumption (mg/kg bw/d) was significantly lower ($p<0.01$) than the control over the dosing period: gd 6-9 (16% lower than control); gd 9-12 (11% lower than control); gd 6-15 (12% lower than the control). At 21.0 mg/kg bw/d, there was a slight though statistically significant reduction ($p<0.01$) in food consumption over the entire 20 day gestation period, which was attributable to the reduction in food consumption over the dosing period.

At termination there were no treatment-related macroscopic abnormalities detected. There was no treatment-related effect on gravid uterine weight or liver weight.

Clinical signs observed at 21.0 mg/kg bw/d dichlorvos

Observation	Gestation day										
	6	7	8	9	10	11	12	13	14	15	16
Tremors											
Fine motor		3	3	2	7	3	1	5	1		
Very slight									1	3	
Slight	5	6	4	2	1	7	9	9	10	12	
Slight to moderate			2								
Moderate	1	4	7	4	4	4	6	6	6	4	
Severe	1									1	
Prone positioning		5	6	1	4	6	7	3	1	5	
Excitability							2				
Leaning/swaying							2				
Lower jaw movement							4				

Results are the absolute numbers of rats affected. Blanks signify 0 rats.

Foetuses: There were no treatment-related effects on the number of resorptions, deaths, sex ratios or foetal bodyweight. Dichlorvos did not cause any external, visceral or skeletal malformations. There was an increase in male foetuses with variations at 21.0 mg/kg bw/d, with this result following a significant linear trend ($p<0.05$), however, none of the pairwise comparisons were significantly different (see Table below). Furthermore, no significant trend was observed for females or for all foetuses (males and females). There were apparent increases in the litter and foetal incidences of slightly enlarged lateral ventricles of the cerebrum (see Table below), a finding that was not statistically significant. An examination of historical control data for SD rats from Charles River Laboratories (compiled by the Middle Atlantic Reproduction and Teratology Association and Midwest Teratology Association, March 1996) revealed that the average (±SD) incidence of cerebral ventricle enlargment in foetuses is 2.573±11.49% (max 87.84%), while the litter incidence is 5.558±17.19% (max 100%). In light of these historical control data, the finding of ventricle enlargement was not considered treatment-related.

Foetal variations

Observation	Dose (mg/kg bw/d)			
	0	0.1	3.0	21.0
Variations/litter (♂)	0.7±0.3 (8.5±3.0%)	0.6±0.2 (7.4±2.0%)	0.6±0.2 (6.6±2.2%)	1.0±0.3 (16.0±4.2%)
Slightly enlarged lateral ventricle (bilateral) per litter	0.5±0.2 (5.9±2.5%)	0.5±0.1 (5.5±1.7%)	0.5±0.2 (7.5±2.8%)	0.8±0.3 (10.4±4.2%)
Foetuses with slightly enlarged lateral ventricle (bilateral)	9 (5.9%)	10 (6.1%)	12 (7.2%)	18 (10.4%)

Conclusions: The NOEL for maternal toxicity was 3.0 mg/kg bw/d, based on the occurrence of cholinergic signs (tremors, prone positioning) and reduced bodyweight and food consumption at 21.0 mg/kg bw/d. The NOEL for foetal and developmental toxicity was 21.0 mg/kg bw/d, the highest dose tested.

8.2 Rabbits

Thorpe E, Wilson AB, Dix KM & Blair DB (1971) Toxicity studies with dichlorvos: teratogenic studies in rats and rabbits given dichlorvos by inhalation. Project No. T507022/3. Lab & Sponsor: Pathology, Toxicology & Chemical Toxicology Divisions of Tunstall Laboratory and the Statistics Unit of Sittingbourne Laboratories, unspecified location. Study duration: unspecified. Report date: July 1971..

Materials and Methods

Female Dutch rabbits (unspecified age; 2-3 kg bw; Hylyne Commercial Rabbits, Hartford, Cheshire UK & Fisons Ltd Pharmaceutical division, Loughborough, Leicestershire, UK) were housed in groups of 4 with single males of proven fertility. Once mating was observed, females were randomly assigned to treatment groups and housed individually within inhalation chambers for 28 days. During this time up to 20 rabbits/group were exposed to atmospheres of dichlorvos [unspecified source and batch/lot No.; technical grade (>97% purity)] at 0, 0.25, 1.25 and 6.25 μg/L (equivalent to 0, 0.25, 1.25 and 6.25 mg/m^3, respectively). In a second experiment, mated female rabbits were exposed to 0, 2 and 4 μg/L dichlorvos (equivalent to 0, 2 and 4 mg/m^3, respectively). Exposure was for 23 hours per day, 7 days per week. No rationale was provided for the dose selection. It was stated that atmospheres of dichlorvos were generated according to a previous unpublished method, however, no study citation or details were provided. Housing conditions were unspecified while food (unspecified) and water (unspecified source) were available *ad libitum*.

Rabbits were observed daily for clinical signs and abnormal behaviour. Any rabbits dying during the study were necropsied. Maternal bodyweight and food consumption were not recorded. At the end of the 28-day exposure period, rats were sacrificed by an unspecified means and blood samples taken for measurement of plasma, RBC and brain ChE activities. Maternal animals were not macroscopically examined for any structural abnormalities or pathological changes. Gravid uterine weights and the number of corpora lutea were not recorded. Numbers of live foetuses, stillbirths and resorptions were recorded. Foetuses were not sexed. Live foetuses were weighed and examined for external malformations. Approximately half of each litter was processed for examination of skeletal abnormalities, with the remainder examined for visceral abnormalities. Unspecified statistical tests were performed on ChE data.

Results

Graphically presented data illustrated that chamber dichlorvos concentrations were relatively stable at 0.25 and 1.25 mg/m^3, but were somewhat variable at 6.35 mg/m^3. In the second experiment, a blockage in the 4 mg/m^3 chamber reportedly lead to an increase in the concentration of dichlorvos (up to 6.6 mg/m^3). Overall, there were insufficient data available to make an independent assessment of the stability and concentration of dichlorvos in the chambers.

At 6.35 mg/m^3, 16/20 rabbits died or were sacrificed in a moribund condition. These rabbits reportedly developed clinical signs from the 6th day of exposure, which included anorexia, lethargy, muscle tremors, mucous nasal discharge and diarrhoea. No clinical signs were observed at 0.25 or 1.25 mg/m^3. In the second experiment, deaths were observed at 2 and 4 mg/m^3 (1 and 6 rabbits, respectively). Clinical signs similar to those observed in the first experiment occurred in these rabbits; 5 of the 6 rabbits in the 4 mg/m^3 group were reportedly affected following the blockage of the chamber filter, which increased the concentration of dichlorvos.

Toxicologically- and statistically-significant inhibition ($p<0.01$) of plasma ChE activity occurred at and above 1.25 mg/m^3 in maternal rats (see Table below). Significant inhibition of RBC and brain ChE activities occurred at and above 0.25 mg/m^3 ($p<0.01$-0.05), however, toxicologically-significant inhibition (ie. >20%) occurred at and above 1.25 mg/m^3.

Effect of dichlorvos on ChE activity in rabbits

ChE	Dichlorvos concentration (mg/m^3)			
	0	0.25	1.25	6.25
Plasma[1]	0.048±0.0034 (0%) n=18	0.041±0.0032 (15%) n=20	0.031±0.0032 (35%)** n=20	0.013±0.0071 (73%)** n=4
RBC[1]	0.138±0.0085 (0%) n=18	0.119±0.0090 (14%)* n=16	0.044±0.0096 (69%)** n=14	0.010±0.0179 (93%)** n=4
Brain[2]	0.440±0.0119 (0%) n=19	0.398±0.0116 (10%)* n=20	0.195±0.0116 (56%)** n=20	0.067±0.0258 (83%)** n=4

1 = results expressed as the mean ± SEM μmoles SH group/min/10 mL; 2 = results expressed as the mean ± SEM μmoles SH group/min/25 mg. The % inhibition relative to the control is shown in parentheses; *p<0.05 (unspecified test); *p<0.01 (unspecified test)

In both experiments, there were no treatment-related effects on pregnancy rates, the number of resorptions, foetal deaths and live litter sizes. In the second experiment, average foetal weight was slightly lower at 4.0 mg/m^3 compared to the control and 2.0 mg/m^3 groups (20.1±0.98 g *versus* 23.1±0.98 and 23.2±1.02 g, respectively). The statistical significance of this finding was unclear, with the study authors concluding that it was due to maternal toxicity. There were no visceral or skeletal abnormalities attributable to dichlorvos.

Conclusions: The NOEL for maternal toxicity in rabbits following 28 days of inhalational exposure to dichlorvos was 0.25 mg/m^3, based on the inhibition of plasma, RBC and brain ChE activities at and above 1.25 mg/m^3. The NOEL for foetal and developmental toxicity was 2 mg/m^3, based on the reduction in average foetal weight at 4.0 mg/m^3. Deficiencies noted in this study were the lack of methodological and reporting detail, and the absence of observational data for maternal animals.

Schwetz BA, Ioset HD, Leong BKJ & Staples RE (1979) Teratogenic potential of dichlorvos given by inhalation and gavage to mice and rabbits. Teratology 20:383-388.

NZW rabbits (Langshaw's Rabbitry, Michigan, USA) were mated and exposed to dichlorvos (Shell Chemical Co.; 96% pure) by vapour inhalation (7 h/d in cages placed in a 3.7 m3 chamber) or oral gavage (in corn oil), during gd 6-15 and 6-18, respectively. The mean inhalational exposure level, monitored daily, was 4.06 (±0.63) mg/m^3, and the oral dose was intended to be the maximum tolerated dose for the species (5 mg/kg bw/d). A control group received the vehicle only. All rabbits were observed and weighed daily and sacrificed gd 29. The number of corpora lutea, live, dead and resorbed foetuses were recorded. All foetuses were weighed, measured and sexed, and were examined for external alterations. One-third of each litter was dissected and examined for visceral anomalies using Staple's method; the heads were fixed in Bouin's fluid and sectioned; and all foetuses were preserved, cleared and stained (alizarin red-S) for skeletal investigation.

Following oral dosing, there were no adverse clinical signs and no effects on bodyweight gain. Eight control and 12 treated litters were delivered by caesarian section on gd 29. No difference in the incidence of litters with resorptions was noted, however there was a 3-fold increase in the number of resorptions/litter, which was not statistically significant. There were no significant differences between treated and control groups in the number of live foetuses, foetal weight and length, or sex ratio, or in the incidence and type of visceral or skeletal anomalies or malformations.

Inhalational exposure did not lead to adverse clinical signs and had no effects on bodyweight gain. Fourteen control and 19 treated litters were delivered by caesarian section on gd 29. There were no significant differences between treated and control groups in the number of live foetuses, resorptions, foetal weight and length, or sex ratio, or in the incidence and type of visceral or skeletal anomalies or malformations.

Therefore, an oral dose of dichlorvos at 5 mg/kg bw/d or inhalational exposure at 4 mg/m^3 was without maternal or developmental toxicity.

Tyl RW, Marr MC & Myers CB (1990b) Developmental toxicity evaluation of DDVP administered by gavage to New Zealand White Rabbits. RTI ID No. 60C-4629-30/40. Lab: Reproductive and Developmental Toxicology Laboratory, Centre for Life Sciences and Toxicology, Chemistry and Life Sciences, Research Triangle Institute, Research Triangle Park, North Carolina, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 19th June 1990 to 2nd August 1990. Report date: 22nd February 1991.

GLP compliant (US EPA; 40 CFR Part 160) and QA study.

Materials and Methods

Dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 96.86% purity) was administered orally to artificially inseminated NZW rabbits (16/group; 6 months of age; 2520-3670 g bw; Hazleton Research Products Inc., Denver, PA, USA) by gavage once daily on gd 7 to 19 at 0, 0.1, 2.5 or 7.0 mg/kg bw/d in deionised or distilled water. The dose volume was 1.0 mL/kg bw. The dose selection was based on a previous rangefinding study in pregnant rabbits (RTI Project No. 60C-4629-30). Prior to experimentation, dosing solutions were analysed for stability and homogeneity, and the concentration of dichlorvos was verified using HPLC. Dosing solutions were prepared 3 times during the study and stored refrigerated.

On receipt, rabbits were quarantined for approximately 2 weeks and housed individually under standard conditions. Food (#5322 Purina Certified Rabbit Chow®) and tap water were available *ad libitum*. Females were induced to ovulate prior to insemination with an intravenous injection of chorionic gonadotropin. Inseminated females were randomly assigned to treatment groups based on bodyweight.

Clinical observations were made once daily on gd 0-6 and 20-30, and twice daily at dosing, 1-2 hours after dosing and on gd 7-19. Bodyweights were recorded on gd 0, 7, 12, 15, 19, 23 and 30. Food consumption was recorded from gd 0-7, 7-9, 9-12, 12-15, 15-19, 19-23 and 23-30. Rabbits were sacrificed by an intravenous injection of Beuthanasia (Veterinary Companies of America, Raleigh, NC, USA) on gd 30, approximately one-and-a-half days before expected parturition. Thoracic and abdominal cavities and organs of maternal rabbits were macroscopically examined. Pregnancy status was confirmed by uterine examination. Body, liver and uterine weights were recorded. Ovarian corpora lutea were counted and uterine contents recorded (implantation sites, resorptions, live and dead foetuses). Maternal livers were sectioned and stored for possible future histopathological examination. Live foetuses were immediately sacrificed by an ip injection of Beuthanasia, weighed and examined for external abnormalities. All foetuses were dissected from the uterus then counted, weighed, sexed and examined for external abnormalities. Approximately half of each litter was examined for visceral malformations and variations. These same foetuses were decapitated, their heads fixed and sectioned then examined for soft tissue craniofacial malformations and variations. All foetuses were eviscerated, fixed, stained and examined for skeletal malformations and variations.

The following parametric statistical tests were performed: ANOVA (following arcsine-square root transformation of all litter-derived percentage data and Bartlett's test for homogeneity of variance) followed by a Dunnett's multiple comparison test to compare each treatment group with the control; General Linear Model analysis to determine the significance of dose-response relationships; one or two-tailed tailed Dunnet's t-test for all pairwise comparisons; χ^2-test for independence for differences among treatment groups (for nominal scale measures), and when significant differences were detected, a one-tailed Fisher's Exact Probability Test for pairwise comparisons was performed.

Results

Analytical chemistry: Analysis of the dosing solutions revealed that they were within ±10% of the nominal concentrations. On one occasion, the low-dose solution was found to contain 113% of the nominal concentration and was therefore not used. It was subsequently reformulated (the next day), analysed and used. Results of a number of previous studies (RTI Project No. 60C-4629), which tested aqueous solutions of dichlorvos at various concentrations, found that solutions of 0.02 and 10 mg/mL were homogenous (3-4% variability between the bottom, middle and top of the formulation). These same solutions were stable for 7 days when stored refrigerated (2-3°C) in the dark or for 6 hours when stored at ambient temperature. The 0.02 mg/mL formulation was subsequently shown to be stable for 24 hours at ambient temperature in a plastic water bottle.

Maternal rabbits: Two and four pregnant rabbits died in the 2.5 and 7.0 mg/kg bw/d groups, respectively. Two females from the 0.1 mg/kg bw/d group were removed from the study because of early delivery. Treatment-related clinical signs were evident in the 7.0 mg/kg bw/d group during the dosing period (gd 7-19) and included ataxia (2-6 rabbits), increased respiration (2), subdued behaviour (2), rapid breathing (2-3), salivation (2-3), fine motor tremors (2) and lurching (2). These clinical signs were not observed in any other group.

Average maternal bodyweight gain was lower than the control at 2.5 and 7.0 mg/kg bw/d over gd 7-19 (67 and 58%, respectively, lower than the control). When maternal bodyweight was corrected for gravid uterine weight, only the bodyweight gain at 7.0 mg/kg bw/d appeared to be lower than the control (~54% lower). None of these effects on bodyweight were statistically significant, which the authors attributed to the high variance typical of the test species. There was a slight but significant reduction in maternal food consumption during the dosing period (gd 7-19), which followed a significant linear trend ($p<0.05$) (43.6±1.1, 46.8±2.5, 41.3±3.3 and 38.4+2.3 at 0, 0.1, 2.5 and 7.0 mg/kg bw/d, respectively). Individual pairwise comparisons revealed that at 7.0 mg/kg bw/d, maternal food consumption was significantly lower ($p<0.05$) than the control only at gd 7-9 (39.5±2.3 *versus* 48.2±1.5 g/kg bw/d, respectively).

Gravid uterine weight was higher at 7.0 mg/kg bw/d (521±34.8 g) compared to the control (459±49.9 g), while absolute and relative liver weights were lower (101.2±3.6/2.60±0.07 *versus* 113.3±6.3/2.99±0.14, respectively). When liver weights were corrected for gravid uterine weight, a significant linear trend ($p<0.05$) was apparent (3.40±0.14, 3.36±0.11, 3.17±0.11 and 3.00±0.08 g at 0, 0.1, 2.5 and 7.0 mg/kg bw/d, respectively). At termination, no treatment-related macroscopic abnormalities were detected in maternal rabbits.

Foetuses: There were no treatment-related effects on the number of resorptions, foetal deaths, sex ratios and bw. dichlorvos did not cause any external, visceral or skeletal malformations or variations.

Conclusions: The NOEL for maternal toxicity was 0.1 mg/kg bw/d, based on the occurrence of mortalities at and above 2.5 mg/kg bw/d. Cholinergic signs and reduced food consumption occurred at 7.0 mg/kg bw/d. The NOELs for foetotoxicity and developmental toxicity were 7.0 mg/kg bw/d, the highest dose tested. There was no evidence that dichlorvos is teratogenic.

9. GENOTOXICITY

The following Tables summarise the findings of *in vitro* and *in vivo* genotoxicity studies submitted and evaluated as part of the current dichlorvos review. A complete written evaluation of those studies indicating positive or equivocal findings follows.

In vitro assays

Assay	Strain or Cell type	Concentration	Batch / Purity	Positive control	Metabolic Activation	Result	Reference
Forward mutation in mammalian cells	Mouse lymphoma L5178Y (TK +/-)	0.004-0.5 μL/mL	Lot No. 11381-23-5 97%	EMS (0.5, 1 μL/mL) 7,12-DMBA (5, 7.5 μL/mL)	-, +	+, +	Ford et al (1986) [QA]
Reverse mutation in bacteria	E Coli WP2	1 drop of a 10% or 10 mg/100 mL aqueous solution	Analytical and technical grade (unspecified purity)	EMS, MMS, MNNG, nitrogen mustard, β-propriolactone (unspecified concentration)	-	-	Dean (1972)*

Results (+ = positive; - = negative or +/- = equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation; EMS = ethyl methanesulfonate; DMBA = 7,12-dimethylbenz(a) anthracene; QA = quality assured study; MMS = methyl methanesulfonate; MNNG = methyl nitroso nitroguanidine; * = inadequate study report

In vivo assays

Assay	Species (Strain)	Dose or concentration	Batch / Purity	Positive control	Result	Reference
Dominant lethal	Mouse (CD-1) 10/group (♂)	0, 1, 3 or 10 mg/kg bw/d ip in corn oil for 5 d	98.4%	Triethylenemelamine (0.5 mg/kg bw, ip)	-	Ford et al (1985a) [GLP, QA]
	Mouse (CD-1) 30 or 35/group (♂)	0, 8, 16, or 32 mg/kg bw/d ip in corn oil for 5 d	97.5%	Triethylenemelamine (0.2 mg/kg bw, ip)	-	Ford & Killeen (1987) [GLP]
Cytogenetic assay	Mice (ICR) Bone marrow & spermatocytes	0, 12.5, 25 or 25 mg/kg bw/d by po gavage in water for 5 d	Lot No. 802097 98.09%	Cyclophosphamide (150 mg/kg bw, po)	-	Putman & Shadly (1992) [GLP, QA]
SCE	Mice (B6C3F1) Bone marrow	0, 3, 10 or 30 mg/kg bw, ip in corn oil	Unspecified	Cyclophosphamide (10 mg/kg bw, ip)	-	Putman (1985) [GLP, QA]
Micronucleus test	Mice (CD-1) Bone marrow	0, 4, 13 or 40 mg/kg bw, ip in corn oil	98.4%	Triethylenemelamine (0.15 mg/kg bw, ip)	-	Ford et al (1985b) [GLP]*

SCE = sister chromatid exchange; Results (+ = positive; - = negative or +/- = equivocal); QA = quality assured study; GLP = statement of compliance with principles of good laboratory practice; * study report only – lab data not supplied with submission

9.1 Evaluation of selected genotoxicity studies

Ford WHF, Killeen Jr JC & Baxter RA (1986) L5178Y TK$^{+/-}$ mouse lymphoma forward mutation assay with dichlorvos. Lab: Microbiological Associates Inc., Bethesda, Maryland, USA. Project ID. T-5211.702003. Sponsor: Dichlorvos Task Force, c/o Fermenta Animal Health Company, Painesville, Ohio, USA. SDS biotech study No. 86-0036. Study duration: 19th August 1986 to 10th October 1986. Report date: 14th October 1986.

QA study.

Materials and Methods: A standard L5178Y TK+/- mouse lymphoma assay was performed on dichlorvos (Dichlorvos Task Force, c/o Fermenta Animal Health Company, Painesville, Ohio, USA; lot No. 11381-23-5; 97.5% purity) at various concentrations ranging from 0.004-0.5 μL/mL. A preliminary toxicity test was performed in the presence and absence of metabolic activation at dichlorvos concentrations of 0.001, 0.01, 0.1, 1.0, 10 and 100 μL/mL. Experiments were performed in the presence and absence of metabolic activation (Aroclor-induced rat liver S9). In addition to a solvent control [(DMSO) n=2], ethyl methanesulfonate [(EMS) 0.5 & 1.0 μL/mL)] and 7,12-dimethylbenz(a) anthracene [(7,12-DMBA), 5.0 and 7.5 μL/mL)] were used as positive controls in the absence and presence of metabolic activation, respectively. Cells were treated for 4 h followed by a two-day expression period. Cells were then placed in cloning medium containing 3 μg/mL triflurothymidine for 10-12 days (n=3). Total numbers of viable and mutant colonies were scored using an automated colony counter, and the median of three readings recorded. The sizes of the colonies were not enumerated. In total, 4 experiments were performed, however, two of these were deemed invalid due to technical problems.

