

CHLORPYRIFOS-METHYL

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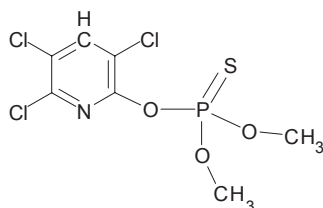
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Explanation

Chlorpyrifos-methyl ([Figure 1](#)) is the International Organization for Standardization (ISO)–approved name for *O,O*-dimethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate (Chemical Abstracts Service [CAS] No. 5598-13-0). Chlorpyrifos-methyl is an organophosphorus compound that acts

against insects. The mechanism of action is inhibition of acetylcholinesterase activity. Chlorpyrifos-methyl was evaluated previously by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) in 1975, 1991 and 1992, when an acceptable daily intake (ADI) of 0–0.01 mg/kg body weight (bw) was established. In 2001, the Meeting concluded that an acute reference dose (ARfD) for chlorpyrifos-methyl was not necessary. Chlorpyrifos-methyl was reviewed at the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues. New studies of dermal and inhalation exposure in rats, genotoxicity in vivo, reproductive toxicity and inhibition of neuropathy target esterase (NTE) had been made available since the last full review in 1992.

Figure 1. Structure of chlorpyrifos-methyl



Most of the pivotal studies met the basic requirements of the relevant Organisation for Economic Co-operation and Development (OECD) or national test guidelines, although the level of detail in some of the reports did not always match current requirements. A number of studies did not contain certificates of compliance with good laboratory practice (GLP). The available studies in human volunteers were considered to have been performed according to contemporary ethical standards. The overall database is considered adequate for deriving reference doses.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Rats

In a 1971 (non-GLP, non-guideline) study, two male Sprague-Dawley rats (~200 g) were gavaged with ^{14}C -radiolabelled chlorpyrifos-methyl (purity >99%; 377 MBq/mmol; labelled at positions 2 and 6 on the pyridyl ring) at a dose level of 16 mg/kg bw (in corn oil). Urine, faeces and expired carbon dioxide were collected for up to 72 h. Blood samples were taken from the tail at intervals throughout the study period. Radioactivity levels were determined by liquid scintillation counting or combustion analysis (faecal samples). Urinary metabolites were identified by thin-layer chromatography (TLC) and autoradiography.

Peak blood radioactivity levels were reported at 5 h, at which point the amount of test material in the blood was calculated to be approximately 3.0% (2.4–3.7%; absolute levels not given) of the administered dose. Levels of radioactivity indicated rapid clearance from blood, and the peak level seen at 5 h had declined by 50% at approximately 9 h following administration, suggesting an initial half-life of approximately 4 h. Excretion of radioactivity was found to be primarily in the urine (Table 1) and largely within 24 h of administration. Radioactivity levels in expired air were minimal.

Table 1. Excretion of radioactivity following a single oral dose of chlorpyrifos-methyl (16 mg/kg bw) to rats

Parameter	Radioactivity excreted in 0–72 h (% of administered dose)
Urine	83–85
Faeces	7–9
Expired air	0.23–0.43
Total residual	0.65–1.3
Total recovery	91–95

From Branson & Litchfield (1970)

Table 2. Residual tissue radioactivity at 72 h following a single oral dose of chlorpyrifos-methyl (16 mg/kg bw) to rats

Tissue	Radioactivity (mg equivalent/kg)	
	Animal A	Animal B
Red blood cells	0.013	0.017
Serum	nd	nd
Liver	0.075	0.108
Spleen	0.039	0.021
Kidneys	0.035	0.074
Adrenals	0.038	0.342
Heart	0.003	0.019
Brain	0.005	0.006
Muscle	0.043	0.164
Fat	0.057	0.156
Bone	0.041	0.634

From Branson & Litchfield (1970)

nd, not detected

Residual tissue radioactivity levels at 72 h were low in all tissues (<1 part per million [ppm]), but varied markedly between the two animals (Table 2) (Branson & Litchfield, 1970).