Results were statistically analysed using Kastenbaum and Bowman tables. The evaluation criteria for a positive response was a reproducible positive dose-response in one or more of the doses in the 10% or greater total growth range and a mutation frequency 2-fold higher than the background level (in the solvent control).

Results and Conclusions: During the preliminary toxicity experiment, cell growth was inhibited at and above 1.0 μL/mL both in the presence and absence of metabolic activation. The following Table summarises the mutagenicity findings for dichlorvos in the two valid experiments; experiment 1 tested dichlorvos at concentrations of 0.013-1.0 L μL/mL, while experiment 2 tested concentrations of 0.0067-0.5 μL/mL. There was a significant ($p<0.05$) concentration-related increase in the mutation frequency per 10^4 cells, which was approximately 2-fold higher in the absence than presence of metabolic activation. This finding was reproducible across the two experiments. According to the performing laboratories evaluation criteria, a positive result occurred at and above 0.043 μL/mL in the absence of metabolic activation, with total growth ranging from 2-52% of the solvent control. In the presence of metabolic activation, dichlorvos concentrations of 0.038, 0.05, 0.089, 0.12, 0.18 and 0.24 μL/mL caused at least a 2-fold increase in mutation frequency relative to the solvent control, with total growth ranging from 11-73%. The positive controls (EMS and 7,12-DMBA) increased the mutation frequency in the absence and presence of metabolic activation, respectively.

Summary of mutagenicity findings for dichlorvos (2 separate experiments)

Concentration (μL/mL)	Mutation Frequency (per 10^4 cells)		Fold Increase (relative to solvent control)		Total Growth (%)	
	-	+	-	+	-	+
0.33	c	t	c	t	c	t
0.24	5.3*	2.6*	13.3	3.7	2	11
0.18	4.1*	1.9*	10.3	2.7	4	30
0.14	3.1*	1.2	7.8	1.7	11	48
0.12	3.0*	1.1*	7.5	3.7	1	11
0.1	2.3*	1.1	5.8	1.6	24	43
0.089	1.8*	0.8*	4.5	2.7	6	23

Concentration (μL/mL)	Mutation Frequency (per 10^4 cells)		Fold Increase (relative to solvent control)		Total Growth (%)	
	-	+	-	+	-	+
0.077	c	0.8	c	1.1	c	71
0.067	1.8*	0.5	4.5	1.7	14	41
0.058	1.0*	0.8	2.5	1.1	52	62
0.05	0.9*	0.6*	2.3	2.0	41	53
0.043	0.9*	0.7	2.3	1.0	46	87
0.038	0.07	0.6*	1.8	2.0	53	73
0.033	0.07	0.9	1.8	1.3	57	88
0.028	0.06	0.5	1.5	1.7	70	87
0.024	0.06	0.8	1.5	1.1	67	89
0.021	0.03	0.5	0.8	1.7	88	89
0.016	0.05	0.6*	1.3	2.0	87	85
0.012	0.03	0.4	0.8	1.3	85	106
0.089	0.04	0.4	0.0	1.3	88	101
Positive control findings						
EMS (no metabolic activation)						
0.5 μL/mL	c, 7.2		c, 18		35, 19	
1.0 μL/mL	16.8, 10.1		33.6, 25.25		5, 5	
7,12-DMBA (metabolic activation)						
5.0 μL/mL	2.7, 2.6		3.9, 6.5		43, 27	
7.5 μL/mL	7.0, c		10, c		5, c	

- = without metabolic activation; + with metabolic activation; c = culture lost due to contamination; t = too toxic to clone; NT = not tested; *p<0.05; Intallicised concentrations were tested in experiment 1

In conclusion, dichlorvos was mutagenic in the L5178Y TK+/- mouse lymphoma assay. Metabolic activation appeared to reduce the mutation frequency, however, the level remained significantly higher than the negative control.

10. NEUROTOXICITY STUDIES

10.1 Hens

Beavers J, Driscoll CP, Dukes V & Jaber M (1988) DDVP: An acute delayed neurotoxic study in chickens. Wildlife International Ltd Project No. 246-103. Lab: Wildlife International Ltd, Easton, Maryland, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 8th August 1988 to 20th September 1988. Report date: 29th December 1988.

GLP compliant (US EPA; 40 CFR Part 160) and QA study. Study based in part on Section 81-7 of US EPA/OPP Pesticide Guidelines (Subdivision F, EPA 540/9-82-025, November 1982).

Materials and Methods

A single oral gavage dose of dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 96.5% purity) was administered to 10 fasted domestic chickens/group (42 weeks old; 1214-1688 g bw; Truslow Farms Inc, Chestertown, Maryland, USA) at 0 or 16.5 mg/kg bw in distilled water. The dose selection was based on an unpublished preliminary LD_{50} study conducted by the performing laboratory, where the LD_{50} was calculated to be 16.5 mg/kg bw. A positive control group was administered a single oral gavage dose of 600 mg/kg bw tri-ortho cresyl phosphate (TOCP) in corn oil. The dose volume for all groups was 8 mL/kg bw. All birds treated with dichlorvos were given a single intramuscular injection of 5 mg/kg bw atropine at the time of dosing, and thereafter at 2 mg/kg bw as required. Samples of dosing solutions were frozen and transported to Hazleton Laboratories (Madison, Wisonsin, USA) for analyses of the stability and concentration of dichlorvos.

Birds had been acclimatised for 7 days prior to dosing and were housed by dosage group in batteries of poultry laying pens under standard conditions. Food (Wildlife International Ltd game bird ration) and tap water were available *ad libitum*. After 21 days, all positive control birds were sacrificed, while the negative control and dichlorvos groups were redosed (after a minimum of 15 hours fasting) and observed for a further 21 days.

Throughout the experimental period, all birds were observed at least twice daily. Mortalities, clinical signs and any abnormal behaviour were recorded. Locomotor activity was evaluated twice weekly and scored based upon an ataxia point system: birds were dropped from a height of approximately one metre and evaluated for landing ability; birds were then encouraged to walk approximately 8 m; birds were forced to hop onto a flat surface approximately 10 cm above the floor, followed by a second surface a further 10 cm higher; birds were then encourage to walk an additional 8 m. Bodyweight was recorded on days -7, 0, 3, 7, 14, 21, 22, 25, 29, 36 and 43. Due to unavoidable food wastage by birds, only average estimated food consumption was determined on days 0-3, 4-7, 8-14, 15-21, 22-25, 26-29, 30-36 and 37-43.

Positive control birds were sacrificed on day 21, while the negative control and dichlorvos groups were sacrificed on day 43. Birds were sacrificed by anaesthesia and perfusion through the heart. No gross necropsy was performed. The following tissues were histopathologically examined: brain, spinal cord and right and left sciatic nerve.

No statistical analysis was performed.
Results

Mortalities and clinical signs: There were no mortalities in any group. dichlorvos-treated birds exhibited cholinergic signs (lethargy or depression, incoordination, lower limb weakness, wing droop, reduced reaction to external stimuli, a ruffled appearance, prostrate posture and a loss of righting reflex) from approximately 30 min after dosing. Birds then recovered over the 21-day observation period. Following the second dose (at day 22), similar clinical signs were observed. Two TOCP-treated birds exhibited transient incoordination or lower limb weakness at day 0, with a third bird exhibiting the same clinical signs over an extended period of time. By terminal sacrifice on day 21, all TOCP-treated birds exhibited incoordination and lower limb weakness.

Bodyweight and food consumption: Bodyweights were unaffected by treatment with dichlorvos or TOCP. The mean (± SD) food consumption of dichlorvos-treated birds was markedly lower than the control group (13 ± 15 *versus* 63 ± 27 g/bird/day, respectively) over days 0-3, with 3/10 birds failing to consume any food over this period (compared to 0/10 in the control). Food consumption remained somewhat lower than the control group over days 4-7 (46 ± 13 *versus* 66 ± 32 g/bird/day, respectively) but had recovered to control levels by days 15-21. TOCP-treated birds also consumed less food over days 0-3 compared to the control (35 ± 34 *versus* 63 ± 27 g/bird/day), with 2/10 birds consuming no food over this period. Food consumption by TOCP-treated birds was comparable to the negative controls over the remainder of the observation period. No statistical analysis was performed on this data by the study authors. Calculations performed by the reviewing toxicologist revealed that the effect of dichlorvos on food consumption at days 0-3 was highly significant (p=0.00008; one-tailed, 2-sample t-test, assuming unequal variances) but the effect at days 4-7 was not. The effect of TOCP on food consumption was significant at days 0-3 (p=0.03; one-tailed, 2-sample t-test, assuming unequal variances).

Locomotor activity: One animal from the control group (#1768) showed locomotor abnormalities at days 7 (incoordination, pronounced ataxia) and 10 (incoordination). Two separate birds refused to hop or walk at day 25, but were not ataxic. Three birds from the dichlorvos group showed locomotor abnormalities over the first 10 days of dosing; at day 3 (#G30, G37; slight to moderate ataxia), 7 (#G37; slight ataxia) and 10 (#G36; slight lameness). Two birds from the TOCP group (#R254, R255) showed locomotor abnormalities (slight-moderate ataxia) on day 3 and 7, respectively. However, the most obvious effect on this group occurred at day 21 when 6/10 birds exhibited abnormal locomotory activity ranging from slight to pronounced ataxia. This finding was clearly treatment-related and indicated that the positive control did in fact cause delayed neurotoxicity.

Histopathology: No control birds displayed any microscopic abnormalities of the brain, spinal cord or bilateral peripheral nerve, while one bird treated with dichlorvos (#G37) exhibited sciatic neural degenerative changes (described as swelling of the axis cylinder and nerve fibre degeneration). In contrast, treatment with the positive control (TOCP) resulted in 5/10 birds with degeneration of the sciatic nerve, particularly the distal segments. The degeneration included swelling of the axis cylinder, nerve fibre degeneration and Schwann cell proliferation. It is somewhat difficult to dismiss the sciatic nerve degeneration in the single bird in the dichlorvos group since the finding was qualitatively the same as the positive control and no such findings occurred in the negative controls. An examination of individual bird data for the positive control group did not reveal a strong correlation between abnormal locomotor activity and sciatic nerve degeneration. In fact, 2/5 birds that exhibited nerve degeneration showed no locomotor effects, while 3/5 birds that showed no degeneration displayed abnormal locomotor activity. In the absence of historical control data, the occurrence of neural degenerative changes in the single dichlorvos-treated hen was considered by the reviewing toxicologist to be of equivocal relationship to treatment.

Conclusions: A single oral dose of 16.5 mg/kg bw dichlorvos resulted in cholinergic signs and significantly reduced food consumption. Histopathological examination revealed sciatic nerve degeneration in one hen in the absence of any clinical signs of delayed neurotoxicity. It was equivocal whether this finding was attributable to dichlorvos treatment. Deficiencies noted in this study were the absence of statistical analysis and macroscopic examination. Furthermore, no biochemical analysis was performed on any birds, such as the measurement of neuropathy target esterase (NTE) or brain ChE activity.

Redgrave VA, Mansell P, Crook D, Begg, SE, Gopinath C, Anderson A & Dawe IS (1994a) DDVP: 28-day neurotoxicity study in the domestic hen. Study No. AVC 1/921405. Lab: Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 28th April 1992 to 3rd February 1994. Report date: 21st October 1994.

Redgrave VA & Mansell P (1994) TOCP: Supplementary data on the delayed neurotoxicity to the domestic hen. Study No. AVC 1a/931884. Sponsor: Huntingdon Research Centre Ltd, Huntingdon,

Cambridgeshire, England. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 14th April 1993 to 22nd July 1994. Report date: 17th February 1994.

Redgrave VA, Cameron DM, Gopinath C & Dawe IS (1994b) TOCP: 28-day neurotoxicity in the domestic hen. Study No. RAD 2/942053. Sponsor: Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, England. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 16th November 1993 to 4th February 1994. Report date: 21st October 1994.

Jortner B (1994) Neuropathological review of studies AVC/1 and RAD/2, Huntingdom Research Centre. Report No. unspecified. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Report date: September 1994.

Hardisty JF (1998) Pathology working group peer review of DDVP 28-day neurotoxicity study in the domestic hen. EPL Project No. 578-001. Lab: Experimental Pathology Laboratories Inc, Research Triangle Park, NC, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Report date: 2nd April 1998.

GLP compliant (UK Compliance Programme, Dept Health & Social Security 1986 and subsequent revision, Dept Health 1989; EC Council Directive 87/18 EEC of 19 December 1986, No. L 15/29; GLP in the testing of Chemicals OECD, ISBN 92-64-12367-9, Paris 1982, subsequently republished OECD Environment Monograph No. 45, 1992; US EPA; 40 CFR Part 160; Japanese Ministry of Agriculture, Forestry and Fisheries, 59 NohSan, Notification No. 3850, Agricultural Product Bureau, 10th August 1984). QA study. Study conducted according to US EPA Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Addendum 10: Neurotoxicity Series 81, 82 and 83, dated March 1991.

Materials and Methods

Dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 97.87% purity) was administered orally by gavage to 12 adult female domestic hens per group (approximately 12-months old; 1945-2295 g bw; Atkinson Bros, Postland, Crowland, Peterborough, Cambridgeshire, England) at 0, 0.3, 1.0 or 3.0 mg/kg bw/d in distilled water for 28 days. The dose selection was based on an unpublished preliminary study conducted by Huntingdon Research Centre Ltd. [The NOEL following 4 consecutive daily doses was 0.2 mg/kg bw/d, with mortalities, cholinergic signs and decreased bodyweight evident at and above 3.0 mg/kg bw/d. Brain ChE activity was inhibited at and above 1.0 mg/kg bw/d.] An additional group of 3 birds was administered 0.1 mg/kg bw/d dichlorvos for measurement of brain ChE activity. A positive control group was administered a daily oral gavage dose of 7.5 mg/kg bw/d TOCP (Coalite Chemical Group, location unspecified; Batch No. S16848; assumed 100% purity) in corn oil for 28 days. The dose volume for all groups was 2 mL/kg bw. Dosing solutions were prepared weekly, with samples analysed for homogeneity and stability (at 6 hours, 4 and 10 days).

Birds had been acclimatised for 14 days prior to dosing and were housed by dosage group in groups of 7 in separate poultry laying pens under standard conditions. Birds were randomly allocated to treatment groups based on bw. Five additional birds were introduced into the study on the day of dosing due to mortalities occurring as a result of aggression. Birds affected by feather pecking were housed individually. Food (HRC Standard Layer Diet) and tap water were available *ad libitum*.

Birds were observed twice daily for 77 days (including the 28-day dosing period) and mortalities and clinical signs recorded. Birds were examined immediately after dosing for signs of delayed locomotor ataxia by allowing them to walk/run approximately one metre and then to jump on to a crate. The degree of ataxia was scored according to a point award system similar to Cavanagh et al (1961). Bodyweights were recorded weekly. Three birds/group were sacrificed by cervical dislocation 4 hours after the fourth dose (day 4) and 48 hours after the final dose, (day 30) for measurement of brain ChE activity, brain neuropathy target esterase (NTE) and spinal cord NTE.

Post mortem examinations were performed on all birds (including decedants). The following tissues were histopathologically examined in 5-6 birds/group sacrificed at day 49 and 77: forebrain, mid and

hind brain, upper cervical spinal cord, lower cervical spinal cord, thoracic spinal cord, lumbar spinal cord, proximal sciatic nerve, distal sciatic nerve (above knee) and tibial nerve (distal branch). Tissues from decedants were not histopathologically examined.

No statistical analysis was performed.

Results

Analytical chemistry: The majority of the dosing solutions were within ±10% of the nominal concentrations. There were 2 instances where particular analytical concentrations differed from the nominal concentrations by -12.4 and +19.4%, with additional formulations prepared to account for these unacceptable deviations. dichlorvos was determined to be stable when stored at ambient temperature for 6 hours and when stored frozen for 4 and 10 days.

Mortalities, clinical signs and bodyweight effects: One bird from the 1.0 mg/kg bw/d group and 4 birds from the 3.0 mg/kg bw/d group died during or immediately after the 28-day dosing period. Pretreatment mortalities in other groups (including the control) were due to aggression.

Treatment-related clinical signs were observed at 1.0 and 3.0 mg/kg bw/d dichlorvos, but did not occur in any other groups (including the positive control). At 1.0 mg/kg bw/d, only 2/21 birds exhibited clinical signs, which included an inability to stand and unsteadiness. At 3.0 mg/kg bw/d, 19/21 birds were quiet or subdued and unsteady. These clinical signs were observed 30 min after dosing and were reported to last for up to 8 hours. By day 30, all surviving birds appeared normal.

Treatment-related effects on bodyweight were confined to the high-dose group (3.0 mg/kg bw/d), where group mean bodyweight gain over the dosing period (days 1-28) was markedly lower than the control (-133 g *versus* +8 g, respectively). No statistical analysis was performed on this data. Over the 28-77 day postdose observation period, the mean bodyweight gain of this group increased compared to the control (+392 g *versus* +129 g, respectively) such that the terminal bodyweight of this group was the same as the control.

Delayed locomotor ataxia: It was reported that there were no clinical signs of neurotoxicity and no evidence of delayed locomotor ataxia in any bird. However, the results of the assessment of ataxia were not provided, including results for the positive control.

Brain ChE activity: There was dose-related inhibition of brain ChE activity, which was toxicologically-significant (ie. ≥20% relative to the control) at and above 1.0 mg/kg bw/d at day 4, and at and above 0.3 mg/kg bw/d at day 30 (see Table below). No statistical analysis was performed on this data.

Brain ChE activity (μmol/g/min) in hens treated with dichlorvos (n=3)

TIME	Dose dichlorvos (mg/kg bw/d)					TOCP
	0	0.1*	0.3	1.0	3.0	7.5 mg/kg bw/d
Day 4	11.15±1.387 (0%)	-	10.34±1.582 (7.3%)	6.24±0.337 (44%)	4.13±1.364 (63%)	10.53±3.137 (5.6%)
Day 30	14.22±0.490 (0%)	-	10.47±0.881 (26.4%)	9.32±0.622 (34.5%)	6.54±0.154 (54%)	14.11+0.587 (0.8%)
Day 30*	12.23±1.211 (0%)	13.07±1.436 (106%)	-	-	-	-

Results expressed as the mean ± 1 SD (% inhibition relative to control); *supplementary group

NTE activity: There was no treatment-related effect on either brain or spinal cord NTE activity. In contrast, the positive control (7.5 mg/kg bw/d TOCP) caused a marked reduction in both brain and spinal cord NTE activity relative to the negative control at day 4 and 30. [Day 4: 1222±216 (TOCP) *versus* 2446±33 nmol/g/min (control) for brain NTE activity; 467±85 (TOCP) *versus* 604±20

nmol/g/min (control) for spinal cord NTE activity; Day 30: 1197±86 (TOCP) *versus* 3075±209 nmol/g/min (control) for brain NTE activity; 236±14 (TOCP) *versus* 520±139 nmol/g/min (control) for spinal cord NTE activity.] No statistical analysis was performed on this data.

Necropsy: Macroscopic examination of deceased hens and those sacrificed at days 49 and 77 revealed no abnormalities that could be attributed to dichlorvos.
Histopathology: At days 49 and 77, TOCP-treated birds (5/6) showed axonal degeneration in the cerebellum ranging from a trace to minimal, while there were no treatment-related abnormalities in the cerebellum of any other group. Relative to the negative control, there was an increased incidence of axonal degeneration in the lower cervical and lumbo-sacral spinal cord of dichlorvos-treated birds, but this did not follow a dose-response relationship and was generally graded as "trace" (see Table below). According to the grading system used by the study authors, a grading of "trace" is not considered as biologically significant. Therefore, the majority of these findings can be disregarded as treatment-related. However, it should be noted that 1/6 and 2/6 dichlorvos-treated birds sacrificed at day 49 were scored as having minimal axonal degeneration in the upper cervical spinal cord, respectively, compared to 0/6 in the negative control group. Single birds in these groups were also graded as having minimal axonal degeneration in the upper cervical spinal cord at day 77, and in the lower cervical region at days 49 and/or 77. While these incidences are slightly higher than the performing laboratories historical control range of 0-10%, the reviewing toxicologist did not consider that they were treatment-related.

Incidence of axonal degeneration in hens

Observation	Dose dichlorvos (mg/kg bw/d)								TOCP 7.5 mg/kg bw/d	
	0		0.3		1.0		3.0			
	d 49	d 77	d 49	d 77	d 49	d 77	d 49	d 77*	d 49	d 77
Cerebellum										
Trace	0	0	0	0	1	0	0	0	3	3
Minimal	0	0	0	0	0	0	0	0	2	3
Spinal cord										
Upper cervical										
Trace	1	5	6	3	3	4	4	3	5	0
Minimal	0	0	0	0	1	1	2	1	0	4
Moderate	0	0	0	0	0	0	0	0	0	2
Lower cervical										
Trace	1	1	4	5	4	5	3	3	4	3
Minimal	0	0	0	0	0	1	1	1	0	0
Moderate	0	0	0	0	0	0	0	0	1	0
Mid-thoracic										
Trace	1	5	3	3	4	2	4	5	5	4
Minimal	0	0	1	0	0	1	0	0	1	2
Lumbo-sacral										
Trace	0	0	3	4	2	3	3	2	3	3
Minimal	0	0	0	0	0	0	0	0	3	2
Moderate	0	0	0	0	0	0	0	0	1	0

n=6 unless specified; * n=5

Neuropathological findings from the current study, as well as those from a supplementary study (No. RAD 2/942053, see below), were reviewed by Jortner (1994) at the request of AMVAC. This review involved a re-evaluation of the histopathology slides for each animal. There was no difference in the types and severity of lesions in the spinal cord of negative control and dichlorvos-treated birds across all dose levels. In contrast, birds treated with 15 or 20 mg/kg bw/d TOCP (Study No. RAD 2/942053) exhibited moderate to marked fibre degeneration in the spinal cord. Jortner concluded that dichlorvos does not cause OP-induced delayed neuropathy (OPIDN). Histopathology slides were re-examined again in 1998 by a pathology working group convened by the sponsor (EPL Project N578-001). This working group confirmed the previous findings and conclusions.

There were no treatment-related histopathological abnormalities detected in any of the other tissues examined.

Results for the positive control group appeared to be consistent with the performing laboratories historical control data for axonal degeneration in the brain and spinal cord, but not necessarily for the peripheral nerve. Furthermore, the absence of clinical signs in TOCP-treated birds led the study authors and sponsor to question the adequacy of the positive control at inducing delayed neurotoxicity. Consequently, a number of supplementary GLP studies were performed to test the ability of TOCP to induce delayed neurotoxicity.

An acute oral toxicity study (No. AVC 1a/931884) was conducted to examine the ability of two different sources of TCOP to induce delayed neurotoxicity in hens. Birds were treated as follows: 5 birds/group were given a single oral gavage dose of 500 mg/bird in corn oil then observed for 28 days; 4 birds/group were given a single oral gavage dose of 1000 mg/bird or 1000 mg/kg bw in corn oil then observed for 21 days; 4 birds/group were given a single oral gavage dose of 500 mg/bird or 500 mg/kg bw in corn oil then observed for 21 days. The incidence and severity of TOCP-induced ataxia was low, with TOCP sourced from Eastman Kodak (Batch No. 806359A; 97.5% purity) more affective than “the original TOCP” obtained from Coalite Chemical Group (Batch No. S16848; assumed 100% purity), which was used in the main study (No. AVC 1/921405) (7/13 *versus* 1/13 birds). It is not surprising that the positive control material sourced from Coalite was ineffective as HRC received it in March 1978, almost 15 years earlier, and assumed that it was stable.

In the second supplementary study (No. RAD 2/942053), TCOP (Coalite Chemical Group Ltd; Batch No. S16848; assumed 100% purity) was administered to hens at 0, 10, 12.5, 15 and 20 mg/kg bw/d for 28-days. Clinical signs of toxicity were observed at and above 12.5 mg/kg bw/d, with treatment-related mortalities occurring at 20 mg/kg bw/d. At 10 mg/kg bw/d, one bird developed ataxia, while there was a dose-related increase in delayed ataxia at and above 12.5 mg/kg bw/d. Inhibition of brain ChE activity and brain and spinal cord NTE activities occurred at all doses. All TOCP-treated birds showed axonal degeneration of the cerebellum and spinal cord, with little difference in the severity of degeneration between the groups. However, birds treated with 20 mg/kg bw/d appeared to be more severely affected and also displayed peripheral nerve degeneration, which was not evident in the majority of other treated birds.

Conclusions: The NOEL in hens following 28-days of repeated oral dosing was 0.1 mg/kg bw/d, based on the occurrence of toxicologically-significant inhibition of brain ChE activity at and above 0.3 mg/kg bw/d. Cholinergic signs occurred at and above 1 .0 mg/kg bw/d. There was no evidence that dichlorvos caused delayed neurotoxicity. The absence of statistical analysis and results of the locomotor assessment were deficiencies of this study.

10.2 Rats

Lamb IC (1993a) An acute neurotoxicity study of dichlorvos in rats. WIL study No. WIL-188003. Lab: WIL Research Laboratories Inc, Ashland, Ohio, USA. Sponsor: AMVAC Chemical Corporation, Los Angeles, California, USA. Study duration: 13th April 1992 to 22nd June 1992. Report date: 15th January 1993.

GLP compliant (US EPA; 40 CFR Part 160) and QA study. Study performed according to US EPA Test Guideline for a Neurotoxicity Screening Battery (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Addendum 10: Neurotoxicity: Series 81-8, March 1991).

Materials and Methods

A single dose of dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 97.87% purity, though assumed to be 100%) was administered orally to non-fasted SD Crl:CD®BR rats (12/sex/group; Charles River Breeding Laboratories Inc, Portage, Michigan, USA) by gavage at 0, 0.5, 35 or 70 mg/kg bw. The vehicle was deionised water and the dose volume was 10 mL/kg bw. Dosing solutions were prepared on the day of administration and analysed by HPLC for homogeneity, stability (at 4, 6 and 8 h) and the concentration of dichlorvos. The dose selection was based on the results of a previous acute range-finding study (WIL study No. WIL-1880022), where 0.5 mg/kg bw did not produce any clinical signs.

Rats were 43-46 days old at the time of dosing and had been acclimatised for at least 11 days. Rats were randomly assigned to each group based on bw, which was 173-250 and 129-178 g for males and females, respectively. On receipt, rats were housed 3/cage/sex for approximately three days and thereafter, individually. Animals were housed under standard conditions, however, relative humidity occasionally exceeded the 70% upper level recommended in OECD test guideline 424 (up to 84%). These exceedances reportedly did not affect the outcome of the study. Purina® Certified Rodent Chow® #5002) and tap water were available *ad libitum*.

Rats were observed twice daily for mortalities and moribundity over 14 days. Clinical signs were recorded daily. Bodyweights were recorded on days -7, 0, 7 and 14. Rats found dead were weighed prior to necropsy. A Functional Observation Battery (FOB) was performed on all rats 7 days prior to dosing and then at 15 min (the approximate time of peak effect), 7 and 14 days. All animals were observed and scored for the standard FOB parameters (see appendix IV). Following the FOB (on day –7, 0, 7 and 14), all rats were assessed for locomotor activity over 40 min using a Digiscan Micro Animal Activity System (Omnitech Electronics Inc, Columbus, Ohio, USA).

Rats found dead were necropsied, which included an examination of the external surfaces, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including the vicera. All surviving animals were sacrificed by CO_2 inhalation on day 14 and then perfused *in situ*. Brain weights and dimensions were recorded. Any gross abnormalities of the brain or spinal cord were recorded, including any abnormal colouration or lesions. The following tissue were histopathologically examined from 5 rats/sex/group in the control and 70 mg/kg bw group: brain (forebrain, centre of cerebrum, midbrain, cerebellum and pons, medulla oblongata), spinal cord (at cervical swellings C3-C8 and at lumbar swellings T13-L4), gasserian ganglion/trigeminal nerves, lumbar dorsal root ganglion T13-L4, lumbar dorsal root fibres at T13-L4, lumbar ventral root fibres at T13-L4, cervical dorsal root ganglion at C3-C8, cervical dorsal root fibres at C3-C8, cervical ventral root fibres at C3-C8, optic nerves, eyes, sciatic nerves (mid-thigh region and at sciatic notch), sural nerves, tibial nerves, peroneal nerves and forelimbs.

Results were analysed using the following statistical tests: one-way ANOVA and Dunnett's t-test (bw, bodyweight changes, brain weights and brain dimensions); Kolmogorov-Smirnov test (histopathological findings); 2-way repeated measures ANOVA, one-way ANOVA, Fisher's Exact test and Dunnett's multiple t-test (FOB and locomotor activity data).

Results

Chemical analysis: Analyses of the homogeneity, stability and concentration of the dosing solutions indicated that they were within 10% of the nominal concentrations.

Mortalities, clinical signs and bodyweight effects: At 70 mg/kg bw, 2 males and 5 females died within 4 h of dosing. There were no other deaths. Necropsy revealed that one of the deceased males probably perished due to an intubation error. There was an apparent dose-related increase in the occurrence of constricted pupils in males, which was not evident in females (see table below). This finding was observed two or more days after dosing and was reported to occur sporadically during the 14-day observation period. In the absence of a consistent effect in males and females, and the sporadic nature of the finding, it was not considered treatment-related. Two high dose females had dried red material around the nose, but this was not considered treatment-related as similar findings occurred

sporadically across all groups in a few animals prior to dosing. There was no treatment-related effect on bw.

Occurrence of constricted pupils in rats given a single oral dose of dichlorvos

Observation	Dose (mg/kg bw)							
	0		0.5		35		70	
	♂	♀	♂	♀	♂	♀	♂	♀
n	12	12	12	12	12	12	10	7
Constricted pupils[1]	1/1	34/10	3/3	28/10	12/5	19/9	10/7	22/7

1 = total occurrence/number of animals

FOB: The pretreatment FOB was unremarkable. Treatment-related effects on FOB findings were restricted to day 0, while there were no effects at day 7 and 14. Significant treatment-related findings at day 0 are summarised in the Table below. The majority of abnormal FOB findings at and above 35 mg/kg bw were different (higher or lower) than historical control values for age, sex and time-matched rats from the performing laboratory.

Home cage observations

At 70 mg/kg bw, there was a significant decrease ($p<0.05$) in rats standing or sitting normally, a significant decrease ($p<0.05$) in females appearing alert and oriented towards the observer, and a significant increase ($p<0.05$) in rats with flattened or extended limbs. The lack of statistical significance in males appearing alert is likely to be attributable to the low incidence of this finding in the control group (25%), which is below the historical control incidence (42%). At 35 mg/kg bw, these FOB parameters were affected to a lesser degree, with only the increase in males with flattened or extended limbs, and the decrease in females appearing alert, significantly different ($p<0.05$) to the control. There was a significant dose-related increase ($p<0.05$) in rats exhibiting clonic convulsions at and above 35 mg/kg bw, manifesting as whole body tremors. There also appeared to be a dose-related increase in the severity of tremors, with a significant increase ($p<0.05$) in slight-moderately coarse tremors at 35 mg/kg bw, and a significant increase ($p<0.05$) in markedly coarse tremors at 70 mg/kg bw.

FOB findings (%) in rats (day 0) given a single oral dose of dichlorvos

Observation	Dose (mg/kg bw)							
	0		0.5		35		70	
	♂ (n=12)	♀ (n=12)	♂ (n=12)	♀ (n=12)	♂ (n=12)	♀ (n=12)	♂ (n=10)	♀ (n=7)
HOME CAGE OBSERVATIONS								
Posture								
Sitting or standing normally	58	50	50	33	17	33	0*	0*
Alert, oriented towards observer	25	50	50	67	0	0*	0	0*
Flattened, extended limbs	0	0	0	0	50*	33	80*	100*
Convulsions - clonic								
Absent	100	100	100	100	17*	17*	10*	0*
Whole body tremors	0	0	0	0	75*	75*	90*	100*
Tremors								
None	100	100	100	100	17*	17*	10*	0*
Slight[1]	0	0	0	0	50*	33	10	0

Observation	Dose (mg/kg bw)							
	0		0.5		35		70	
	♂ (n=12)	♀ (n=12)	♂ (n=12)	♀ (n=12)	♂ (n=12)	♀ (n=12)	♂ (n=10)	♀ (n=7)
Moderately coarse[2]	0	0	0	0	25	42*	20	13
Markedly coarse[3]	0	0	0	0	8	8	60*	75*
Extremely coarse[4]	0	0	0	0	0	0	0	13
HANDLING OBSERVATIONS								
Salivation								
None	100	100	100	100	50*	50*	20*	0*
Slight	0	0	0	0	8	33	10	29
Severe	0	0	0	0	42*	17	70*	71*
Fur appearance								
Normal	100	100	100	100	50*	50*	30*	0*
Slightly soiled	0	0	0	0	50*	50*	70*	100*
Respiratory character								
Normal	100	100	100	100	92	100	70	71
Rales	0	0	0	0	8	0	20	14
Gasping	0	0	0	0	0	0	10	14
Skin								
Pink	100	100	100	100	92	92	80	71
Pale	0	0	0	0	8	8	20	29
Eye prominence								
Normal	75	67	92	67	58	50	10*	14
Exophthalmus	25	33	8	33	42	50	90*	86
Muscle tone								
Normal	100	92	100	100	58*	67	70	86
Soft and flabby	0	8	0	0	42*	33	30	14
OPEN FIELD OBSERVATIONS								
Time to 1st step (s)[5]	0.5±0.16	0.5±0.1	0.6±0.33	0.5±0.1	0.9±0.5	0.9±0.6	31.7± 50.27**	18.3± 39.96
Mobility								
Normal	100	100	100	100	17*	25*	10*	0*
Slightly impaired	0	0	0	0	17	8	0	0
Moderately impaired	0	0	0	0	66*	67*	10	43*
Totally impaired	0	0	0	0	0	0	80*	57*
Gait								
Normal	100	100	100	100	17*	17*	10*	0*
Hindlimbs splayed or dragging, unable to support weight	0	0	0	0	0	0	80*	57*
Ataxia, excessive sway	0	0	0	0	83*	83*	10	43*
Clonic convulsions								
Absent	100	100	100	100	17*	17*	10*	0*
Whole body tremors	0	0	0	0	83*	83*	90*	100*
Clonic convulsions	0	0	0	0	0	0	10	14
Tremors								
None	100	100	100	100	17*	17*	10*	0*
Slight[1]	0	0	0	0	33	33	10	0
Moderately	0	0	0	0	50*	42*	0	0

Observation	Dose (mg/kg bw)							
	0		0.5		35		70	
	♂ (n=12)	♀ (n=12)	♂ (n=12)	♀ (n=12)	♂ (n=12)	♀ (n=12)	♂ (n=10)	♀ (n=7)
coarse[2]								
Markedly coarse[3]	0	0	0	0	0	8	10	43*
Extremely coarse[4]	0	0	0	0	0	0	70*	*57
Gait score								
Normal	100	100	100	100	17*	17*	10*	0*
Slight impairment	0	0	0	0	17	17	10	0
Considerable impairment[6]	0	0	0	0	58*	50*	0	0
Marked impairment[7]	0	0	0	0	8	17	0	29
Severe impairment[8]	0	0	0	0	0	0	*80	71*
Arousal								
Low, somewhat stuperous	0	0	0	0	67*	67*	80*	100*
Normal	83	100	100	100	33.3*	25*	20*	0*
Rearing	8.8±2.66	13.8± 9.41	9.1±3.12	13.5± 3.87	1.5± 1.73**	1.3± 2.63**	1.6± 3.5**	0**
SENSORY OBSERVATIONS								
Approach response								
No reaction	0	0	0	0	8	0	30	57*
Slow approach	100	100	100	100	92	100	70	43*
Touch response								
No reaction	0	0	0	0	33	33	40*	71*
Rat may slowly turn and walk away	100	92	100	100	67	67	60*	29*
Tail pinch response								
No reaction	0	0	0	0	58*	42*	90*	86*
Rat may slowly turn and walk away	42	42	8	33	25	25	10	0
More energetic response, with or without vocalisation	58	50	92	67	17	33	0*	14
Olfactory orientation								
No reaction	0	0	0	0	0	0	30	29
Reaction present	100	100	100	100	100	100	70	31
Pupil response								
No pupil response	0	8	0	08	42*	42	80*	100*
Pupil response	100	92	100	92	58*	58	20*	0*
Air righting reflex								
Normal	100	100	100	100	75	75	30*	14*
Slightly uncoordinated	0	0	0	0	17	25	30	14
Lands on side	0	0	0	0	8	0	30	57*
Lands on back	0	0	0	0	0	0	10	14
NEUROMUSCULAR OBSERVATIONS								
Hindlimb extensor strength								
	Hindlimb	0	0	0	8	17	0	43*

Observation	Dose (mg/kg bw)							
	0		0.5		35		70	
	♂ (n=12)	♀ (n=12)	♂ (n=12)	♀ (n=12)	♂ (n=12)	♀ (n=12)	♂ (n=10)	♀ (n=7)
	resistance absent							
Reduced hindlimb resistance	0	0	0	8	33	25	70*	43*
Hindlimb resistance present	100	100	100	92	58*	28*	30*	14*
Grip Strength (g)								
Forelimb[5]	495±76	421±100	517±53	452±59	444±65	399±101	343±110**	271±99**
Hindlimb[5]	369±76	331±69	331±51	311±67	356±65	297±90	267±98**	202±81**
Rotarod performance (s)[5]	98±40	111±33	94±44	85±51	25±12	35±31	15±31**	5±4**
PHYSIOLOGICAL OBSERVATIONS								
Catalepsy (s)[5]	0.7±0.41	0.9±1.45	0.8±0.61	0.4±0.09	2.7±2.9	3.4±3.62	26.9±28.85**	10.3±12.3**
Body temp (°C)[5]	38.8±0.31	39.4±0.35	38.4±0.39	39.4±0.45	36.5±0.96**	36.6±1.25**	35.7±1.06**	35.2±0.37**
LOCOMOTOR ACTIVITY (counts)								
Total activity[5]	1448±463	1842±733	1475±335	1680±582	654±181*	767±240*	725±289*	836±213*
Ambulatory activity[5]	759±270	1017±369	819±214	864±298	317±80*	379±142*	393±163*	460±218*

1 = 1.5 mm; 2 = 3mm, slight impairment of locomotion; 3 = 4.5 mm, moderate/marked impairment of locomotion; 4 = 6 mm, locomotion impossible; 5 = mean ± 1 SD; 6 = without falling; 7 = fall every 4-6 steps, 8 = cannot walk without falling; * $p<0.05$, Fisher's exact test; ** $p<0.01$, Dunnett's t-test

Handling observations

There was a significant dose-related increase ($p<0.05$) in salivation and the occurrence of slightly soiled fur at and above 35 mg/kg bw, with the severity of salivation increasing with dose (see Table above). There were a number of other findings at 35 and/or 70 mg/kg bw that were not all statistically significant but appeared to be related to treatment. These included respiratory abnormalities (rales, gasping), pale skin, exophthalmus ($p<0.05$ at 70 mg/kg bw in males) and decreased muscle tone ($p<0.05$ at 35 mg/kg bw in males).

Open field observations

The time to first step was increased by approximately 30-60-fold at the highest dose, with the effect on males significantly different to the controls ($p<0.01$). Mobility was significantly impaired at and above 35 mg/kg bw, with the severity of impairment increasing with dose (moderate impairment at 35 mg/kg bw, total impairment at 70 mg/kg bw). Abnormal gait was observed at and above 35 mg/kg bw, with a significant increase ($p<0.05$) in ataxia predominating at 35 mg/kg bw, while splayed hindlimbs or dragging, and rats unable to support their bw, observed at 70 mg/kg bw. Gait scores confirmed the significant dose-related increase in abnormal gait at and above 35 mg/kg bw, with severe impairment observed at 70 mg/kg bw. Previous homecage observations of whole body tremors and tremors were confirmed during open field observations, with the severity of tremors clearly increasing with dose. There was a significant ($p<0.05$) dose-related increase in stupor at and above 35 mg/kg bw. Rearing was significantly reduced ($p<0.05$-0.01) at and above 35 mg/kg bw, with no rearing observed in females at 70 mg/kg bw.

Sensory observations

The approach response of high-dose females was significantly lower ($p<0.05$) than the control. Touch response was decreased at and above 35 mg/kg bw, with the effect statistically significant at 70 mg/kg bw. There was a significant ($p<0.05$) dose-related decrease in tail pinch response at and above 35 mg/kg bw. Pupil response was significantly reduced ($p<0.05$) at and above 35 mg/kg bw in males and at 70 mg/kg bw in females. There was a significant loss ($p<0.05$) of air righting reflex at 70 mg/kg bw. Olfactory response was also reduced at 70 mg/kg bw, but this finding was not statistically significant.

Neuromuscular observations

There was a significant dose-related reduction ($p<0.05$) in hindlimb extensor strength at and above 35 mg/kg bw. Forelimb and hindlimb grip strength, and rotarod performance were significantly reduced at 70 mg/kg bw ($p<0.01$).

Physiological observations

Catalepsy was significantly increased ($p<0.01$) at 70 mg/kg bw (~10-fold). There was a significant dose-related reduction ($p<0.01$) in body temperature at and above 35 mg/kg bw.

Locomotor activity

Over a 40 min observation period, total and ambulatory activities were significantly reduced ($p<0.05$) at and above 35 mg/kg bw. While this activity was lower than the control over the entire 40 min period, statistically significant effects predominated over the first and second 10 min subsessions.

Necropsy: No treatment-related macroscopic abnormalities were detected in any rats sacrificed on day 14. One male and one female high-dose rat that died on day 0 were found to have a reddened cortico-medullary junction in each kidney. The 4 other high-dose females that died were unremarkable, while the other male had red foamy contents in the lungs and trachea due probably to intubation trauma. There was no treatment-related effect on brain weight, length or width.

Histopathology: There were no treatment-related histopathological abnormalities.

Conclusions: The NOEL in rats following a single oral gavage dose of dichlorvos was 0.5 mg/kg bw, based on clinical signs of neurotoxicity (FOB) at and above the next highest dose of 35 mg/kg bw. These signs were transient, only occurring within 15 min of dosing. There was no evidence that dichlorvos caused delayed neurotoxicity.

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GLP compliant (US EPA; 40 CFR Part 160, 16th October 1989) and QA study. Study performed according to US EPA Test Guideline for a Neurotoxicity Screening Battery (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Addendum 10: Neurotoxicity: Series 82-7, October 1982 & March 1991).

Materials and Methods

Dichlorvos (AMVAC Chemical Corporation, Los Angeles, CA, USA; lot No. 802097; 97.87% purity, though assumed to be 100%) was administered orally to non-fasted SD Crl:CD®BR rats (15/sex/group; Charles River Breeding Laboratories Inc, Portage, Michigan, USA) by gavage at 0, 0.1, 7.5 or 15 mg/kg bw/d for 13 weeks. The vehicle was deionised water and the dose volume was 10 mL/kg bw. The dose selection was based on the results of a previous 90-day oral toxicity study in rats (unspecified study No.), where the NOEL was 0.1 mg/kg bw/d based on the occurrence of cholinergic signs and inhibition of plasma, RBC and brain ChE activities at 15 mg/kg bw/d. Ten and 5 rats/sex/group were designated for ChE and neuropathological evaluation, respectively. Dosing solutions were prepared weekly and stored frozen and protected from light. Analysis of the homogeneity and stability (over 10 days of refrigeration or when stored frozen) in the vehicle was performed prior to study initiation. The concentrations of dichlorvos in the dosing solutions were analysed at weeks 0, 1, 2, 3, 7 and 11 by HPLC.