In a 1976 (non-GLP, non-guideline) study, 10 male rats (strain not specified; ~330 g) were gavaged with a single dose of approximately 30 mg/kg bw of [¹⁴C]chlorpyrifos-methyl (radiopurity >99%; 25.5 kBq/rat) in 0.5 ml ethanol. Urine and faeces were collected for up to 48 h, when rats were terminated. Radioactivity was quantified by scintillation counting (urine) or combustion analysis (faeces). Overall recovery was low (<80%). Radioactivity was found to be excreted primarily in urine (mean 64% of the administered dose) and largely within 24 h of administration. No tissue residues were detected at 48 h (Bakke & Price, 1976).

A briefly reported study of the comparative kinetics of chlorpyrifos (Dowco 179) and chlorpyrifos-methyl (Dowco 214) in rats showed chlorpyrifos to be more rapidly absorbed (time to peak concentration in blood [$T_{\max}$] = 3 h) than chlorpyrifos-methyl ($T_{\max}$ = 5 h). Both compounds had similar peak concentrations in blood ($C_{\max}$ values). Chlorpyrifos was excreted more rapidly than chlorpyrifos-methyl, but plasma levels of radiolabel from both compounds were very low at 24 h post-dosing (Branson & Litchfield, 1970).

Table 3. Recovery of radioactivity from rat skin samples ($n = 6$) exposed to [^{14}C]chlorpyrifos-methyl after 28 h

Sample	Mean recovery of radioactivity (%)	
	Concentrate	1% aqueous dilution
Receptor fluid	3.32 $\pm$ 0.29	1.08 $\pm$ 0.48
Skin	23.40 $\pm$ 6.46	32.55 $\pm$ 3.06
Surface wash	66.63 $\pm$ 8.31	60.12 $\pm$ 5.12
Cell wash	4.37 $\pm$ 1.32	4.11 $\pm$ 1.29
Additional vial wash	0.17 $\pm$ 0.15	0.26 $\pm$ 0.18
Total mass balance	97.9	98.1

From Perkins (1995)

(b) Dermal route

The dermal absorption of Reldan 22 (224 g/l chlorpyrifos-methyl, solvent based) was assessed in vitro using full-thickness male Fischer 344 rat skin. Single (10 μl) 28 h applications of ^{14}C -labelled test material (undiluted or 1% aqueous dilution) were made to the epidermal surface of skin discs held in occluded flow diffusion cells at 31 °C. Receptor fluid (tissue culture medium containing 6% polyethylene glycol 20 oleyl ether) was collected at 2 h intervals. The solubility of chlorpyrifos-methyl in the receptor fluid was confirmed to be higher than the applied dose. For the concentrate, total recovery of radioactivity was high (92.27–99.32%). A lag time for penetration of 4–6 h was observed; this was followed by linear absorption over the following 24 h. For the spray strength dilution, total recovery of radioactivity was also high (95.05–100.89%). Distribution of radioactivity was consistent for all of the six replicates. A lag time for penetration of 8–10 h was observed; this was followed by linear absorption over the remainder of the study. The linear absorption rate was calculated to be 10.4 $\mu\text{g}/\text{cm}^2$ per hour for the concentrate and 0.039 $\mu\text{g}/\text{cm}^2$ per hour for the 1% dilution. Dermal penetration was low for both formulation types (Table 3) (Perkins, 1995).

1.2 Biotransformation

Rats

The urinary metabolites of chlorpyrifos-methyl were investigated by TLC using urine samples collected as part of a previously described study (Branson & Litchfield, 1970). The primary metabolite was identified by co-chromatography as 3,5,6-trichloro-2-pyridinol (TCP). Additional non-mobile material was stated to be conjugated TCP. Metabolites were not quantified, and faecal metabolites were not investigated (Branson & Litchfield, 1970).