Rats were 43-days old at study initiation and had been acclimatised for 19 days. Rats were randomly assigned to each group based on bw, which was 158-240 and 122-177 g for males and females, respectively. On receipt, rats were housed 3/cage/sex for approximately 3 days and thereafter, individually. Animals were housed under standard conditions. Purina® Certified Rodent Chow® #5002 and tap water were available *ad libitum*.

Rats were observed twice daily for mortalities and moribundity. Clinical signs were recorded daily, except days when the FOB was performed. Rats were also observed at the time of peak effect, which was approximately 15 min after dosing (determined in studies WIL-188002 and WIL-188003). Individual bodyweights and food consumption were recorded weekly, beginning one-week prior to the initiation of dosing.

FOB was performed on 10 rats/sex/group (5 each from the ChE and neuropathological evaluation groups) pretreatment and then at week 4, 8 and 13 of dosing. These rats were scored for the standard FOB parameters (see appendix IV). Following the FOB, the same 10 rats/sex/group were assessed for locomotor activity over 40 min using a Digiscan Micro Animal Activity System (Omnitech Electronics Inc, Columbus, Ohio, USA).

Rats found dead or sacrificed in a moribund condition were subjected to a *post mortem* examination covering the external surfaces, all orifices and the cranial, thoracic, abdominal and pelvic cavities, including the vicera. Tissues and organs (unspecified) were retained for possible future histopathological examination.

Plasma and RBC ChE activities were measured in the 10 rats/sex/group designated for ChE analysis, pretreatment (unspecified time) and then during weeks 4, 8 and at study termination. At study termination, these same rats were sacrificed by CO_2 anaesthesia, exsanguinated and their brain weights recorded. The following brain regions were weighed and analysed for ChE activity: olfactory region, cerebellum, hippocampus, cerebral cortex, brain stem and midbrain. The 5 rats/sex/group designated for neurohistopathological examination were similarly sacrificed, and the following tissues microscopically examined in the control and 15 mg/kg bw groups: brain (forebrain, centre of cerebrum, midbrain, cerebellum and pons, medulla oblongata), spinal cord (at cervical swellings C3-C8 and at lumbar swellings T13-L4), gasserian ganglion/trigeminal nerves, lumbar dorsal root ganglion T13-L4, lumbar dorsal root fibres at T13-L4, lumbar ventral root fibres at T13-L4, cervical dorsal root ganglion at C3-C8, cervical dorsal root fibres at C3-C8, cervical ventral root fibres at C3-C8, optic nerves, eyes, sciatic nerves (mid-thigh region and at sciatic notch), sural nerves, tibial nerves, peroneal nerves and forelimbs.

Results were analysed using the following statistical tests: one-way ANOVA and Dunnett's t-test (bw, bodyweight changes, brain weights and brain dimensions, food consumption and ChE activity); Kolmogorov-Smirnov test (histopathological findings); 2-way repeated measures ANOVA, one-way ANOVA, Fisher's Exact test and Dunnett's multiple t-test (FOB and locomotor activity data).

Results

Analytical chemistry: The test material was inadvertently stored incorrectly at room temperature for approximately 7 weeks before it was transferred to a desiccator and refrigerated. A second test sample was subsequently received and stored properly.

The 3 dosing solutions were homogenous as concentrations were within 10% of the nominal values at the top, middle and bottom of each solution. For the low-dose solution, a loss of approximately 20-25% of the nominal concentration was seen when stored refrigerated or frozen for 10 days. A subsequent test found that storing the low-dose solution frozen for 7 days did not lead to an unacceptable loss of concentration.

Periodic analysis revealed that the concentrations of dichlorvos in the dosing solutions were within 10% of the nominal concentrations.

Mortalities and clinical signs: One male in the 0.1 mg/kg bw/d group was sacrificed in a moribund condition at week 12 apparently due to mechanical trauma occurring during the FOB. All remaining rats survived until study termination. Treatment-related clinical signs occurred at and above 7.5 mg/kg bw/d, but predominated at 15 mg/kg bw/d and included cholinergic signs (tremors, salivation and lacrimation), the presence of a wet clear material on the forelimbs, rales, exophthalmus and chromodacryorrhea (see Table below). A few females (< 4/15) had a wet red, orange or yellow material, and a dried red material, around the mouth at 15 mg/kg bw/d. The occurrence of salivation in a few animals at 0.1 mg/kg bw/d was not considered to be toxicologically significant as the incidence was the same or only slightly higher than the control group. Clinical signs were reported to occur at 15 min postdose (the time of peak effect) throughout the 13-week dosing period. Females appeared to be marginally more sensitive than males to dichlorvos treatment in terms of clinical signs.

% Incidence of clinical signs in rats 15 min after oral dosing with dichlorvos (n=15)

OBSERVATION	Dose (mg/kg bw/d)							
	0		0.1		7.5		15	
	♂	♀	♂	♀	♂	♀	♂	♀
CNS effects								
Whole body tremors	0	0	0	0	13	27	100	100
Repetitive movement of mouth & jaws	0	0	0	0	13	20	87	100
Tremors - hindlimb	0	0	0	0	0	47	93	100
Tremors - forelimb	0	0	0	0	0	47	100	100
Eartwitch	0	0	0	0	0	13	73	93
Body/integument								
Wet clear material on forelimbs	0	0	0	0	0	0	47	80
Cardio-pulmonary								
Rales	0	0	0	0	0	0	20	47
Eyes/ears/nose								
Exophthalmus (both eyes)	0	0	0	0	0	20	67	100
Lacrimation (both eyes)	0	0	0	0	0	0	47	87
Chromodacryorrhea (both eyes)	0	0	0	0	0	0	7	~30
Oral/dental								
Salivation	7	0	7	13	13	20	100	100

Bodyweight effects and food consumption: There was no treatment-related effect on bodyweight in males. The mean bodyweight of females in the 15 mg/kg bw/d group was marginally lower the control group for most of the dosing period, with the difference increasing over the later stages of the study. However, the mean bodyweight ($\pm$1 SD) of the 15 mg/kg bw/d group was significantly lower ($p<0.05$) than the control only at week 13 (258$\pm$19.6 *versus* 282$\pm$33.6, respectively). An examination of bodyweight changes in females from the 15 mg/kg bw/d group revealed significantly reduced bodyweight gain ($p<0.05$) compared to the control group over weeks 0-2, 0-5, 0-7 and 0-9, and more significant differences ($p<0.01$) over weeks 0-10, 0-11, 0-12 and 0-13.

There was no treatment-related effect on food consumption.

FOB and locomotor activity: Cage side, handling, open field, sensory, neuromuscular and physiological observations at weeks 3, 7 and 12 revealed no treatment-related effects. There was no treatment-related effect on motor activity.

ChE activity: Results of the analysis of ChE activity are summarised in the Table below. Toxicologically significant (ie. >20%) inhibition of plasma ChE activity occurred at and above 7.5 mg/kg bw/d in both sexes at weeks 3, 7 and 13, but did not follow an obvious dose-response relationship. The majority of these findings were significantly lower than the control group ($p<0.01$-0.05). In males, toxicologically significant inhibition of RBC ChE activity occurred at and above 7.5 mg/kg bw/d only during week 3, reaching statistical significance at 15 mg/kg bw/d ($p<0.05$). In females, toxicologically significant inhibition of RBC ChE activity occurred at and above 7.5 mg/kg bw/d during weeks 7 and 13, however, no results were statistically significant due possibly to the high variability within the groups (particularly the control group). Significant inhibition ($p<0.01$-0.05) of ChE activity occurred in the brain cortex of both sexes at and above 7.5 mg/kg bw/d, but as the level of inhibition was well below 20% compared to the control, these findings were not considered to be toxicologically significant. ChE activity in the brainstem was also significantly reduced in males at 15 mg/kg bw/d ($p<0.05$), but the magnitude of this effect was small and also not considered to be toxicologically significant.

ChE activity in dichlorvos-treated rats

ChE	Dose (mg/kg bw/d)							
	0		0.1		7.5		15	
	♂	♀	♂	♀	♂	♀	♂	♀
Plasma								
Week 3	388±80 (0%)	1268±247 (0)	422±130 (+9%)	1753±717 (+38%)*	246±24 (37%)**	704±103 (44%)**	257±46 (34%)**	722±168 (43%)**
Week 7	377±89 (0%)	1721±368 (0)	435±163 (+15%)	2292±852 (+33%)	264±58 (30%)	778±155 (55%)**	244±63 (35%)	844±304 (51%)**
Week 13	418±110 (0%)	2025±367 (0)	409±193 (+2%)	2829±1057 (+40%)	241±24 (42%)	884±161 (56%)**	212±37 (49%)	855±561 (58%)**
RBC								
Week 3	658±113 (0%)	718±287 (0%)	563±162 (14%)	836±144 (+16%)	508±251 (23%)	628±215 (14%)	426±188 (35%)*	692±286 (4%)
Week 7	608±217 (0%)	750±510 (0%)	643±141 (+6%)	602+374 (20%)	536±224 (12%)	462±151 (38%)	558±131 (8%)	437±207 (42%)
Week 13	610±139 (0%)	696±235 (0%)	583±226 (+4%)	712±400 (2%)	499±159 (18%)	469±273 (32%)	543±203 (11%)	455±240 (35%)
Brain								
Brainstem	13220± 1865 (0%)	14249± 2180 (0%)	13757± 1326 (+4%)	14355± 1216 (0%)	11693± 1802 (12%)	12496± 1668 (12%)	11073± 1512 (16%)*	12833± 1651 (10%)
Cortex	3410± 372 (0%)	3483± 326 (0%)	3384± 441 (0%)	3699± 179 (0%)	2991± 208 (12%)*	3086± 288 (11%)**	2905± 242 (15%)**	3131± 190 (10%)*

Results expressed as International Unit/L ± 1 SD (% inhibition relative to the control); * $p<0.05$; ** $p<0.01$

Necropsy: There were no treatment-related macroscopic abnormalities. The absolute weight of different brain regions in dichlorvos-treated rats were not significantly different to the control group. Relative brain region weights were unaffected in males, while the relative weight of the olfactory lobes in females was significant higher ($p<0.05$) than the control at 15 mg/kg bw/d (0.049±0.0045 *versus* 0.042±0.0058 g/100g, respectively). This result was attributable to the lower terminal bodyweight of this group compared to the control (256±22 versus 288±40 g, respectively). There was no treatment-related effect on brain dimensions.

Neurohistopathology: There were no treatment-related neurohistopathological effects.

Conclusions: The NOEL in rats following 13 weeks of daily oral dosing was 0.1 mg/kg bw/d, based on the occurrence of cholinergic signs and inhibition of plasma ChE activity at and above 7.5 mg/kg bw/d. Statistically significant inhibition of RBC and brain ChE activities also occurred at and above 7.5 mg/kg bw/d but was below 20% of the control group. At 15 mg/kg bw/d, females had a lower bodyweight gain than the control. There was no evidence that dichlorvos caused delayed neuropathy

11. HUMAN STUDIES

11.1 Oral Administration

Rider JA (1967) Determination of the minimal incipient toxicity of dichlorvos in humans. Report and Study no. unspecified. Lab: Gastrointestinal Research Laboratory, Franklin Hospital, San Francisco, CA, USA. Sponsor: Shell Chemical Company, Agricultural Chemicals Division, New York, New York, USA. Report date: October 1967.

Materials and Methods

Dichlorvos (unspecified source, Batch/Lot No. & purity), formulated in corn oil, was administered to "healthy young men" in gelatine capsules at 1.0, 1.5, 2.0 or 2.5 mg/d for 28 days (equivalent to 0.014, 0.021, 0.029 and 0.036, respectively, assuming an average bodyweight of 70 kg). The doses were split between two capsules, which were ingested at 8 am and 3 pm. Few details were given regarding the subjects (such as age and bodyweight) other than that they were serving sentences at the Californian Medical Facility, Vacaville, California, USA. Each subject was physically examined and interviewed prior to the commencement of dosing. At each dose, 4 or 5 subjects received the capsules containing dichlorvos, while 2 control subjects received capsules containing only corn oil. An additional group of subjects received 0 (n=2) or 1.5 mg/d (n=10) dichlorvos over 60 days followed by a recovery period of 74 days. Analysis of the concentration and stability of dichlorvos was not performed.

All subjects were interviewed on a weekly basis and any symptoms recorded. Baseline plasma and RBC ChE activities were analysed over a 14-28 day period prior to dosing using the potentiometric method of Michel (1949), which measures the ΔpH/h. Plasma and RBC ChE activities were analysed 24 hours after the first dose and then twice weekly throughout the study. In the additional group of subjects dosed with 1.5 mg/d, plasma and RBC ChE activities were measured during the 74-day recovery period. The following clinical chemistry parameters were measured prior to treament and on a weekly basis for 4 weeks: SGOT, alkaline phosphatase, prothrombin time, thymol turbidity and total bilirubin. The following haematology parameters were measured prior to treatment and on a weekly basis for 4 weeks of dosing: Hct, Hb, RBC, WBC and WBC-DC. The following urinary parameters were measured prior to treament and on a weekly basis for 4 weeks of dosing: pH, colour, character, specific gravity, albumin, sugar, leucocytes, erythrocytes, epithelial cells, casts, crystals, amorphous and other.

No statistical analysis was performed.

Results

There were no treatment-related symptoms. There was no treatment-related effect on any haematology, clinical chemistry or urinary parameter. RBC ChE activity was unaffected by treatment.

It was reported that there was no effect on plasma ChE activity at 1 or 1.5 mg/d following 4 weeks of dosing, however, no data were provided to substantiate this finding. At 2.0 mg/d, mean plasma ChE activity was depressed by $\geq$20% relative to pretreatment activity from the second week of administration, reaching a maximum of 29% 3 days after treatment ended (see Table below). However, the concurrent control group also showed a reduction in plasma ChE activity (up to 12%) relative to its mean baseline activity. When the data were corrected to account for this reduction in activity in the controls, the level of inhibition in the dichlorvos group was approximately 20% from the second week of dosing (maximum of 25% at day 19). This was considered a toxicologically significant level of inhibition despite the absence of statistical analysis to support the finding. Three days after the cessation of dosing, plasma ChE activity remained depressed by 22%.

Plasma ChE activity in male subjects treated with 2.0 mg/d dichlorvos

Time	Control (n=2)	2.0 mg/d (n=4)	Corrected % inhibition[1]
Baseline activity	0.827[2]	0.813[2]	-
Day 1	0.795 (4%)	0.8975 (0.8-1.03) (-10%)	6
Day 5	0.7385 (11%)	0.716 (0.667-0.760) (13%)	2
Day 9	0.7335 (11%)	0.624 (0.596-0.687) (24%)	13
Day 12	0.7285 (12%)	0.59 (0.49-0.677) (28%)	16
Day 15	0.815 (2%)	0.634 (0.596-0.677) (23%)	21
Day 19	0.840 (-2%)	0.627 (0.556-0.76) (23%)	25
Day 22	0.7485 (10%)	0.599 (0.556-0.656) (25%)	15
Day 26	0.815 (2%)	0.632 (0.576-0.760) (23%)	21
Day 3 post-treatment	0.7685 (7%)	0.582 (0.51-0.677) (29%)	22

Results expressed as the mean ΔpH/h, with the % inhibition relative to pretreatment activity contained in parentheses. The range is shown for the dichlorvos group in parentheses; 1 = % inhibition in the control group subtracted from the % inhibition of the dichlorvos group; 2 = average of 4 measurements for each subject

At 2.5 mg/d (see Table below), toxicologically-significant inhibition of plasma ChE activity occurred after 2 weeks of dosing (ie. >20% relative to pretreatment activity). In fact, treatment was stopped after 20 days because the level of inhibition had reached 30%. Following a 15-day washout period, plasma ChE activity returned to pretreatment levels.

Plasma ChE activity in male subjects treated with 2.5 mg/d dichlorvos

Time	Control (n=2)	2.5 mg/d (n=5)
Baseline activity	0.886[1]	0.645[1]
Day 1	0.925 (-6)	0.786 (0.656-1.02) **(-10%)**
Day 5	0.0.98 (-11%)	0.710 (0.52-1.07) **(11%)**
Day 8	0.945 (-7%)	0.459 (0.596-0.960) **(13%)**
Day 12	0.98 (-11%)	0.601 (0.449-0.91) **(16%)**
Day 15	0.915 (-3%)	0.473 (0.347-0.646) **(27%)**
Day 19	0.955 (-2%)	0.4525 (0.377-0.586) **(30%)**
Day 3 post-treatment	0.870 (2)%	0.468 (0.398-0.566) **(27%)**
Day 12 post-treatment	0.910 (-3%)	0.636 (0.576-0.737) **(1%)**
Day 16 post-treatment	0.995 (-12%)	0.621 (0.51-0.677) **(4%)**

Results expressed as the mean ΔpH/h, with the % inhibition relative to pretreatment activity contained in parentheses. The range is also shown for the dichlorvos group in parentheses; 1 = average of 4 measurements for each subject

In the supplementary group of subjects treated with 1.5 mg/g dichlorvos (see Table below), progressive inhibition of plasma ChE activity occurred from the second week, reaching a maximum of 41% at the end of the 60-day treatment period (relative to pretreatment activity). However, the magnitude of this effect was partially negated by the up to 24% reduction in plasma ChE activity in the concurrent controls over the same time. When the data were corrected to account for the declining activity in the control group, inhibition of plasma ChE activity in the dichlorvos subjects was 27% after 16 days and continued to be depressed by 13-21% for the remainder of the dosing period. During the 74-day post-treatment period, plasma ChE activity recovered to control levels within 2 weeks. The author indicated that over a period of approximately 3 weeks (corresponding to post-treatment days

31-48), there was a general decrease in plasma ChE activity in the performing laboratory. This was apparently remedied when a fresh acid cleaning solution was employed to clean the laboratory glassware used for the ChE assay.

Plasma ChE activity in additional male subjects treated with 1.5 mg/d dichlorvos

Time[1]	Control (n=2)	1.5 mg/d (n=10)	Corrected % inhibition[2]
Baseline activity	715	0.915	-
Day 1	0.6235 (23%)	0.852 (0.596-1.18) (7%)	-16
Day 8	0.6775 (5%)	0.775 (0.566-1.04) (15%)	10
Day 15	0.850 (0%)[3]	0.674 (0.459-0.920) (27%)	27
Day 22	0.685 (4%)	0.720 (0.51-0.98) (22%)	18
Day 29	0.665 (7%)	0.687 (0.418-0.88) (26%)	19
Day 35	0.618 (13%)	0.682 (0.469-0.93) (26%)	13
Day 42	0.6795 (5%)	0.676 (0.418-0.83) (26%)	21
Day 50	0.63 (12%)	0.622 (0.439-0.81) (33%)	21
Day 59	0.5475 (24%)	0.547 (0.377-0.750) (41%)	17
Day 4 post-treatment	0.558 (22%)	0.594 (0.408-0.78) (35%)	13
Day 10 post treatment	0.6045 (15%)	0.711 (0.5-0.97) (23%)	8
Day 17 post-treatment	0.568 (21%)	0.77 (0.586-1) (16%)	-5
Day 24 post-treatment	0.6145 (14%)	0.772 (0.556-1.01) (16%)	2
Day 34 post-treatment	0.4715 (34%)	0.626 (0.469-0.8) (32%)	-2
Day 41 post-treatment	0.5675 (21%)	0.7125 (0.53-0.88) (24%)	3
Day 48 post treatment	0.4915 (31%)	0.675 (0.459-0.87) (28%)	-3
Day 74 post-treatment	0.588 (18%)	0.747 (0.52-0.89) (21%)	3

Results expressed as the mean ΔpH/h, with the % inhibition relative to pretreatment activity contained in parentheses. The range is shown for the dichlorvos group in parentheses; 1 = only results for every second time point are shown; 2 = % inhibition in the control group subtracted from the % inhibition of the dichlorvos group; 3 = data for one control subject only compared to his own baseline activity of 0.851.

Conclusions: The NOEL was 1.0 mg/d, based on toxicologically-significant inhibition of plasma ChE activity at and above 1.5 mg/d. In the absence of bodyweight data for the test subjects it is assumed that the average bodyweight for young healthy men is 70 kg. Therefore the NOEL, using this figure, is 0.014 mg/kg bw/d.

Gledhill AJ (1996) A study to investigate the effect of a single oral dose of dichlorvos on erythrocyte cholinesterase inhibition in healthy male volunteers. CTL Report No. CTL/P/5393. CTL Study No. XH6064. Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK and Medeval Limited, University of Manchester, Manchester Science Park, Manchester, UK. Sponsor: AMVAC Chemical Corporation, unspecified location. Study duration: 5th November 1996 to 19th November 1996. Report date: 25th March 1997.

Gledhill AJ (1997a) First supplement to dichlorvos: A study to investigate the effect of a single oral dose of dichlorvos on erythrocyte cholinesterase inhibition in healthy male volunteers. CTL Report No. CTL/P/5393. CTL Study No. XH6064. Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK and Medeval Limited, University of Manchester, Manchester Science Park, Manchester, UK. Sponsor: AMVAC Chemical Corporation, unspecified location. Study duration: 5th November 1996 to 19th November 1996. Report date: 8th October 1997.

Morris T (1996a) A study to investigate the effect of a single oral dose of dichlorvos on erythrocyte cholinesterase inhibition in healthy male volunteers (STUDY PROTOCOL) CTL Study No. XH6064. Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK and Medeval Limited, University of Manchester, Manchester Science Park, Manchester, UK. Sponsor: AMVAC Chemical Corporation, unspecified location. Study duration: 5th November 1996 to an unspecified time. Report date: unspecified.

GLP compliant [UK GLP Compliance Programme, Dept Health 1989; OECD Principles of GLP (1982) ISBN 92-64-12367-9 (OECD Environment Monograph No. 45, 1992); EC Council Directive 87/18 EEC and 88/320/EEC; US EPA (40 CFR Part 160)]. This study was quality assured and conducted according to the Declaration of Helsinki (1964) including all amendments up to and including the Hong Kong revision (1989). The study protocol was approved by the Medeval Independent Ethics Committee.