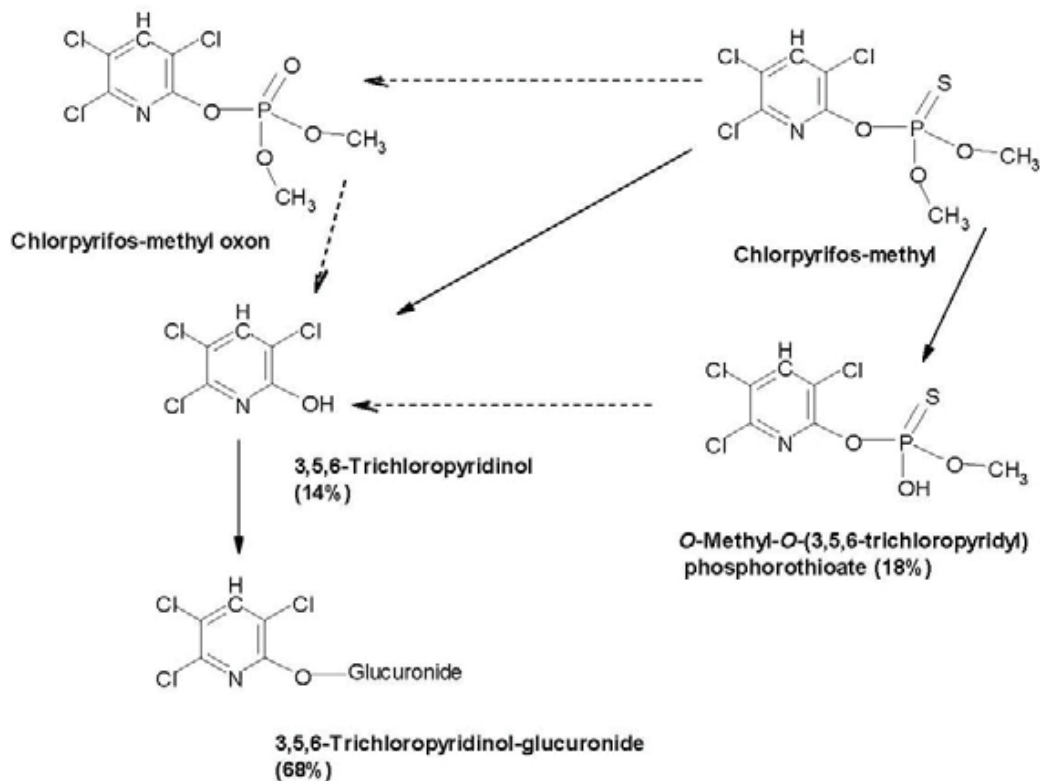
Urinary metabolites from the study of Bakke & Price (1976) were identified by gas-liquid chromatography, TLC and mass spectrometry as TCP (13.8%), its glucuronide conjugate (68.6%) and the desmethyl metabolite *O*-methyl-*O*-(3,5,6-trichloropyridyl) phosphorothioate (17.8%). Faeces were not analysed, as they contained less than 5% of the administered radiolabel (Bakke & Price, 1976).

The above results, although minimally reported, are broadly consistent with data for the closely related compound, chlorpyrifos (see [Annex 1](#), reference 86).

A normal metabolic reaction with phosphorothioates is oxidative desulfuration or isomerization to give the oxon form, which is the more potent inhibitor of acetylcholinesterase. Chlorpyrifos-methyl oxon was not identified in the rat metabolism studies with chlorpyrifos-methyl.

A proposed metabolic pathway is shown in [Figure 2](#).

Figure 2. Proposed metabolic pathway of chlorpyrifos-methyl in rats (% of administered radiolabel in parentheses)



2. Toxicology studies

2.1 Acute toxicity

(a) Lethal doses

Chlorpyrifos-methyl was of low acute toxicity via the oral, dermal and inhalation routes (Table 4).

(b) Dermal and ocular irritation and dermal sensitization

Chlorpyrifos-methyl was a slight transient irritant to rabbit skin (Jones, 1984) and eyes (Jones, 1985c). In a guinea-pig skin sensitization study using a Buehler protocol, chlorpyrifos-methyl was not a skin sensitizer (Jones, 1985d), but positive results were seen when a Magnusson & Kligman maximization protocol was used (Wilson, 2000).

2.2 Short-term studies of toxicity

(a) Oral route

Mice

Chlorpyrifos-methyl (purity 91.8%; batch No. AGR 209075) was administered to ICR(Crj:CD-1) mice (12 of each sex per group) in the diet at a level of 0, 1, 5, 10, 1000 or 10 000 ppm for 28 days. Blood samples were taken at termination for the measurement of haematological and

Table 4. Acute toxicity studies with chlorpyrifos-methyl

Test substance	Species	Strain	Sex	Route	LD ₅₀ (mg/kg bw)	LC ₅₀ (mg/l air)	Purity (%); batch	Vehicle	Reference
OP2	Rat	SD	M & F	Oral	2814	—	95.5; EK 8429097	Corn oil	Lackenby (1985)
Reldan F	Rat	SD	M & F	Oral	>5000	—	95.5	Polyethylene glycol	Jones (1985a)
Chlorpyrifos- methyl	Mouse	OF-1	M & F	Oral	2843	—	96.9	0.5% CMC	Clouzeau (1992)
Reldan F	Rat	SD	M & F	Dermal	>2000	—	95.5; EK 840929097	Polyethylene glycol	Jones (1985b)
Reldan F	Rat	HC/ CFHB	M & F	Inhalation (4 h, nose only)	—	>0.67 ^a	Not stated; EK 82092 8086	None; vapour (55 °C)	Hardy & Jackson (1984)

CMC, carboxymethyl cellulose; F, female; LC₅₀, median lethal concentration; LD₅₀, median lethal dose; M, male; SD, Sprague-Dawley

^a Maximal technically achievable concentration.

clinical chemistry parameters (five of each sex per group) and assessment of cholinesterase activity (six of each sex per group). The method of determination of cholinesterase activity was an automated 5,5'-dithiobis(2-nitrobenzoic) acid (DTNB) procedure based on the method of Ellman et al. (1961). The brain, pituitary, thyroid, heart, thymus, liver, kidneys, spleen, adrenals and gonads were weighed at necropsy. A comprehensive list of tissues from animals of the control, 1000 and 10 000 ppm groups were subjected to histopathological examination.

Dietary analyses revealed satisfactory stability, homogeneity and concentration of test material. Achieved intakes were 0, 0.12, 0.65, 1.3, 122 and 523 mg/kg bw per day in males and 0, 0.14, 0.75, 1.5, 139 and 318 mg/kg bw per day in females. All animals administered 10 000 ppm were found dead or killed in extremis on days 5–9. The report authors cite starvation of these animals due to the severe unpalatability of test material in the diet. There were no deaths or clinical signs in the lower dose groups. Body weights of 1000 ppm animals of both sexes were slightly lower throughout the study period; values attained statistical significance for males at week 4. Overall food consumption, water consumption and food conversion efficiency were slightly (but not statistically significantly) decreased at 1000 ppm.

Urinalysis and haematology did not reveal any treatment-related findings. Clinical chemistry findings indicative of mild hepatotoxicity were noted at the top dose level in both sexes. Plasma cholinesterase activity was significantly lower at 10 ppm and above in males and at 5 ppm and above in females. Erythrocyte cholinesterase activity was significantly lower at 10 ppm and above in males and at 1000 ppm only in females. Brain cholinesterase activity was significantly reduced at 1000 ppm in both sexes (Table 5).