Materials and Methods

A single 70 mg dose of dichlorvos (AMVAC Chemical Corporation, unspecified location; lot No. 6080028074; 98%) formulated in corn oil, was administered orally to six healthy male volunteers in gelatine capsules. This dose was equivalent to approximately 1 mg/kg bw for a 70 kg male. No control group was used. The dose selection was based on a previous published study (Slomka & Hine 1981), where the NOEL for plasma and RBC ChE inhibition following a single oral dose formulated in PVC was 245 mg (3.5 mg/kg bw). Furthermore, a recently completed unpublished study by Medeval reportedly found that a single 35 mg dose in corn oil did not inhibit plasma or RBC ChE activities. Capsules were taken with 150 mL distilled water. Dose formulations and capsules were analysed for dichlorvos by GC. The stability of dichlorvos had reportedly been established in a previous study (unspecified).

Subjects were sourced from a volunteer panel held by the performing laboratory. All volunteers were Caucasian, 20-30 years of age, 172-190 cm in height and weighed 67-80 kg. Volunteers were subjected to a thorough medical examination prior to predose measurements of RBC ChE activity, and at the end of the study (day 14). These examinations included an analysis of the following: standard haematology and clinical chemistry parameters; hepatitis B and HIV status; RBC ChE activity; heart rate, blood pressure and ECG; and urinalysis including the analysis for illicit drugs.

The baseline RBC ChE activity for each subject was determined 3 times/week for 3 weeks prior to dosing. Subjects were fasted from midnight prior to dosing. Blood samples were collected immediately prior to dosing then on days 1, 3, 5 or 6, 7 and 14, for measurement of RBC ChE activity. Urine was collected 24 hours before and then at 12 hours, 1, 2, 3, 4 and 5 or 6 days postdose. Body temperatures were recorded predose and at 2, 4, 8, 12 and 24 hours postdose. Symptoms reported by subjects were recorded. Prestudy and test parameters were statistically analysed using a paired t-test (all data) or a permutation test (ChE data).

Results

The concentration of dichlorvos in the dosing formulation was 105% of the nominal concentration, while the weight of dichlorvos contained in the capsules was approximately 98% of the nominal content.

No treatment-related symptoms were reported by the subjects and there was no effect on body temperature. Results of the analysis of RBC ChE activity are summarised in the Table below. Mean RBC ChE activity was significantly lower ($p<0.01$) than mean pretreatment activity at days 5 or 6, 7 and 14, however, the level of inhibition was only 10-12% and therefore not considered toxicologically significant. Subjects 1, 2, 3, 4 and 6 had significantly lower RBC ChE activity ($p<0.01$-0.05) on single occasions at 3, 7 or 14 days postdose, with the level of inhibition ranging from 9-18% of pretreatment activity. Given the delay in the occurrence of these significant findings relative to the time of dosing, and their apparent random occurrence, they were not considered treatment-related.

RBC ChE activity (IU) in male volunteers following a single oral dose of dichlorvos

Time (postdose)	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Mean
Predose	23214	18754	19048	17393	16584	16361	18559
Day 1	21020 (9%)	16280 (13%)	18550 (3%)	16740 (4%)	17180 (-4%)	14970 (9%)	17457 (6%)
Day 3	22170 (4%)	17400 (7%)	18550 (3%)	17570 (-1%)	16840 (-2%)	14830 (9%)*	17893 (4%)
Day 5 or 6	20910 (10%)	16120 (14%)	17300 (9%)	15320 (12%)	15530 (6%)	15460 (6%)	16773** (10%)
Day 7	19830* (15%)	15940 (15%)	16620** (13%)	15440 (11%)	15440 (7%)	15050 (8%)	16386** (12%)
Day 14	20800 (10%)	15440* (18%)	17390 (9%)	14940* (14%)	15580 (6%)	15070 (8%)	16537** (11%)

Results expressed in international units (IU) with the % inhibition of mean baseline activity in parentheses; *$p<0.05$; **$p<0.01$

Results of the medical examinations prior to predose measurements of RBC ChE activity and at the end of the study were not reported. Urine was not analysed because the analytical method for measuring dimethyl phosphate (a dichlorvos metabolite) “could not be adequately reproduced at the Central Toxicology Laboratory”.

Conclusions: The NOEL following a single oral dose of dichlorvos to male volunteers was 1 mg/kg bw, based on the absence of RBC ChE inhibition, clinical symptoms and effects on body temperature at this dose.

Gledhill AJ (1997b) Dichlorvos: A single blind, placebo controlled, randomised study to investigate the effects of multiple oral dosing on erythrocyte cholinesterase inhibition in healthy male volunteers. CTL Report No. CTL/P/5392. CTL Study No. XH6063. Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK and Medeval Limited, University of Manchester, Manchester Science Park, Manchester, UK. Sponsor: AMVAC Chemical Corporation, unspecified location. Study duration: 21st October 1996 to 27th November 1996. Report date: 24th March 1997.

Gledhill AJ (1997c) First supplement to dichlorvos: A single blind, placebo controlled, randomised study to investigate the effects of multiple oral dosing on erythrocyte cholinesterase inhibition in healthy male volunteers. CTL Report No. CTL/P/5392. CTL Study No. XH6063. Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK and Medeval Limited, University of Manchester, Manchester Science Park, Manchester, UK. Sponsor: AMVAC Chemical Corporation, unspecified location. Study duration: 21st October 1996 to 27th November 1996. Report date: 9th October 1997.

Morris T (1996b) A single blind, placebo controlled, randomised study to investigate the effects of multiple oral dosing of dichlorvos on erythrocyte cholinesterase inhibition in healthy male volunteers. (STUDY PROTOCOL) CTL Study No. XH6063. Lab: Zeneca Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, UK and Medeval Limited, University of Manchester, Manchester Science Park, Manchester, UK. Sponsor: AMVAC Chemical Corporation, unspecified location. Study duration: 5th November 1996 to an unspecified time. Report date: unspecified.

GLP compliant [UK GLP Compliance Programme, Dept Health 1989; OECD Principles of GLP (1982) ISBN 92-64-12367-9 (OECD Environment Monograph No. 45, 1992); EC Council Directive 87/18 EEC and 88/320/EEC; US EPA (40 CFR Part 160)]. This study was quality assured and conducted according to the Declaration of Helsinki (1964) including all amendments up to and including the Hong Kong revision (1989). The study protocol was approved by the Medeval Independent Ethics Committee.

Materials and Methods

Dichlorvos (AMVAC Chemical Corporation, unspecified location; lot No. 6080028074; 98%), formulated in corn oil, was administered orally to six healthy fasted male volunteers in gelatine capsules at 7 mg for 21 days (equivalent to approximately 0.1 mg/kg bw/d for a 70 kg male). A control group of three healthy males received 21 daily doses of the placebo (gelatine capsules containing corn oil). The dose selection was based on a previous published study (Slomka & Hine 1981), where the NOEL for plasma and RBC ChE inhibition following multiple oral dosing for up to 21 days was 0.3 mg/kg bw (PVC vehicle). Furthermore, a recently completed unpublished study by Medeval reportedly found that 21 mg dichlorvos administered daily for 21 days resulted in 20-40% inhibition of RBC ChE activity. In the absence of a NOEL in this study, 7 mg was chosen for the current study. Capsules were taken with 150 mL distilled water. Dose formulations and capsules were prepared on four separate occasions and analysed for stability and dichlorvos content by GC.
Subjects were sourced from a volunteer panel held by the performing laboratory. All volunteers were Caucasian, 19-34 years of age, 170-182 cm in height and weighed 61-89.7 kg. Volunteers were subjected to a thorough medical examination within 14 days of predose measurements of RBC ChE activity, and at the end of the study. These examinations included an analysis of the following: standard haematology and clinical chemistry parameters; hepatitis B and HIV status; RBC ChE activity; heart rate, blood pressure and ECG; and urinalysis including the analysis for illicit drugs. Analysis for illicit drugs was also performed twice during the study.

Of the nine subjects, volunteers 5-9 were dosed one week after volunteers 1-4. On the day of dosing, volunteers were randomly assigned to treatment or placebo groups. Venous blood was taken during the 14-day predose period (days -14, -12, -10, -7, -5 and -3) then immediately prior to dosing on days 1, 2, 4, 7, 9, 11, 14, 16, 18 and 25 (subject 1-4), 28 (subject 5, 6 and 8), 29 (subject 7) or 30 (subject 9). RBC ChE activity was analysed in blood samples as soon as possible after collection. No plasma ChE activity was measured. Urine was collected at days 0, 2, 4, 7, 9, 11, 14, 16, 18, 21, 23 and 25 (volunteers 1-4) and stored frozen for possible future analysis. Any symptoms were recorded.

The following statistical tests were performed: repeated measures ANOVA and two-sided Student's t-test (comparison of group mean ChE activity at each time point); paired t-test (comparison of prestudy and subsequent group means for each treatment at each time point); and permutation test (comparison of prestudy and subsequent ChE activity for each individual at each timepoint).

Results

Dosing formulations contained 97.1 or 100% of the nominal concentration of dichlorvos. The weight of dichlorvos in the gelatine capsules ranged from 87.6-100.4% of the nominal level. Dichlorvos was stable in corn oil over 17 days, ranging from 89.6 to 96.9 % of the nominal concentration.

Symptoms reported by subjects in the control and dichlorvos groups are summarised in the Table below, but given their sporadic nature, none were attributable to treatment.ptoms reported by subjects following repeated oral dosing of dichlorvos or a placebo

Subject	Treatment	Time	Description
1	Placebo	Day 1	Dizziness
		Day 10-16	Cough
2	Dichlorvos	Day 0-4	Tiredness
4	Dichlorvos	Day 1	Intermittent nausea
		Day 1-22	Tiredness
5	Placebo	Day 3-7	Forgetfulness and tiredness
		Day 16-18	Flatulence
		Day 4 to study termination	Backache
6	Dichlorvos	Day 4	Nosebleed
8	Dichlorvos	Day 10-11	Mild headache

Results of the analysis of RBC ChE activity are summarised in the Table below. There was clearly a time-related increase in the inhibition of RBC ChE activity, which was not evident in the placebo group. Mean RBC ChE activity was significantly lower ($p<0.01$) than the placebo at days 7, 11, 14, 16, 18 and 21 postdose. Given the high statistical significance of this result and that, the pattern of inhibition was consistent with the dosing regime, the inhibition of RBC ChE activity was attributable to dichlorvos treatment.

Group mean RBC ChE activity (IU)

Day	Dichlorvos (n=6)		Placebo (n=3)	
	Mean ± 1 SD	% inhibition[1]	Mean ± 1 SD	% inhibition[1]
Predose	17738±1714	0	18484±1347	0
1	17628±1915	1	17930±1404	3
2	16817±1547	5	18180±1565	2
4	16933±1597	5	18740±1771	-1
7	16182±1759	9**	18530±1888	0
9	16708±2504	6	18460±1007	0
11	16037±1654	10**	19210±1036	-4
14	15333±1250	14**	18490±1642	0
16	15192±1063	14**	17707+2470	4
18	14855±1199	16**	18260±2299	1
Study termination (day 21)	-	17[2]	-	3[2]

1 = relative to pretreatment baseline values; 2 = no statistical analysis performed; - data not provided; ** Statistically different to the placebo group at $p<0.01$;

Conclusions: The LOEL for inhibition of RBC ChE activity following 21 days of repeated oral dosing was 0.1 mg/kg bw/d, the only dose tested. The absence of the analysis of plasma ChE activity was a deficiency of this study, which limited its regulatory value (ie. as the basis for the Australian ADI for dichlorvos).

11.2. Inhalational or dermal exposure

Zavon MR & Kindel EA Jr (1966) Potential hazard in using dichlorvos insecticide resin. Organic Pesticides in the Environment: Advances in Chemistry Series 60: 177-187.

Four female and 6 male volunteers (aged 21-52 and 24-47 years, respectively) handled Vapona Resin Vaporiser (unspecified source; 20% dichlorvos) for 30 min/d for 5 days 'in the manner required for household or farm use'. A 2.5 x 5-inch resin strip was afixed directly to the volar surface of the forearm for 30 min/day for 5 consecutive days. Plasma and RBC ChE activities were measured prior to exposure and after 3 and 5 days. There was no treatment-related effect on plasma or RBC ChE activity.

Two volunteer families had Vapona Resin Vaporisers installed in their homes for 6 months. During the first 4 months of observation, new vaporisers were installed monthly. The first residence was a 5-room

air-conditioned apartment, with 5 vaporisers installed in a total of 5088 cubic feet. The second residence was a detached house, with 8 vaporisers installed in 8000 cubic feet. No effects on plasma or RBC ChE activities were observed. Air monitoring conducted one month after installation of the last vaporiser, detected dichlorvos air levels of 0.097 and 0.087 μg/L (97 and 87 μg/m^3 respectively) in the first and second residence, respectively.

Ueda K & Nishimura M (1967) Effect of vapona/strips to human beings. No Report/Study No. Lab/Sponsor: Japanese Environmental Sanitary Association. Report date: November 1967.

Blood samples were collected from 47 hospital patients (unspecified location) who were inhalationally exposed to dichlorvos via vapona strips (source and formulation details unspecified) installed at the recommended rate of one strip/28 m^3. No effect on plasma or RBC activities was reported.

A separate study was conducted using six healthy male subjects aged 21-57 years old. Two subjects were exposed to dichlorvos for 20 hours/day for 2 days in a room containing 10 vapona strips/51.5 m^3 (ie. 5-times the recommended level). The subjects had entered the room 24 hours after the strips were installed and blood samples were collected after 48 hours for measurement of plasma and RBC ChE activities. Two control subjects were kept in an untreated room. There was no effect on RBC ChE activity, while plasma ChE activity was reduced to approximately 80% of pre-exposure activity. A separate group of two subjects showed greater levels of ChE inhibition after spending 48 hours confined to a room containing 17 strips/46.8 m^3 (ie. 10-times the recommended dosage). Plasma ChE activity was suppressed to 80, 70 and 60% of pre-exposure activity after 12, 24 and 48 hours of confinement, respectively. Pre-exposure levels of activity returned after 7 days. Less marked effects were observed for RBC ChE but no data were presented. Air levels of dichlorvos were 2.2 and 0.8 μg/L (mg/m^3) after 13 and 48 hours, respectively, in the room containing 10 strips. Air levels of dichlorvos were 7.1 and 2.4 μg/L (mg/m^3) after 3 and 48 hours, respectively, in the room containing 17 strips.

Cavagna G, Locati G & Vigiliani EC (1970) Exposure of new born babies to vapona insecticide. European Journal of Toxicology 3(1): 49-57.

Materials and Methods: Plasma and RBC ChE activities were measured in babies that had been exposed to Vapona pest strips (18.8% w/w dichlorvos; unspecified source) installed in hospital wards. The study was conducted on 89 healthy babies born at the University of Milan Medical Clinic from healthy women having a normal pregnancy and delivery. There were four experimental groups. Group 1 consisted of 25 babies who were born of women exposed to dichlorvos during labour and puerperium, with the babies exposed for 5 days after birth (unspecified exposure conditions). Group 2 consisted of 22 babies exposed for 5 days after birth in a well-ventilated nursery, where approximately 1 strip was installed every 40 m^3 (7 strips/277 m^3 total). The room was ventilated by 2 doors that were kept open for most of the time, and a mechanical ventilation system that produced 2 air changes/hour. An air conditioning system kept the temperature at 22-24°C, with the relative humidity at 70-90%. Group 3 consisted of 22 babies exposed for 5 days after birth in a poorly ventilated nursery, where approximately 1 strip was installed every 30 m^3. This nursery actually consisted of two rooms of 145 and 90 m^3. Both rooms had air conditioning, which did not always operate, and there was no change of air. The temperature in both rooms was 25-27°C and the relative humidity was 50-75%. Group 4 was a control group of 20 babies.

Babies in Groups 2 and 3 were exposed to dichlorvos continuously for the first 24 hours after birth and for approximately 18 h/d thereafter. Air was sampled twice per day in the well-ventilated nursery (ie. Group 2) and 4 times daily in the poorly ventilated nursery (ie. Group 3). In the well ventilated nursery, the insecticidal activity of the air was also analysed by determining the time required to kill *Drosophila Melanogaster* (Cavagna et al 1968).

Milk was collected from mothers exposed to dichlorvos and analysed for its ability to inhibit plasma ChE activity in plasma with known ChE activity. For babies, plasma and RBC ChE activities were measured at birth and 5 days after birth.

Results: Babies from Group 1 (ie. those exposed *in utero* and then for 5 days after birth) stayed in rooms where the dichlorvos concentration ranged from 0.095 to 0.25 mg/m^3. No time-weighted average air concentration was reported for this group. In the well ventillated nursery (Group 2; 1 strip/40 m^3) the air concentration of dichlorvos reached a maximum of 0.128 mg/m^3 after 2 days, decreasing to 0.027 mg/m^3 on day 3 due to auxilliary ventilation, increasing to 0.087 mg/m^3 on day 4 then declining to 0.042 mg/m^3 on day 5. The mean air concentration of dichlorvos for Group 2 was approximately 0.053 mg/m^3. The insecticidal activity of the air in this nursery was proportional to the air concentration of dichlorvos and decreased over time (time of death for *Drosophila Melanogaster* at day 2, 3 and 5 was 55, 80 and 120 min, respectively). In the poorly ventilated nursery (Group 3; 1 strip/30 m^3), which consisted of two rooms, the air concentration of dichlorvos ranged from 0.11-0.28 mg/m^3 in one room and 0.11-0.23 mg/m^3 in the other. The mean air concentration in both rooms was approximately 0.15 mg/m^3. The study authors estimated that the intake of dichlorvos in Group 3 babies was approximately 0.036 mg/kg bw/d, based on exposure for 18 h/d and assuming an inhaled volume of air of 1 m^3/24 h.

No adverse health effects were reported. No anticholinesterase activity was detected in mothers milk. Analysis of plasma and RBC ChE activities in babies is summarised in the Table below. There was no significant effect on either plasma or RBC ChE activities, noting that plasma ChE activity was somewhat lower in Group 3 babies at birth (24%) and at 5 days after birth (16%) relative to the unexposed group. However, given the large variability in both plasma and RBC ChE activities, these findings are not considered biologically significant.

Plasma and RBC ChE activities in babies exposed to dichlorvos pest strips

Group (n)	At birth		5 days after birth	
	Plasma	RBC	Plasma	RBC
Not exposed to dichlorvos (20)	15.7±3.46 (10-22)	29.16±6.16 (18-45)	16.1±3.6 (12-23)	29.10±6.24 (16-42.5)
Born of mothers exposed to dichlorvos (25)	16.17±5.01 (9-31)	32.3±6.4 (22-45)	16.5±5.56 (9-28)	32.19± 5.9 (24-48)
Exposed to dichlorvos at a mean concentration of 0.053 mg/m^3 (22)	16.17±4.71 (10-27)	29.9±7.61 (14-40)	17.1±4.79 (11-28.5)	28.1±8.12 (14-41)
Exposed to dichlorvos at a mean concentration of 0.15 mg/m^3 (22)	12± 2.86 (6.5-19.6)	28.2±4.3 (16-35.5)	13.5±3.4 (9-18.3)	32±3.2 (23-36)

Results expressed as the mean mM NaOH/100 mL/min ± standard deviation (range).

Conclusion: In babies, plasma and RBC ChE activities were unaffected by exposure to dichlorvos at approximately 0.15 mg/m^3 for 5 days (estimated to be 0.036 mg/kg bw/d).

Hunter CG (1970a) Dichlorvos: inhalational exposures with human subjects. Part 1. Report no. TLGR.0061.70. Lab/Sponsor: Shell Research Centre (Tunstall Laboratory), Sittingbourne, UK. Study duration: unspecified. Report date: September 1970.

Twenty-six male and 6 female laboratory staff were exposed to dichlorvos vapour (approximately 1 mg/m^3) in a 'large' inhalation chamber for 2-7.5 h. Males were 21-57 years old and weighed 53-94 kg. Females were 19-25 years old and weighed 57-93 kg. Dichlorvos was supplied by Shell Chemical Co. and had a purity of 94.6%. Subjects were dressed conventionally and were allowed to sit or stand during exposure. Average ventilation rates were approximately 8 and 10 L/min in females and males, respectively. Analyses of plasma and RBC ChE activity were made before, immediately and 16-18 h after exposure.

Some subjects considered they could detect dichlorvos odour in the air but no symptoms were reported. No effects were seen on EEG, ECG or heart rate, respiratory function, kidney or urinalysis (protein, sugars, ketone bodies, osmolar clearance, free water and phosphorus reabsorption index), or

haematology. Plasma ChE activity was depressed by >20% in some individuals after >6 h exposure. However, mean values remained within 20% of pre-exposure activity. There were no marked effects on RBC ChE activity. Three men were exposed for 5.5-8 h/day on consecutive days; two showed no effects on plasma ChE activity but the third showed activity depressed by 21, 23 and 37% on days 2, 3 and 4 respectively. Graphical representation of mean single-exposure data showed a strong dose-response relationship for plasma ChE activity, declining as dose (expressed as mg min/m^3) increased. At 20% inhibition, the dose was approximately 580 mg min/m^3, whilst at zero inhibition, the dose was 200 mg min/m^3. The latter value constituting the NOEC for the inhibition of plasma ChE activity.

Hunter CG (1970b) Dichlorvos: inhalational exposures with human subjects. Part II. Report No. TLGR.0067.70 Lab/Sponsor: Shell Research Centre (Tunstall Laboratory), Sittingbourne, UK. Study duration: unspecified. Report date: October 1970.

Seven male laboratory staff (25-56 years old; 71-86 kg bw) were exposed to dichlorvos vapour in glass bell jars at concentrations of 1-53 mg/m^3 for 10-240 minutes. Dichlorvos was supplied by Shell Chemical Co. (94.6% purity) and the vapour was produced in a saturator. The total flows of air and vapour were 20-25 L/min. Clinical and laboratory examinations were made before, during and 16-18 hours after exposure. Clinical observations included pupil size and response, electrocardiograms (ECGs), electroencephalograms (EEGs) and measurement of airway pressure changes. Blood and urine samples were collected before and after exposure; blood samples were analysed for plasma and RBC ChE activities, inorganic phosphates and glucose; urine was analysed for inorganic phosphates, organic and ester phosphates, and glucose.

One subject (a smoker) complained of upper airway irritation and tightness of the chest and consequently exposure was terminated after 9 minutes at 52 mg/m^3. This subject showed no signs of airway obstruction. Other subjects reported throat dryness and occasional rhinorrhoea, which were not considered treatment-related. There was no effect on pupil size, visual acuity, EEG, ECG or airway pressure. There was no effect on blood glucose and urinary analysis was unremarkable. RBC ChE activity was somewhat reduced (9-16%) at exposures greater than 1450 mg/min/m^3 relative to pre-exposure activity, however, this was not considered treatment-related. Graphically-presented data illustrated a linear relationship between exposure (mg min/m^3) and the inhibition of plasma ChE activity. Exposures greater than approximately 2000 mg min/m^3 reduced plasma ChE activity by more than 20% (measured at the end and 16 hours after exposure) relative to pre-exposure activity. The level of inhibition was approximately 90% in the subject exposed to the highest level of 5100 mg min/m^3.

Leary JS, Keane WT, Fontenot C, Feichtmeir EF, Schultz D, Koos BA, Hirsch L, Lavor EM, Roan CC & Hine CH (1974) Safety evaluation in the home of polyvinyl chloride resin strip containing dichlorvos (dichlorvos). Arch Environ Health 29:308-314.

Over a period of two years, three studies were conducted in a number of residences in Tucson Arizona to determine the safety of resin strips containing dichlorvos. Details of these studies are summarised in the Table below. In total, 84 adults and 55 children were involved in the studies.

Summary of three residential exposure studies on dichlorvos

Study	Samples	Exposure conditions	Observations
1	20% vapona (18.5% dichlorvos) in PVC resin strips (100, 10x2.5x0.25 inches and 20g, 2x2.5x0.25 inches (3 homes)[a] Control: Non-active PVC strip (2 homes)	7.5-18 strips/home Used according to label directions – ie. 1/1000 ft^3/90 days Strips replaced every 3 months for 12 months	Plasma & RBC ChE activity (pretreatment; weekly for 1st month; fortnightly for next 2 months; monthly thereafter) Haematology: Hb, Hct, reticulocyte count, platelet count & WBC count Illness, abnormal health patterns, time spent in house, medications used Temperature & humidity Age & sex (study 2) Air analysis: samples collected from kitchen (pre-exposure; d 1, 2, 3, 4, 6, 7, 10, 13, 16, 21 & 28 at 1/1000 ft^3; d 1, 2, 3, 4, 6, 7, 10 & 18 1/500 ft^3; 17 days post-exposure) (study 3) Food analysis: samples collect on same days as air analysis; meals were prepared and exposed (Study 3) Plasma & RBC ChE activity (pretreatment; same days as air & food sampling; 21 & 75 days after strips removed) (Study 3) Physical examination performed on day of strip installation, d 7 & 28 of standard exposure & d 13 of exagerated exposure (Study 3) Clinical chemistry: Ca, P, glucose, BUN, uric acid, cholesterol, protein, bilirubin, AP, LDH, SGOT (pretreatment;d 3 & 13 of standard epxosure) (Study 3)
2	20% vapona (18.5% dichlorvos) in PVC resin strips (10x2.5x0.25 inches) (4 homes)	4-18 strips/home 1/1000 ft^3 Replaced monthly for 6 months	
	20% vapona (18.5% dichlorvos) in PVC resin strips (10x2.5x0.25 inches) enclosed in a sealed plastic wrapper permeable to dichlorvos (4 homes)		
	20% vapona (18.5% dichlorvos) in PVC resin strips (6.5x2.5x0.33 inches) (4 homes)		
	Control: Non-active PVC strip (3 Homes)		
3	10-inch commercial strips containing 20% vapona (4 homes) Placebo strips (2 homes)	Study conducted over winter under minimum ventillation conditions 8-18 strips/home 1/1000 ft^3 New strips placed in kitchen and dining areas at 1/500 ft^3 All strips removed after 2 weeks	

a = 2 families also used 2x2 inch dichlorvos strips in all clothes closets

The time spent indoors was approximately 50-60% in the dichlorvos-treated houses and 60-70% in the control houses. Higher indoor occupancy did not occur during the winter months, which the authors attributed to the mildness of winters in Arizona. In study 2, a 20-year old female reported headaches approximately after one month of exposure. Tranferal of the dichlorvos strip from her room into another room coincided with the cessation of these headaches, however, the authors reported that there was no change to the overall rate of application (in the house). Blood samples collected during

this period revealed no effect on plasma or RBC ChE activity in this subject. No symptoms were reported in any of the other subjects in studies 1, 2 or 3. Pets did not display any abnormal signs.

In study 1 and 3, no inhibition of plasma or RBC ChE activity was evident in any of the subjects. In study 2 there was a marginal effect on plasma ChE activity over the 6 month exposure period, which was significantly inhibited (no p value; statistical test unspecified) by 15-30% compared to the control. Given that a decrease in plasma ChE activity also occurred in residents from the control homes over the same time period (though of a smaller magnitude) relative to pretreatment activity, the apparent treatment-related effect on plasma ChE activity was considered to be equivocal. Haematology findings were unreported.

In study 3, the concentration of dichlorvos in air reportedly reached a maximum of 0.12-0.13 mg/m^3 within several days, declining to a steady state level of 0.08-0.09 mg/m^3 from days 13 to 28. When exposure was increased to 2 strips per 500 ft^3 (equivalent to 14.16 m^3), a maximum level of 0.16 mg/m^3 was reached in 2 days, decreasing to 0.11 mg/m^3 13 days later. It was reported that no dichlorvos was detected in air 17 days after the removal of all strips. At an application rate of 1 strip/1000 ft^3 (equivalent to 28.32 m^3), dichlorvos levels in food reached a mean of 0.11-0.12 ppm from day 2-16, with the lowest level of 0.055 ppm detected at day 28.

Gold RE & Holcslaw (1984) Dermal and respiratory exposure of applicators and residents to dichlorvos-treated residences. In: Dermal exposure related to pesticide use: discussion of risk assessment (RC Honeycutt, G Zwerg & N Ragsdoleet eds). American chemical Society Symposum Series No. 273.

Materials and Methods

Two applicators were monitored for their exposure to dichlorvos during the treatment of twenty single-family residences with a 0.5% water-emulsion spray prepared from Vaponite 2EC (unspecified source and batch/lot No.; 24.7%). The application rate was 0.19 g/m^2 dichlorvos (38.7 mL/m^2 spray) and the average residence size was 103$\pm$33 m^2. Temperature and humidity were 26.1°C and 82%, respectively. The spray was applied along the baseboards, doorways, windows, all entrances, under the sink, stove and refrigerator, shelves, cabinets and around plumbing and utility installations. Residents were advised not to re-enter their premise for two hours.

Both applicators wore a long-sleeved polyester jumpsuit, hard hat, respirator and rubber gloves. Dermal exposure pads were fitted on the clothing and the skin beneath the clothing at the following sites: head (under the top of the hard hat), forearm (above the wrists), leg (above the ankle), chest and back. Following pesticide application, exposure pads were removed and stored frozen to reduce volatilisation and degradation of dichlorvos. Each applicator washed their hands in ethanol:water (50:50), with this wash kept for analysis. Inhalational exposure was monitored using a personal air pump fitted with an impinger filled with water:ethylene glycol (5:15). Blood and urine samples were collected from the applicators prior to treatment and then at 7 and 30 h for applicator one, and at 8, 24, 30, 48 and 56 h for applicator two. Blood and urine samples were also collected from one resident at each premises prior to and then 24 hours after treatment. To monitor environmental exposure, dermal exposure pads were placed on top of the refrigerator, stove, kitchen table and floor and collected two hours post-treatment. Air samplers were also operated in each premises for 24 hours prior to treatment and then at 0-2 and 2-24 hours post-treatment.

Following acetone extraction (dermal exposure pads), dichloromethane extraction (personal air pump and hand wash samples) or hexane extraction (urine), dichlorvos was analysed by GC. Plasma and RBC ChE activities were measured in blood samples. Following derivatisation and extraction, dichloroacetic acid (a metabolite of dichlorvos) was measured in urine by GC.

Results

Applicators: Individual applicator data were not provided. Average dermal exposure was 0.499$\pm$0.274 μg/cm^2/h according to the exposure pads fitted to the clothing, 0.102$\pm$0.062 μg/cm^2/h for the exposure pads fitted to the skin under the clothing and 0.024$\pm$0.021 μg/cm^2/h for the hands. These findings

indicate the movement of dichlorvos through the clothing and rubber gloves. Potential inhalational exposure was 0.021±0.019 μg/L (21±19 μg/m^3). Total dermal exposure was 0.028 mg/kg bw/h, while potential inhalational exposure was 0.037 mg/h or 0.0004 mg/kg bw/h assuming a ventilation rate of 1740 L/h. The authors calculated that the toxic dose of dichlorvos was 0.028±0.021 %/h, with a worst case estimate for an unprotected applicator of 0.11 %/h.

Results of ChE analysis were incompletely reported and only a textual description of the findings was provided. Applicator one was reportedly forced to stop applying dichlorvos due to illness, with the nature of this illness unconfirmed although "pesticide poisoning" could not be discounted. He reportedly had a 59% reduction in plasma ChE activity at 7 hours post-treatment but this had "rebounded significantly" by 30 hours. Applicator two had a 21% reduction in plasma ChE activity at 2 h, which returned to the baseline activity at 48 hours. The authors reported that RBC ChE activity was difficult to interpret due to the inconsistent response to dichlorvos. Applicator two was observed to have a general decline in RBC ChE activity, which correlated with increased exposure to dichlorvos. No dichloroacetic acid was detected in the urine of either applicator.

Residents: A proportion of residents (15%) reported a headache following re-entry into their premises. The greatest level of dichlorvos detected by air samplers was during the first 2 hours after application (0.548±0.297 μg/L). However, over the 24 hour post-application period, the level of dichlorvos was 0.21 μg/L (210 μg/m^3). Residents who spent a mean of 15.8 hours in their treated residences were calculated to be exposed to 0.08 mg/kg bw. The authors indicated that the potential respiratory exposure to residents was an order of magnitude greater than the applicators. The mean level of dichlorvos detected on the exposure pads situated within the residences was 0.319±0.183 μg/cm^2/h up to 2 hours post-application.

Results of ChE measurements were not provided, however it was stated that there was a slight (7.9%) though significant reduction ($p<0.05$) in plasma ChE activity in residents at 24 hours post-application (relative to pre-application activity). RBC ChE activity was reportedly decreased by 5.3-37.5%, a result that was not statistically significant. No dichloroacetic acid was detected in the urine of residents.

Conclusions: This study suggested that toxicologically-significant exposure of applicators and residents can occur during and immediately following the application of dichlorvos. Although this study had some deficiencies (such as the lack of reporting detail and the small sample sizes), it had qualitative value for risk assessment purposes.

12. OTHER STUDIES

12.1 Methylation of DNA

Wooder MF, Wright AS & King LJ (1976) In vivo alkylation studies with dichlorvos at practical use concentrations. Report No. TU/28/76. Lab/Sponsor: Department of Biochemistry, University of Surrey, Guildford, Surrey, UK and Shell Research Ltd, Tunstall Laboratory, Sittingbourne Research Centre, Sittingbourne, Kent, UK. Report date: 29th October 1976.

In two replicate experiments, each consisting of 2 groups of 5 male CFE rats, animals were exposed for 12 h to [^{14}C-methyl]dichlorvos (113 Ci/mol) at 0.064 μg/L (equivalent to 0.064 mg/m^3). Following exposure, rats were decapitated and soft tissue was processed for extraction of DNA and RNA. Analysis revealed methylation of the $\underline{N}_7$ atom of quinine moieties. The limits of detection of methylation were one methyl group per 5.7×10^{11} and per 2.0×10^9 nucleotide units for DNA and RNA respectively. Thus, dichlorvos did not appear to methylate rat nucleic acids following inhalation at low (practical use) concentrations.

Wright AS, Hutson DH & Wooder MF (1979) The chemical and biochemical reactivity of dichlorvos. Arch Toxicol 42: 1-18

This review paper discussed the chemical structure, reactivity and metabolic fate of dichlorvos, particularly in relation to its possible genotoxicity. On the basis of comparing mammalian and bacterial assays reported in the literature, the authors concluded that the mutagenicity of dichlorvos in bacterial assays was due to methylation of DNA under the conditions of the tests. However, the methylation of mammalian DNA could not be demonstrated *in vivo*. Conversely, methylation by the known alkylating mutagen, methyl methansulfonate, was evident. The failure to detect methylation by dichlorvos *in vivo* was attributed to highly efficient enzyme-catalysed biotransformation relying on the phosphorylating reactivity of dichlorvos. The metabolic pathways for dichlorvos had been characterised largely in the rat but were also reported to be common to pig, mouse, hamster and humans.

12.2 Forestomach irritation studies

Benford DJ (1991) Investigation of the genotoxic and/or irritant effects of dichlorvos on mouse forestomach. Study No. 26/89/TX. Report No. RI90/0405. Lab: Toxicology Unit, The Robens Institute of Health and Safety, University of Surrey, Surrey, England. Sponsor: Temana International Ltd., Slough, Bucks, England. Study duration: 19th February 1990 to 24th September 1991. Report date: 25th September 1991.

Benford DJ, Price SC, Lawrence JN, Grasso P & Bremmer JN (1994) Investigations of the genotoxicity and cell proliferative activity of dichlorvos in mouse forestomach. Toxicology. 92:203-215

GLP compliant [UK GLP Compliance Programme, Dept Health 1989; US EPA (40 CFR Part 792, 29th November 1983 and subsequent amendments); US FDA (title 21 CFR Part 5B, 22nd December 1978 and subsequent amendments)]. QA study.

Materials and Methods

Dichlorvos (Ciba-Geigy, unspecified location; Batch No. op.910111; 99.8% purity) was administered as a single oral dose to fasted B6C3F1 mice (approximately 20 g bw, age unspecified; Bantin and Kingman Ltd., Hull, UK) by gavage at 0, 10, 20, 40 or 100 mg/kg bw. At each dose, 4 separate groups of 5 mice/sex were assigned to one of 4 sacrifice times of 2, 4, 12 or 48 hours. The vehicle was corn oil and the dose volume was 10 mL/kg bw. Doses were the same as those used in a previous NTP carcinogenicity study (Chan 1989), where forestomach tumours occurred in the same mouse strain. Positive control groups of 5 mice/sex were treated with either 300 mg/kg bw butylated hydroxyanisole

(BHA) in aqueous gum tragacanth or 200 mg/kg bw *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in corn oil. BHA is a non-genotoxic promoter of forestomach tumours and is used as an antioxidant in food, while MNNG is a genotoxic forestomach carcinogen.

Mice had been acclimatised for at least 3 days prior to dosing and were fed CRM diet (Labsure, Cambridge, England). Details of housing and feeding conditions were unspecified, although it was stated that mice were maintained according to the current standard operating procedures of the performing laboratory. Mice were randomly assigned to treatment groups based on an unspecified criterion. Following dosing, food was returned to the 48-h group, while all other groups remained fasted. Male mice from the 2-h groups were initially dosed without food withdrawal, however, preliminary studies indicated that more consistent results were achieved with food withdrawal. Consequently, this part of the experiment was repeated (with food withdrawal), however there were only sufficient spare male mice (25) to test the negative and positive controls, and the 40 and 100 mg/kg bw doses of dichlorvos.

Mice were sacrificed by cervical dislocation at 2, 4, 12 and 48 hours postdose, stomachs removed and examined for any gross abnormalities. Stomachs were opened, contents removed and the forestomachs divided into 4 longitudinal sections. One strip was fixed, stained and histopathologically examined. The remaining three strips were incubated with ^{3}H-thymidine and then fixed for autoradiographic examination. UDS was assessed in the 2 and 4 hour groups by manually scoring the occurrence of nuclear grains, using light microscopy. The total number of fields with at least 2 positive cells ($\geq$ 3 grains) was scored, with the number of nuclear grains subsequently scored in 50 epithelial cells within the most positive field. Replicative DNA synthesis (RDS) was assessed in the 12 and 48-hour groups by determining the proportion of s-phase cells in 500 cells of the squamous epithelium of each strip (s-phase cells were defined as having numerous closely associated grains, while cells in repair contain discrete grains).

Data were analysed by a Student's t-test. UDS results were considered positive when the mean nuclear count of each treated mouse was significantly greater than the negative (solvent) control.

Results

Mortalities: Mortalities occurred in 3 males from the 100 mg/kg bw group within 2 hours of dosing, with another male from this same group sacrificed in a moribund condition at 1.5 h postdose. Mortalities also occurred in the MNNG (one mouse/sex) and BHA (one male) groups.

Pathology: Macroscopic examination revealed no treatment-related abnormalities in females at any dose. At 100 mg/kg bw, one male had dilation of the blood vessels in the stomach at 12 h postdose. In the 2 h group, one and two males had haemorrhage in the stomach at 40 and 100 mg/kg bw dichlorvos, respectively. Bloating, fluid, dilation of the blood vessels (males only) and haemorrhage occurred in the stomachs of mice treated with either of the two positive controls at various times after dosing. Males appeared to be more affected than females and MNNG appeared to be marginally more effective at inducing these abnormalities than BHA.

Histopathological examination of the mouse forestomach revealed a number of treatment-related abnormalities mainly at 12 and 48 hours postdose, which are summarised in the Table below. Two hours after dosing, there was little evidence of any treatment-related effects; submucosal oedema occurred in 2 or 3 rats per group in females at and above 40 mg/kg bw compared to no rats in the control or lower dose groups. Four hours after dosing, dichlorvos had clearly induced epithelial hypertrophy in females (but not males) at 40 and 100 mg/kg bw, with this finding not detected at lower doses or in the negative control. Oedema of the submucosa and lamina propia were consistently observed in both sexes at most doses of dichlorvos after 4, 12 and 48 hours of dosing. While the lack of a dose-response effect makes the interpretation of these findings somewhat difficult, the absence or very low incidence ($\leq$1/5 mice) of these findings in the negative control suggests that they were treatment-related. Twelve hours after dosing, epithelial cell hypertophy occurred in all but one male at every dose compared to its absence in the concurrent negative control. At 48 hours, hypertrophy was evident in both sexes across all dose groups; males appeared to be predominantly affected at 10, 20

and 40 mg/kg bw but not at 100 mg/kg bw, while all but 2 females were affected across all dose groups. Diffuse hyperplasia was elevated in both sexes at all doses at 12 and 48 hours. Focal hyperplasia was somewhat elevated in females at 12 hours at 10 and 20 mg/kg bw.

Both positive controls caused histopathological abnormalities in the mouse forestomach. MNNG induced oedema of the submucosa and lamina propia (2, 4, 12, 24 h), epithelial cell hypertrophy (4, 24 h), infiltration of inflammatory cells (2, 12, 24 h) and increased mitotic counts (12 h). BHA caused oedema of the submucosa and lamina propia (4, 12, 24 h), epithelial cell hypertrophy (4, 12, 24 h), focal (12 h) and diffuse hyperplasia (12, 24 h) and increased mitotic counts (4, 24 h). The times at which these effects occurred varied between males and females. The effects seen with BHA, notably the hyperplasia, were consistent with the histopathological effects caused by dichlorvos.

Histopathological findings in mice at 4, 12 and 48 postdose

Findings	Dose (mg/kg bw)									
	0		10		20		40		100	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
4 h postdose										
Submucosal oedema	0	0	3	5	4	4	5	1	0	0
Lamina propia oedema	0	1	3	5	2	3	5	1	3	2
Epithelial cell hypertrophy	0	0	0	0	1	0	0	5	1	5
Mitotic counts	0	4	2	4	1	1	4	2	4	0
12 h postdose										
Submucosal oedema	0	0	2	0	0	1	1	0	5	5
Lamina propia oedema	0	0	4	3	5	3	4	0	4	3
Epithelial cell hypertrophy	0	0	5	0	5	1	4	0	5	0
Focal hyperplasia	0	0	1	3	1	3	0	1	0	1
Diffuse hyperplasia	0	0	4	2	4	1	4	2	5	4
48 h postdose										
Submucosal oedema	1	0	3	1	5	2	3	4	5	0
Epithelial cell hypertrophy	1	0	3	5	2	3	3	5	1	5
Diffuse hyperplasia	1	0	3	4	5	2	5	4	4	4

Results are the absolute numbers of mice affected (n=5)

UDS: Dichlorvos did not significantly increase the number of nuclear grains relative to the negative control at 2 or 4 h postdose (ie. the amount of ^{3}H-thymidine incorporated into the DNA of non-dividing cells) and therefore did not induce UDS. At 4 h postdose, females from the 100 mg/kg bw group had a significantly reduced ($p<0.05$) mean number of nuclear grains compared to the control (0.23±0.07 *versus* 0.53±0.26, respectively). The mean % of cells in repair (ie. the number of cells with >3 nuclear grains) was also significantly ($p<0.05$) reduced in this same group (2.00±0.47 *versus* 5.53±2.84 in the control). The biological significance of these reductions is unclear.

MNNG significantly increased ($p<0.05$) the mean number of nuclear grains in males at 4 h postdose (2.58±1.11 *versus* 0.55±0.26 in the control). Non-significant increases occurred at 2 h (both sexes) and in females at 4 h. BHA caused a significant increase ($p<0.05$) in the mean number of nuclear grains only in males at 2 h (0.30±0.09 *versus* 0.12±0.08 in the control). In the absence of a similar

effect at 4 h or in females, and given the fact that this apparent increase was within the control range, this finding is not considered biologically significant.

RDS: There appeared to be no treatment-related effect on the % of cells in s-phase, although the high level of intragroup variability made the interpretation of these findings somewhat difficult. For example, at 12 h in males, the mean % of s-phase cells was elevated across all treatment groups relative to the control (1.32±1.89, 0.91±1.29, 1.35±1.43 and 1.40±1.20% at 10, 20, 40 and 100 mg/kg bw compared to 0.76±0.97% in the control). However, none of these findings were statistically significant due to the large SD, and there was no dose-response relationship. In females at this same time, the % of s-phase cells was lower than the control at every dose but only the effect at 10 mg/kg bw was statistically significant ($p<0.05$). These observations in males and females at 12 h were not evident at 48 h postdose. While MNNG and BHA caused an increase in the mean % s-phase cells in males at 12 h relative to the control (3.04±2.65 and 1.02±1.41%, respectively) these findings were not statistically significant.