Mean absolute and relative liver and adrenal weights were significantly increased in 1000 ppm males (~25%); relative liver weight was also significantly increased in 1000 ppm females (~12%). Emaciation of all 10 000 ppm decedents was noted at necropsy, together with splenic atrophy, hepatocellular atrophy and pancreatic acinar cell atrophy. Adrenal cortical cell swelling in the zona fasciculata was noted in all 1000 ppm males; similar findings were not reported in females.

A no-observed-adverse-effect level (NOAEL) of 10 ppm, equal to 1.3 and 1.5 mg/kg bw per day in males and females, respectively, can be determined for this study, based on decreased brain cholinesterase activity and adrenal and liver pathology at 1000 ppm (Yoshida et al., 1985).

Table 5. Clinical chemistry parameters and cholinesterase activity in mice receiving chlorpyrifos-methyl in the diet for 28 days

Parameter	Males					Females				
	Dietary concentration (ppm)									
	0	1	5	10	1000	0	1	5	10	1000
GPT (U/l)	13	11	11	10	17	8	14	7	11	12*
Cholesterol (mg/dl)	93	104	108	100	149	82	70	84	86	127**
Plasma cholinesterase ^a										
U/ml	3.59	3.54	2.66	1.69*	0.15***	5.40	4.85	4.46*	4.14**	0.14***
% of control	—	99	74	47	4	—	90	83	77	3
Red blood cell cholin- esterase ^a										
U/ml	0.15	0.16	0.15	0.10*	0.00***	0.13	0.13	0.12	0.11	0.00***
% of control	—	107	100	67	0	—	100	92	85	0
Brain cholinesterase ^a										
U/ml	0.28	0.25	0.24	0.25	0.08***	0.23	0.22	0.24	0.24	0.06***
% of control	—	89	86	89	29	—	96	104	104	27

From Yoshida et al. (1985)

GPT, glutamic-pyruvic transaminase; U, unit; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

^a Activity at termination.

Rats

In a 1975 (non-GLP) study, Sprague-Dawley rats (five of each sex per dose level) were gavaged with Dowco 214 (purity and batch not specified), suspended in 1% gum tragacanth, at 0, 0.2, 1 or 5 mg/kg bw per day, 6 days/week, for 6 weeks. Blood was sampled from the orbital sinus after 30 days of dosing for the determination of cholinesterase activity according to the pH stat method of Nabb & Whitfield (1967). Aortal blood samples were taken at termination for the assessment of haematological and clinical chemistry parameters. Liver samples were taken for the determination of mixed-function oxidase (*N*-demethylase and biphenyl hydroxylase) activity. Homogenized whole-brain samples were assayed for acetylcholinesterase activity.

Overall body weight gain in top-dose females was slightly reduced (81% of controls). No effects were seen in other groups. No effects were seen on haematological or clinical chemistry parameters at any dose level. No consistent effects were seen on mixed-function oxidase activity. Plasma and erythrocyte cholinesterase activities were reduced at 1 mg/kg bw per day and higher. A trend to decreasing brain cholinesterase activity was noted in all dose groups, with reductions of greater than 20% at 5 mg/kg bw per day (Table 6). None of the decreases in cholinesterase activity were reported to be statistically significant.

A NOAEL of 1 mg/kg bw per day can be determined for this study, based on decreased (>20%) brain cholinesterase activity at 5 mg/kg bw per day, although this was not statistically significant. The study report does not identify the relative times of cholinesterase measurement and the final dose (Griffin & Coulston, 1975).

Table 6. Cholinesterase activity in a 6-week study in rats administered chlorpyrifos-methyl in the diet