Conclusions: Dichlorvos did not induce UDS or RDS in the mouse forestomach up to 100 mg/kg bw, a dose where mortalities and macroscopic stomach abnormalities (dilation of blood vessels and haemorrhage) occurred in males. Dichlorvos caused a range of histopathological forestomach effects including oedema, epithelial cell hypertrophy and hyperplasia at and above 10 mg/kg bw. The induction of hyperplasia was similar to that caused by the BHA, a known promoter of forestomach tumours in mice.

Benford DJ (1992) Investigation of irritant effects of dichlorvos on mouse forestomach. Study No. 14/91/TX. Report No. RI91/0405. Lab: Toxicology Unit, The Robens Institute of Health and Safety, University of Surrey, Surrey, England. Sponsor: Temana International Ltd., Slough, Bucks, England. Study duration: 5th August 1991 to 18th October 1991. Report date: 16th November 1992.

GLP compliant [UK GLP Compliance Programme, Dept Health 1989; US FDA (title 21 CFR Part 5B, 22nd December 1978 and subsequent amendments)]. QA study.

This study was essentially a repeat of the above study of Benford (1991) except that UDS was not examined.

Materials and Methods

The materials and methods were the same as those described in the previous evaluation, with the following differences:
Groups were designated for sacrifice at 8, 10 and 48 hours after dosing;
The 8 and 10 h groups were assessed for replicative DNA synthesis (RDS) and epithelial cell proliferation (^{3}H-thymidine incorporation), while the 48 h group was examined for histopathological effects;
Forestomachs were dissected into 5 longitudinal strips; and
RDS was assessed by determining the proportion of s-phase cells in 1000 cells of the squamous epithelium of each strip.

Results

Mortalities: There were five deaths in the 10 hour groups at 10 (one female), 40 (one mouse/sex) and 100 mg/kg bw (2 males) dichlorvos. One male from the 10 h MNNG group was sacrificed in a moribund condition 10 hours after dosing, with autopsy revealing a swollen and bleeding stomach. No clinical signs were reported.

Pathology: At autopsy (48 hours), the majority of males treated with dichlorvos (all doses) or BHA had “pronounced” forestomachs, a finding which was not evident in females or in the concurrent negative control group. In the majority of males, MNNG caused bloating of the forestomach and the presence of a clear liquid, foci of blood and a small nodule. In females, MNNG caused bloating (2/5 mice), red patches (3/5) and thickening of the forestomach (3/5 mice).

Treatment-related histopathological abnormalities were generally consistent with those observed in the previous study (Benford 1991) and included perinuclear vacuolation (males), submucosal and lamina propria oedema (both sexes), focal and diffuse epithelial cell hypertrophy (both sexes), focal hyperplasia (both sexes) and diffuse hyperplasia (females). These findings were seen at most doses, with the numbers of mice affected generally increasing with dose. The forestomach of the majority of mice treated with BHA showed oedema, cell hypertrophy and hyperplasia, consistent with the effects seen with 40 and 100 mg/kg bw dichlorvos. However, the severity of the effects seen with dichlorvos was less than with BHA (scored as present in dichlorvos-treated mice and present to moderate in BHA-treated mice). MNNG failed to cause hyperplasia of the forestomach, although oedema and hypertrophy were induced in most mice.

RDS: Results of the measurement of RDS (induction of s-phase cells) at 10 hours are summarised in the Table below. It should be noted that intragroup variability was large and there were problems with strips being too small or disintegrating during processing. Consequently, there were reduced numbers of samples from each mouse available for scoring.

Dichlorvos did not induce RDS at 8 hours, but there was a significant increase ($p \leq 0.05$) in the proportion of s-phase cells at 10 hours at 10 and 40 mg/kg bw in males and 20 and 100 mg/kg bw in females. Given the similar magnitude of the increase (3-4-fold in males and 4.25-4.5-fold in females) it did not appear that females were more affected than males as conlcuded by the author. The toxicological significance of these findings is somewhat difficult to interpret given the lack of a dose-response effect and the large intragroup variability. In males, the increase in s-phase cells at 10 and 40 mg/kg bw is probably not treatment-related. At 10 mg/kg bw at 10 hours, the proportion of s-phase cells is the same as that detected at 8 hours, which was not statistically different to the control. Examination of individual mouse data also revealed that the significant increase at 10 mg/kg bw is attributable to a single outlying animal. The statistical significance of the increase at 40 mg/kg bw may be due to the decreased proportion of s-phase cells in the control (from 0.4% at 8 hours to 0.2% at 10 hours). Examination of individual animal data did not identify any outliers in the 40 mg/kg bw group and therefore the finding should be viewed as equivocal. There is a marginally stronger case for a treatment-related effect in females at 20 and 100 mg/kg bw given the consistent proportion of s-phase cells in the negative control at 8 and 10 hours (0.3 and 0.4%, respectively), and that all dose levels had higher proportions of s-phase cells than the control (albeit the absence of a dose-response effect). An examination of individual mouse data revealed no obvious outliers in the 20 mg/kg bw group, however, there was an obvious outlier in the 100 mg/kg bw group. Overall, the reviewing toxicologist considers that the findings in males and females are equivocal.

BHA significantly increased ($p \leq 0.05$) the proportion of s-phase cells in males by 7- and 14-fold at 8 and 10 hours respectively. In females, s-phase cells were significantly elevated ($p \leq 0.05$) at 10 hours by 6.25-fold. MNNG was less affective than BHA at inducing RDS, with a significant increase ($p \leq 0.05$) only seen in males.

Induction of s-phase cells in mouse forestomach with dichlorvos after 10 hours

Treatment	% S-phase cells	
	Males	Females
Control	0.2±0.2	0.4+0.5
10 mg/kg bw dichlorvos	0.6±0.6*	0.8±0.6 (n=4)
20 mg/kg bw dichlorvos	0.2±0.1	1.7±1.1*
40 mg/kg bw dichlorvos	0.8±0.3* (n=4)	0.6±0.2 (n=4)
100 mg/kg bw dichlorvos	0.3±0.1 (n=3)	1.8±1.3*
MNNG (200 mg/kg bw)	0.9±0.6*	0.9±0.5
BHA (300 mg/kg bw)	2.8±2.5*	2.5±1.6*

Results expressed as the mean ± 1 SD % (n=5 unless specified); *$p \leq 0.05$;

There was an increase in epithelial cell proliferation (^{3}H-thymidine incorporation) in the forestomach of mice (both sexes) 10 hours after dosing with dichlorvos. However, this result was considered

equivocal due to the high intragroup variability and lack of a dose-response effect. For example, in females the mean (±SD) levels of ^{3}H-thymidine incorporation at 10 hours were 2026±1282, 2393±2111, 7696±10313 and 6021±4770 dpm/μg DNA at 10, 20, 40 and 100 mg/kg bw, respectively, compared to 728±715 dpm/μg DNA in the control group. An examination of individual animal data indicated that these apparent increases could have been due to outlying animals in each group with abnormally high readings. In addition, a substantial number of samples were lost (in some groups 4/5 samples) due to vials exploding following removal from liquid nitrogen. BHA increased ^{3}H-thymidine incorporation at 8 and 10 hours in both sexes (581±717 and 4921±3414 dpm/μg DNA in males and females, respectively, at 10 hours compared to 97±71 and 728±715, respectively in the controls).

Conclusions: Treatment of mice with a single oral gavage dose of dichlorvos resulted in histopathological effects on the epithelium of the forestomach similar to that caused by BHA including oedema, epithelial cell hypertrophy and hyperplasia. However, the severity of the effects seen with dichlorvos was less than with BHA. There was an equivocal increase in RDS and cell proliferation.

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APPENDIX I: History of public health considerations of dichlorvos in Australia

Date	Activity/outcome
March 1962	DPSC: Dichlorvos scheduled in S5 of the SUSDP
February 1963	DPSC: Fly sprays containing 1% Nankor and 0.5% dichlorvos to remain in S5
April 1964	DPSC: Concluded that OPs irrespective of their structural formula and degree of toxicity should not be included in insecticide products for use as household sprays
September 1964	DPSC: Concern that there could be rapid release of dichlorvos from PVC pest strips if overheated. Vapona slow-release strips scheduled in S6 of the SUSDP
July 1965	DPSC: S6 entry for dichlorvos confirmed
December 1965	DPSC: Concluded that in suitable circumstances toxic levels of dichlorvos vapour could be released from Vapona strips. S6 entry for dichlorvos confirmed
March 1966	DPSC: Oral toxicity comparable to other OPs in S6 of the SUSDP. S6 entry for dichlorvos confirmed.
December 1968	DPSC: New S5 for dichlorvos when impregnated in plastic resin strip material containing 20% or less dichlorvos
February 1970	DPSC: Noted letter from US DA advising manufacturers that dichlorvos pest strips were no longer permitted for use in food preparation areas and that the label should bear the statement “Do no use in kitchens, resturants or areas where food is prepared or served”. The following statement was also made mandatory: “Do not use in nurseries or rooms where infants, ill or aged persons are confined”.
November 1970	DPSC: Amend S5 entry for dichlorvos by the addition of “and when in aerosol preparations containing 1% or less dichlorvos”
February 1971	DPSC: New Appendix A entry for dichlorvos impregnated pest strips containing 20% or less dichlorvos (a) avoid usage in infant nurseries; (b) do not use in food cupboards; (c) do not use in places with the chronically ill
August 1971	DPSC: Appendix A entry amended to (a) Do not use in food preparation or food storage areas; (b) Do not use in nurseries and sick rooms where people may be continuously exposed
November 1972	DPSC: Confirmed S6 entry for all dichlorvos other than resin impregnated strips
June 1973	DPSC: Scheduling of Sectovap Pest Killer Lantern did not proceed due to the absence of information on the release potential of dichlorvos under all conditions including sucking by infants, and proof that fatal poisoning cannot occur
August 1974	DPSC: Sectovap Pest Killer Lantern to remain in S6 as the release rate of dichlorvos was too high to warrant a S5 classification
November 1974	DPSC: New S7 entry for dichlorvos. Amended S6 entry for dichlorvos “Dichlorvos in preparations containing 50% of less dichlorvos except when included in schedule 5”.
March 1976	DPSC: Confirmed poison’s schedules for dichlorvos. Concluded that labelling requirements for aerosol preparations were adequate
May 1978	DPSC: S5 entry amended by adding the words “in aerosol packs containing 10 grams or less of dichlorvos”
May 1981	DPSC: S5 entry amended as follows: DICHLORVOS (i) when impregnated in plastic resin strip material containing 20% of less dichlorvos; (ii) sustained release resin pellets for veterinary use containing 20% or less dichlorvos; and (iii) in aerosol lacks containing 10 grams or less of dichlorvos
May 1982	DPSC: In the light of a review article on the carcinogenicity of dichlorvos and correspondence between the author and the Shell Company it was agreed that that there was no justification to recommend further restrictions on the availability of dichlorvos but that the compound should be kept “under review”.
May 1982	PACC: On the basis of data provided to Shell Chemical Company on the carcinogenicity of dichlorvos, the Committee agreed that dichlorvos did not appear

Date	Activity/outcome
	to pose a carcinogenic risk to humans.
May 1983	DPSC: Amendment to Appendix A warning statement to read “Dichlorvos when impregnated in plastic resin strip material containing 20 per cent or less dichlorvos. New entry in Appendix B (First Aid Instructions): Dichlorvos when in sustained release resin pellets for veterinary use containing 20 per cent or less dichlorvos (a); and Dichlorvos in aerosol packs containing 10 g or less of dichlorvos (o). The committee further recommended an amendment to warning statement 21 to read “An anticholinesterase compound (to appear immediately below the approved name or thelast of declared ingredients on the label)”
August 1984	DPSC: Amendment to S5 dichlorvos entry to read DICHLORVOS (a) when impregnated in plastic resin material containing 20 per cent or less dichlorvos; (b) in sustained release resin pellets containing 20 per cent or less of dichlorvos for the treatment of animals; (c) in pressurised spray packs containing 10 grams or less of dichlorvos
November 1987	DPSC: Following consideration of a review of NTP chronic mouse and rat studies, the committee concluded that the occurrence of forestomach tumours may be irrelevent where inactivation in the body can occur. It was noted that COT would be reviewing this data.
Nov 1987	PACC: The ommittee noted the dichlorvos Working Party conclusion, which based on the review of available information did not identify any specific health hazards associated with currently approved uses of dichlorvos, including its use in household pest-strips, or trichlorfon. If there was a significant expansion of the use of trichlorfon carcinogenicity studies to meet current standards should be required. The Committee agreed to consider an ADI when the additional data on carcinogenicity had become available and had been reviewed.
February 1988	PACC: The Committee expressed concern over the increased incidence of forestomach tumours in mice. However, it was noted that major exposure in humans was via inhalation and the studies reporting these forestomach lesions had utilised gavage dosing. In addition, dichlorvos was a mild irritant and this may have influenced the result. The occurrence of panreatic tumours in rats was questionable in view of the use of corn oil as a vehicle, as a number of previous studies utilising corn oil as vehicle had demonstrated a similar effect. The committee noted that there was no evidence of any adverse effects in humans following long term exposure to dichlorvos. The committee agreed to establish an ADI of 0.005 mg/kg bw/d based a NOEL of 0.05 mg/kg bw.d in a 15 week rat study.
March 1988	SCOT: The committee considered that the findings of the NTP studies could not be dismissed but their significance was difficult to assess as the final reports were not yet available. The committee noted that the results of the NTP studies are at variance with previous oral studies but this may be due to the method of administration. The committee considered that inhalation studies would be more relevant to human exposure and recommended that the sposnors be asked to undertake such studies. Thte committee recommended that the DPSC review the scheduling of dichlorvos in the light of current uses with a view to minimising human exposure. The committee noted that there are no standards that appear to apply to the advertising of domestic insecticide preparations and recommended that the PHC consider the need for such standards.

Date	Activity/outcome
May 1988	DPSC: Amendment of paragraph (a) of the S5 entry for dichlorvos to read "when impregnated in plastic resin strip material containing 20 per cent or less of dichlorvos". Amendment of S6 entry for dichlorvos to read "DICHLORVOS in preparations containing 50 per cent or less of dichlorvos except when included in schedule 5 The Committee noted the draft report from the Committee on Toxicity dealing with the working party on dichlorvos and NTP carcinogenicity studies in rats and mice. The Committee "felt" that although carcinogenicity had not been proven, the widespread use of a genotoxic substance which may be a carcinogen was not recommended. The committee recommended that more data on user exposure levels and a chronic inhalational study would prove useful in reviewing the scheduling of dichlorvos.
February 1989	DPSC: The committee considered an overall review of the carcinogenicity potential for dichlorvos prepared by the Environmental Health Branch of the Commonwealth Department of Health. Also considered was an executive summary prepared by the US subsidary of Shell in response to US EPA concerns. On the basis of this information the committee concluded that the evidence for carcinogenicity was weak and was found only under two conditions, both of which are inappropriate to human exposure patterns. Bayer was requested to forward to the DPSC all human epxosure studies not previously provided.
February 1989	PACC: The committee did not believe that the NTP rat study, which is confounded by the high and variable historical control incidence of the observed tumours, and the use of corn oil gavage administration when considered in the light of previous studies, indicates that there is a carcinogenic hazard to humans.
Jun 1989	SCOT: Although administration of dichlorvos may prove carcinogenic in animals under certain test conditions, it is not likely to be carcinogenic under conditions of exposure relevant to humans. Based on inhalation studies in humans an NEL for ChE inhibition is approximately 0.03 mg/kg bw/d. Recommended that the report be referred for consideration by DPSC.
June 1991	DPSSC: Following the consideration of an evaluation of supplementary data, the committee had no objection to the clearance of the clearance of dichlorvos sourced from Bayer and Ciba-Geigy.
Nov 1991	PACSC: Agreed to clearance of dichlorvos sourced from Bayer and Ciba-Geigy.
Nov 1991	SCOT: The Committee concluded that new genotoxicity data was consistent with previous information and provided clear evidence that genotoxicity was induced where metabolism was not a factor. The weight-of-evidence from lon term oral bioassays and genotoxicity studies support the conclusion that dichlorvos is carcinogenic in experimental animals and should be regarded as if it poses a carcinogenic hazard to humans. There was insufficient data to determine the carcinogenic hazard posed to humans exposed to dichlorvos via the dermal or inhalational routes. A clear understanding of the mechanism of carcinogenicity of dichlorvos is not available. It was noted that it may be useful to perform a 2-stage skin carcinogenicity study to determine whether genotoxic activity of dichlorvos in skin could subsequently lead to skin tumours. The DPSC should review use patterns with a view to minimising dermal and inhalation exposure.
Aug 1992	PACSC: The Committee noted that new genotoxicity data had been reviewed by SCOT and that the DPSSC was to review the use patterns for dichlorvos with a view to minimising dermal and inhalational exposure.
Nov 1992	DPSSC: Decided that it was undesirable that people be exposed to dichlorvos and recommended that First Aid and Safety Directions Panel undertake a review of the labels of registered dichlorvos products in relation to safety directions.
Nov 1994	NDPSC: Consideration of the rescheduling of dichlorvos from S6 for a product containing 80 g/kg dichlorvos and 800 g/kg naphthalene for use in "wheelie bins". The sponsor was requested to provide further data on the slow release characteristics of the block under both alboratory and operating conditions.

Date	Activity/outcome
	Information on the stability of the product would also be required.
Feb 1996	ACPH: Noted that concerns about the carcinogenic potential of dichlorvos have been assuaged by the interpretation that the adverse effects are not likely to affect humans or pose a hazard if used in accordance with safety directions. Recommended that the NRA review the labels of dichlorvos products to discourage situations in which long-term human exposures could arise.
Oct 1998	ACPH: Considered that equivocal carcinogenicity findings in rodents following oral gavage administration were not necessarily relevant to the human risk assessment of dichlorvos. Supported a revision to the existing ADI of 0.0005 to 0.001 mg/kg bw/d on the basis of the NOEL of 0.013 mg/kg bw/d for plasma ChE inhibition in human volunteers in a 28-day oral dosing study.

ACPH = Advisory Committee on Pesticides and Health; PACC = Pesticide and Agricultural Chemicals Committee; PACSC = Pesticide and Agricultural Chemicals Standing Committee; DPSC = Drugs and Poisons Schedule Committee; DPSSC = Drug and Poisons Schedule Standing Committee; SCOT = Standing Committee on Toxicity; NDPSC = National Drug and Poisons Schedule Committee

APPENDIX II: List of clinical Chemistry, Haematology & Urinalysis Parameters

Clinical Chemistry	Haematology	Urinalyses
albumin alkaline phosphatase bilirubin (total) calcium chloride cholesterol (total) cholinesterase activity creatinine (blood) gamma-glutamyl transpeptidase globulin glucose (blood) LDH (serum lactate dehydrogenase) phosphorus potassium protein (total) SGPT (serum alanine aminotransferase) SGOT (serum aspartate aminotransferase) sodium triglycerides urea nitrogen (blood) CPK (creatinine phosphokinase)	clotting parameters (clotting time, prothrombin time) erythrocyte count haematocrit (packed cell volume) haemoglobin leucocyte differential count leucocyte total count platelet count reticulocyte count MCH MCHC MCV blood smear	appearance specific gravity glucose ketones sediment (microscopic) occult blood pH protein volume bilirubin urobilinogen reducing substances

APPENDIX III: Organs for weight determination and histopathological examination

Organs Weighed	Tissues Examined		
Adrenals	Adrenals	heart	prostate
Brain	aorta	ileum	rectum
Gonads	blood smear	jejunum	salivary gland
Heart	bone	kidneys	seminal vesicle
Kidneys	bone marrow	lacrimal gland	skin
Liver	brain (3 levels)	liver	spinal cord (cervical
Spleen	cecum	lungs	thoracic, lumbar)
Thyroid	colon	lymph nodes	spleen
(w/parathyroid)	duodenum	mammary gland	sternum
	epididymes	muscle (smooth)	stomach
	eyes	muscle (skeletal)	testes
	eyes (optic nerve)	nerve (peripheral)	thymus
	gall bladder	oesophagus	thyroid (w/parathyroid)
	Harderian glands	ovaries	trachea
	head - 3 sections	pancreas	urinary bladder
	(nasal cavity, para-	pituitary	uterus
	nasal sinus, tongue,		vagina
	oral cavity, naso-		Zymbal's gland
	pharynx, inner-ear)		gross lesions

APPENDIX IV: Reproductive and Developmental Indices

I

number of males/females with confirmed mating*
Male/female mating index (%) = x 100
number of males/females placed with females/males
* defined by females with vaginal sperm or that gave birth to a litter or with pups/fetuses in utero

number of males proving their fertility*
Male fertility index (%) = x 100
number of males placed with females/males
* defined by a female giving bith to a litter or with pups/fetuses in utero

number of females pregnant*
Female fertility index (%) = x100
number of females mated**
* defined as the number of females that gave birth to a litter or with pup/fetuses in utero
** defined as the number of females with vaginal sperm or that gave birth to a litter or with pups/fetuses in utero

number of females with live pups on the day of birth
Gestation index (%) = x 100
number of females pregnant*
* defined as the number of females that gave birth to a litter or with pups/fetuses in utero

number of liveborn pups at birth
Live birth index (%) = x 100
total number of pups born

number of live pups on day 4* after birth
Viability index (%) = x 100
number of liveborn pups on the day of birth
* before standardisation of litters (i.e. before culling)

number of live pups on day 21 after birth
Lactation index (%) = x 100
number of live pups on day 4* after birth
* after standardisation of litters (i.e. after culling)

number of live male or female pups on day 0/21
Sex ratio = x 100
number of live male and female pups on day 0/21

number of pregnant animals
Conception rate (%) = x 100
number of fertilised animals

number of corpora lutea – number of implantations
Preimplantation loss (%) = x 100
number of corpora lutea

number of implantations – number of live foetuses
Postimplantation loss (%) = x 100
number of implantation

APPENDIX V: Standard FOB parameters

Observations	Parameters
Home cage observations	Posture, convulsions, faeces consistency, biting, palpebral (eyelid) closure
Handling observations	Ease of removal from cage, lacrimation/chromodacryorrhea, piloerection, palpebral closure, red/crusty deposits, eye prominence, ease of handling, salivation, fur appearance, respiratory rate/character, mucous membranes/eye/skin colour, muscle tone
Open field observations	Mobility, rearing. convulsions/tremors, grooming, bizarre/stereotypic behaviour, time to first step, gait, arousal urination/defecation, gait score, backing
Sensory observations	Approach response, startle response, pupil response, forelimb extension, air righting reflex, touch response, tail pinch, eye blink response, hindlimb extension, olfactory orientation
Neuromuscular observations	Hindlimb extensor strength, hindlimb foot splay, grip strength-hind and forelimb, rotarod performance
Physiological observations	Catalepsy, body temperature, bodyweight

APPENDIX VI: Summary of *in vitro* and *in vivo* gentoxicity findings for dichlorvos.

The summary Tables below are a modified and edited version of that published in the 1993 JMPR toxicology monograph on dichlorvos (Pesticide Residues in Food – 1993; WHO 1994). Many of the studies cited in these Tables are from early published papers and did not provide full details on methodology or experimental results. These studies were not amenable to independent assessment and so are of limited regulatory value.

In vitro studies on microorganisms

Test system	Test object		Concentration	Purity	Results	Reference
Mitotic non-disjunction and crossing over	*A. nidulans* strain p (plate test)		≤ 0.8 mg/mL	?	+ Results given as +ve or -ve with no other information	Morpurgo et al, 1979
	A. nidulans strain p (plate test)		28 mg/disc	? ('pure std')	+	Bignami et al, 1977
Recombinant assay	B. subtilis	H17 Rec+	2 mg/plate	?	-	Shirasu et al, 1976
		M45 Rec-	"		+	
Forward mutation	A. nidulans	strain 35	14 mg/disc	? ('pure std')	+	Bignami et al, 1977
	E. coli	B	5-25 mmol/L	95%	+ Positive control MMSA yielded positive response	Wild, 1973
		Gal RS	?	?	+	Fahrig, 1974*
		K12(5-MT)	0.3-3.2 mmol/L	?	+ Positive controls β-propiolactone, MNNG, MMSA were positive	Mohn, 1973
	S. coelicolor (spot test)	A 3(2) his A1	5.6 mg/disc	99.9%	+ Positive controls used to calibrate assay	Carere et al, 1978 a,b
Reverse mutation	E. coli	B/r WP2	5 mg/plate	> 97%	+ Plus/minus S9; 20 mM cysteine did not attenuate mutagenic response	Moriya et al, 1978
	S. typhimurium	TA 1535	5 mg/plate	> 97%	+ Negative with S9; 20 mM cysteine abolished mutagenic response	
	E. coli	WP2 hcr	up to 5 mg/plate	?	+ Plus/minus S9; 20 mM cysteine did not attenuate mutagenic response	Moriya et al, 1983*
	S. typhimurium	TA 98	up to 5 mg/plate	?	-	
		TA 100	up to 5 mg/plate	?	+	
		TA 1535	up to 5 mg/plate	?	?	

Test system	Test object		Concentration	Purity	Results	Reference
		TA 1537, 1538	up to 5 mg/plate	?	-	
	E. coli	B/r Wp2( ); SR714	0.04-2.3 mmol/L	99%	+ Plus/minus S9; 20 mM cysteine did not attenuate mutagenic response	Houk & DeMarini, 1987*
		CM 561	0.2%	99%	+ Positive control MMSA - yielded positive response	Bridges et al, 1973
		CM 571; CM 611	0.2%	99%	- Positive control MMSA - yielded negative response	
		WP2	0.2%	99%	+ Positive control MMSA - yielded positive response	
		WP2 uvr A	0.2%	99%	+ Positive control MMSA - yielded positive response	
		WP2	1 drop analytical or technical grades or 10% aq sol/plate	?	- Positive controls MMSA, MNNG, β-propiolactone, nitrogen mustard, yielded positive responses	Dean, 1972a
		K12HfrH	0.1% v/v	97.5%	+	Voogd et al, 1972
	C. freundii	425	0.05% or 0.1% v/v	97.5%	+ At 0.1%	
	E. aerogenes		0.1% v/v	97.5%	+	
	K. pneumoniae		0.05% or 0.1% v/v	97.5%	+	
	S. typhimurium	64-320	0.05% or 0.1% v/v	97.5%	+	
	S. marcescens	Hy/alpha 13 Hy/alpha 21	1.25-5 mg/disc	?	+ Positive controls TMPA, EMSA, DMPA yielded +ve responses	Dean, 1972a
	E. coli	WP2 (fluctuation test)	5 µg/mL	?	+ Positive control MMSA yielded positive response	Green et al, 1976
		WP2 hcr+/hcr-	20-25 µl/disk	diluted 50% commercial formulation	+ Positive controls MNNG, NMU, acridium chloride yielded positive responses	Nagy et al, 1975

Test system	Test object		Concentration	Purity	Results	Reference
		CM 881	≥ 0.1 µg/mL	?	+ No results given. Paper concerned assay development	Bridges, 1978
	S. typhimurium	TA 1535	5 mg/plate		+	Shirasu et al, 1976
		TA 1536	5 mg/plate	?	- (JMPR recorded this result as positive)	
		TA 1537, 1538	5 mg/plate	?	-	Shirasu et al, 1976
	E. coli	WP2 hcr+/hcr-	5 mg/plate	?	+	
		WP2 uvr A, WP 67	5-10 µL/plate[2]	?	+ Results given as +ve with no other information	Hanna & Dyer, 1975
	CM 561, CM571 CM 611, WP12		5-10 µL/plate[2]	?	- Results given as -ve with no other information	
	S. typhimurium	TA 1530, 1535	5-10 µlLplate	?	+ Results given as +ve with no other information	
		his C117, G46	5-10 µL/plate	?	- Results given as -ve with no other information	
		TA 1531, 1532, 1534 TA 1536, 1537, 1538	5-10 µL/plate	?	- (results given as +ve or -ve with no other information)	
	P. aeroginusa	PAO 38	0.08 mol/L	?	+	Dyer & Hanna, 1973*
	S. typhimurium	his C117	0.03 mol/L	?	+	
	S. typhimurium	TA 98	?	?	- Plus/minus S9	Braun et al, 1982*
		TA 100	?	?	+ Plus/minus S9	
		TA 1535, 1536 TA 1537, 1538	?	?	- Plus/minus S9	
		TA 98	0.1-6.7 mg/plate[3]	99%	- Plus/minus S9	Chan, 1989; Zeiger et al, 1988
		TA 100	0.1-6.7 mg/plate[3]	99%	+ Plus/minus S9	
		TA 1535, 1536 TA 1537, 1538	2.8 mg/plate	99.9%	- Plus/minus S9	Carere et al, 1978 a,b

Test system	Test object		Concentration	Purity	Results	Reference
		TA 1535 (Liquid test)	1.5 mg/ml	99.9%	+	
	Schizosaccharmoyces pombe ade 6		1.5-14 mmol/plate[4]	> 99%	+ Plus/minus S9	Gilot-Delhalle et al, 1983*
Gene conversion	S. cerevisiae	D4	0.25-8 mg/ml	> 97%	+ From 4 mg/ml; +ve control EMSA yielded positive response	Dean et al, 1972
		D4	6-40 mmol/l	?	+ Positive control MMSA yielded positive response	Fahrig, 1973, 1974*
		632/4	?	?	-	Guerzoni et al, 1976*
DNA strand breaks	E. coli	K-12CR34Co1E 1	1 mg/ml	?	+ Positive controls MMSA, MNNG, yielded positive responses	Griffin & Hill, 1978*
	E. coli	WP67	0.1% v/v	99%	+	Bridges et al, 1973
Growth inhibition	E. coli	W3110 pol A+/pol A-	6.4 mmol/l	?	+	Rosenkranz, 1973
	P. mirabilis	PG 273; PG 713	?	?	+ From 4 mg/ml	Braun et al, 1982*

+ = positive for genotoxicity; - = negative for genotoxicity * = not independently reviewed by the Australian authorities but cited in JMPR 1994; 1 = Without metabolic activation except where noted; 2 = toxic dose; 4 = The LD50 was 5.5 mmol/l; 5 = Toxic effect at 3.3 mg/plate. MMSA = methyl methanesulfonate; EMSA = ethyl methanesulfonate; MNNG = N-methyl-N'-nitro-nitroso guanidine; NMU = N-nitroso-N-methyl urethane; TMPA = trimethyl phosphate; DMPA = dimethyl propiolactone.

In vitro on mammalian cells

Test system	Test object	Concentration	Purity	Results	Reference
Viral transformation	Syrian hamster embryo cells/adenovirus SA7	0.05-0.45 mmol/l cytotoxic	?	+ Positive controls (benzo(a)pyrene 0.001-0.002 mmol/l) yielded expected responses.	Hatch et al, 1986*
Gene mutation	Chinese hamster V79 cells (azaguanine/ouabain resistance)	up to 1 mmol/l	?	-	Wild, 1975*
		1.25-5 mmol/l	?	- (JMPR recorded this as a positive result). Positive controls (ethyl methane sulfonate 20 mmol/l) yielded expected responses.	Aquilina et al, 1984
	Mouse lymphoma L5178Y cells (trifluorothymidine resistance)	6.25-50 nl/ml[1]	99%	+ From 12.5 nl/ml. Positive controls (methyl methane sulfonate 5 nl/ml) yielded expected responses.	Chan, 1989
		12.5-200 nl/ml[2]	99%	+ From 100 nl/ml. Positive controls (methyl methane sulfonate 5 nl/ml) yielded expected responses.	Chan, 1989
DNA strand breaks	Chinese hamster V79 cells	0.2%	?	+ Negative at lower concentrations. Positive controls (methyl methane sulfonate 0.025%) yielded expected responses.	Green et al, 1974
Sister chromatid exchange	Primary rat tracheal epithelial cells	5-160 µg/ml[3]	93.9%	+ From 10 µg/ml. Positive controls (N-methyl-N'-nitro-N-nitroso guanidine 0.25-1 µg/ml, 50% mortality at 0.5 µg/ml) yielded expected responses.	Lin et al, 1988*
	Chinese hamster ovary cells	0.03-0.1 mmol/l	98%	+	Nishio & Uyeki, 1981*
		0.1-0.5 mmol/l	> 98%	+	Tezuka et al, 1980*
		0.3-1000 µg/ml	?	+ From 40 µg/ml.	Wang et al, 1988*
		1-500 µg/ml	?	+ From 10 µg/ml without S9 and from 300 µg/ml with S9. Positive controls (0.005 µg/ml mitomycin C, 1.5 µg/ml cyclophosphamide) yielded expected responses.	Chan, 1989
Sister chromatid exchange	Human lymphocytes	2.5-10 µg/ml	99%	-	Nicholas et al, 1978*
	Human fetal lung fibroblasts		99%	-	
Chromosomal aberrations	Rat tracheal epithelial cells	5-160 µg/ml[3]	93.9%	+ From 80 µg/ml. Positive controls (0.25-1 µg/ml N-methyl-N'-nitro-N-nitroso guanidine, 50% mortality at 0.5 µg/ml) yielded expected responses.	Lin et al, 1988*

Test system	Test object	Concentration	Purity	Results	Reference
	Chinese hamster lung fibroblasts	?	?	+	Ishidate et al, 1981*
	Chinese hamster V79 cells	0.1-0.5 mmol/l	> 98%	+ At 0.5 mmol/l.	Tezuka *et al,* 1980*
	Chinese hamster ovary cells	16-1000 µg/ml	99%	+ At 160 µg/ml with and without S9. Positive controls (0.25 µg/ml mitomycin C, 50 µg/ml cyclophosphamide) yielded expected responses.	Chan, 1989
	Human lymphocytes	1-40 µg/ml 5-500 µg/ml	>99% 99.8%	Cytotoxicity was observed at 5-40 µg/ml. +	Dean, 1972b Procco & Fini, 1980
	EUE human cells	6.5-650 mmol/l	?	+ Positive controls (N-methyl-N'-nitro-N-nitroso guanidine) yielded expected responses.	Aquilina et al, 1984
DNA (sedimentation coefficient)	Calf thymus DNA	0.1%	?	+	Rosenkranz & Rozenkranz, 1972*
DNA (thermolabile regions)		45 mmol/l	99%	-	Olinski et al, 1980*
DNA (resistance to micrococcal)	Chinese hamster ovary cells	10 mmol/l	?	+	Nishio & Uyeki, 1982*

+ = positive for genotoxicity; - = negative for genotoxicity; * - not independently reviewed by the Australian authorities but cited in JMPR 1994. 1 Growth inhibition from 12.5 nl/ml; 2 Growth inhibition from 100 nl/ml; 3 50%

In vivo studies

Test system	Test object	Concentration	Purity	Results	Reference
Crossover / recombination	Drosophila melanogaster ++++/dp b cn bw	0.035% (approx. LD50)	?	-	Jayasuriya & Ratnayaka, 1973*
Sex-linked recessive lethal mutation	Drosophila melanogaster	0.035% (approx. LD50)	?	-	Jayasuriya & Ratnayaka, 1973*
	Oregan K	0.6-600 nmol/l	95%	- Positive controls (25 mmol/l ethyl methanesulfonate) yielded expected responses.	Sobels & Todd, 1979

Test system	Test object	Concentration	Purity	Results	Reference
	Oregan K	0.01-0.1 ppm in food	? 100 EC commercial formulation	-	Kramers & Knapp, 1978
Chromosomal aberration	Drosophila melanogaster	1 ppm in food	?	+	Gupta & Singh, 1974*
Autosomal recessive levels	Drosophila melanogaster	0.75 ppm in food for 18 months	?	+	Hanna & Dyer, 1975
Host-mediated assay	*Salmonella typhimurium* G46 His- in mice (NMRI)	25 mg/kg SC	?	-	Buselmayer et al, 1972*
	Salmonella typhimurium (64-320) in mice (Swiss)	8-10 mg/kg PO	97.5%	-	Voogd et al, 1972
	Serratia marcescens (a 21 Leu-) in mice (NMRI)	25 mg/kg SC	?	-	Buselmayer et al, 1972*
	Sacchoromyces cerevisiae (D4) in mice (CF1)	0, 50 or 100 mg/kg PO or 60, 99 mg/m^3 inhalation for 5 h	> 97%	- Positive controls (400 mg/kg PO ethyl methanesulfonate) yielded expected responses.	Dean et al, 1972
Dominant lethal	Female mice (CF1)	0, 25 or 50 mg/kg PO	?	- Positive controls (100 mg/kg IP methyl methanesulfonate) did not indicate sensitivity of the assay.	Dean & Blair, 1976
		inhalation at 0, 2 or 8 mg/m^3 from weaning to 11 wks of age	?	-	Dean & Blair, 1976
	Male mice (ICR/Ha Swiss)	0, 5 or 10 mg/kg/d PO for 5 d (8 wks of mating)	?	-	Epstein et al, 1972*
		Single IP injection of 13 or 16.5 mg/kg (8 wks of mating)	?	-	Epstein et al, 1972*
Dominant lethal (continued)	Male mice (Q)	2 ppm in drinking water, 5 d/wk for 7 wks	99%	-	Degraeve et al, 1984a*

Test system	Test object	Concentration	Purity	Results	Reference
		10 mg/kg IP	99%	- Positive controls in 2nd and 5th week of mating.	Moutschen-Dahmen et al, 1981*
	Male mice (Balb/c)	6 mg/kg IP single dose or 5 daily doses	? 50% commercial formulation	- Positive controls (cyclophosphamide 40 mg/kg IP) yielded only weakly positive responses.	Dzwonkowska & Hübner, 1991
	Male mice (CF1)	inhalation at 30 or 55 mg/m^3 for 16 h	> 97%	- Positive controls (200 mg/kg IP ethyl methanesulfonate and 1000 mg/kg IP trimethyl phosphate) yielded expected responses.	Dean & Thorpe, 1972b
		inhalation at 2.1 or 5.8 mg/m^3 for 23 h/d for 4 wks	> 97%	- Positive controls (200 mg/kg IP ethyl methanesulfonate and 1000 mg/kg IP trimethyl phosphate) yielded expected responses.	Dean & Thorpe, 1972b
Sister chromatid exchange	Male mice (B6C3F1) peripheral lymphocytes	5-35 mg/kg IP	99%	- Positive controls (2-acetylaminofluorene, ethylmethane sulfonate and N-nitroso morpholine) yielded expected results.	Kligerman et al, 1985*
Sister chromatid exchange continued	Male mice (B6C3F1) bone marrow cells	6.25-25 and 10-40 mg/kg IP	99%	- Positive controls (ethyl methane sulfonate 100 mg/kg PO) yielded expected results.	Chan, 1989
Micronucleus test	Mice (Swiss Webster)	0.0075-0.015 mg/kg/d IP for 2 or 4 d	?	- Positive controls (cyclophosphamide 30-240 mg/kg IP) yielded expected positive responses.	Paik & Lee, 1977*
Micronucleus test (in vitro/in vivo)	Mice (HRA/Skh, hairless) skin keratinocytes	skin painting, single dose of 0-228 µg (in 100 µl acetone)	? tech.grade	+ Positive control (urethane 5 mg) yielded a positive response. Recovery of plated cells in vitro after a single topical application of dichlorvos was about 50% of controls. A statistically significant dose related increase in micronuclei was reported in dichlorvos treated groups.	Tungul et al, 1991
Chromosomal aberrations	Chinese hamster both sexes, bone-marrow cells	15 or 2x10 mg/kg PO	> 97%	- Positive controls (endoxan 100-200 mg/kg IP or 50 mg/kg PO) yielded expected responses.	Dean & Thorpe, 1972a

Test system	Test object	Concentration	Purity	Results	Reference
	Male mice (Q) bone-marrow cells	2 ppm in drinking water, 5 d/wk for 7 wks	99%	-	Moutschen-Dahmen et al, 1981*; Degraeve et al, 1984b*
	Male mice (Q) bone-marrow cells	10 mg/kg IP	99%	-	Degraeve et al, 1984b*
	Male mice (B6C3F1) bone marrow cells	6.25-25 and 10-40 mg/kg IP	99%	- Positive controls (ethyl methane sulfonate 300-375 mg/kg PO) yielded expected responses.	Chan, 1989

Test system	Test object	Concentration	Purity	Results	Reference
Chromosomal aberrations (continued)	Female Syrian golden hamster bone-marrow cells	0, 3, 6, 15, or 30 mg/kg IP	? 50% commercial formulation	+ LD50 = 30 mg/kg IP Statistically significant increases were seen at the 2 highest doses but there was no dose related response. Positive controls (cyclophosphamide 40 mg/kg) yielded expected responses.	Dzwonkowska & Hübner, 1986
	Mice (CF1) both sexes, bone-marrow cells	inhalation at 64-72 mg/m^3 for 16h	> 97%	- Positive controls (endoxan 100-200 mg/kg IP or 50 mg/kg PO) yielded expected responses.	Dean & Thorpe, 1972a
		inhalation at 5 mg/m^3 23 h/d for 21 days			
	Male Chinese hamsters bone-marrow cells	inhalation at 28-36 mg/m^3 for 16h.			
	Mice (Q) spermatocytes	2 ppm in drinking-water 5 d/wk for 7 wks	99%	-	Moutschen-Dahmen et al, 1981*; Degraeve et al, 1984a*
	Chinese hamsters spermatocytes	15 mg/kg PO	> 97%	- Positive controls (endoxan 100 mg/kg PO) caused chromatid but not chromosomal aberrations.	Dean & Thorpe, 1972a
	Mice (Q) spermatocytes	10 mg/kg IP	99%	-	Moutschen-Dahmen et al, 1981*; Degraeve et al, 1984a*

Test system	Test object	Concentration	Purity	Results	Reference
	Mice (CF1) spermatocytes	inhalation at 64-72 mg/m^3 for 16h	> 97%	- Positive controls (endoxan 200 mg/kg IP) caused chromatid but not chromosomal aberrations.	Dean & Thorpe, 1972a
		inhalation at 5 mg/m^3 23h/d for 21 days	> 97%	- Positive controls (endoxan 200 mg/kg IP) caused chromatid but not chromosomal aberrations.	Dean & Thorpe, 1972a
	Chinese hamster spermatocytes	inhalation at 28-36 mg/m^3 for 16 h	> 97%	- Positive controls (endoxan 100 mg/kg IP) caused chromatid but not chromosomal aberrations.	Dean & Thorpe, 1972a
	Mice (CF1) spermatogonia	2 ppm in drinking-water, 5 d/wk for 7 wks	99%	-	Moutschen-Dahmen et al, 1981*; Degraeve et al, 1984b*
		10 mg/kg IP	99%	-	Moutschen-Dahmen et al, 1981*; Degraeve et al, 1984b*
DNA strand breaks	Rats (Wistar), both sexes rat liver cell DNA	10 mg/kg IP	99.8%	- Positive controls (methyl methanesulfonate 30-60 mg/kg IP) yielded expected responses.	Wooder & Creedy, 1979*
Unscheduled DNA synthesis	Male rats (F344) hepatocytes	0, 2, 10 or 35 mg/kg PO	?	-	Mirsalis et al, 1989*
	Mice (B6C3F1) (both sexes) forestomach rats	0, 10, 20, 40 or 100 mg/kg PO	> 99%	- Positive controls (N-methyl-N'-nitro-N-nitroso guanidine 200 mg/kg PO) yielded weakly positive results. Dichlorvos treatment induced hyperplasia similar to that induced by 300 mg/kg PO butylated hydroxyamisole (a non-genotoxic carcinogen).	Benford et al, 1994

+ = positive for genotoxicity; - = negative for genotoxicity; * - not independently reviewed by the Australian authorities but cited in JMPR 1994. 1 Growth inhibition from 12.5 nl/ml; 2 Growth inhibition from 100 nl/ml; 3 50%

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