Cholinesterase sample	Time	Cholinesterase activity ($\mu\text{mol}/\text{min}$ per milligram protein, mean $\pm$ SD)							
		Dose (mg/kg bw per day)							
		0		0.2		1		5	
		M	F	M	F	M	F	M	F
Plasma	Pretest	0.9 $\pm$ 0.1	1.8 $\pm$ 0.9	0.6 $\pm$ 0.1	1.7 $\pm$ 0.6	0.5 $\pm$ 0.0	1.8 $\pm$ 0.4	0.7 $\pm$ 0.1	1.4 $\pm$ 0.2
	Day 30	0.5 $\pm$ 0.1	2.4 $\pm$ 1.5	0.5 $\pm$ 0.1	2.1 $\pm$ 0.7	0.4 $\pm$ 0.1	1.4 $\pm$ 0.4	0.4 $\pm$ 0.1	0.9 $\pm$ 0.2
	(%) ^a	—	—	100	88	80	58	80	68
Red blood cells	Pretest	1.1 $\pm$ 0.1	1.6 $\pm$ 0.2	1.8 $\pm$ 0.8	2.5 $\pm$ 0.2	1.9 $\pm$ 0.3	2.2 $\pm$ 0.7	1.7 $\pm$ 0.7	2.6 $\pm$ 1.0
	Day 30	1.9 $\pm$ 0.2	2.1 $\pm$ 0.3	2.1 $\pm$ 0.3	2.1 $\pm$ 0.2	1.5 $\pm$ 0.3	2.0 $\pm$ 0.1	1.1 $\pm$ 0.2	1.8 $\pm$ 0.5
	(%) ^a	—	—	111	100	79	95	58	86
Brain	Termination	0.092 $\pm$ 0.013	0.114 $\pm$ 0.010	0.081 $\pm$ 0.011	0.108 $\pm$ 0.006	0.083 $\pm$ 0.017	0.102 $\pm$ 0.013	0.070 $\pm$ 0.010	0.091 $\pm$ 0.010
	(%) ^a	—	—	88.0	94.7	90.2	89.5	76.1	79.8

From Griffin & Coulston (1975)

F, female; M, male; SD, standard deviation

^a% of day 30 control.

Fischer 344 rats (10 of each sex per group) were administered Reldan (purity ~95%; batch No. AGR 219561) in the diet at variable dose levels calculated to be equivalent to 0, 0.1, 1, 10 or 250 mg/kg bw per day for 13 weeks. Additional recovery groups (10 of each sex) at 0 and 250 mg/kg bw per day were similarly treated, then administered control diet for an additional 4 weeks. Blood samples for the analysis of haematological and clinical chemistry parameters were taken from the orbital sinus immediately prior to termination. Samples for urinalysis were also taken prior to termination. Plasma and erythrocyte cholinesterase activities were measured in blood samples taken during week 6 and at termination. Brain cholinesterase activity was determined in half-brain samples taken at necropsy. Cholinesterase activity was measured using an automated procedure based on the method of Ellman et al. (1961). A functional observational battery (FOB) was performed on days 10, 30, 86 and 115. The adrenals, brain, heart, kidneys, liver and gonads were weighed at necropsy. The adrenals, brain, spinal cord, peripheral nerve, liver, kidney, lungs and gross lesions from all animals were examined microscopically. A comprehensive list of tissues from the control and top dose group were similarly examined.

Dietary analyses revealed acceptable homogeneity, stability and concentration of test material. No animals died during the study period. Food consumption was consistently lower in both sexes at the top dose level; however, values did not attain statistical significance. Food consumption during the recovery period was comparable in both groups. Body weights and weight gains of top-dose animals of both sexes were significantly lower than those of controls throughout the study period (Table 7). No consistent effects were seen at lower dose levels. Body weights of top-dose females remained significantly lower than those of controls after the recovery period; however, body weight gain was increased in both sexes.

Plasma cholinesterase activity was significantly decreased at 1 mg/kg bw per day and higher in both sexes at weeks 6 and 13 and in 0.1 mg/kg bw per day males at week 13 only. Week 13 values for treated groups of males were slightly but consistently lower than week 6 values; similar effects were not seen in females. Following the recovery period, activities showed evidence of considerable recovery. Erythrocyte cholinesterase activity was significantly decreased in both sexes at 10 mg/kg bw per day and higher at week 6 and in 250 mg/kg bw per day females only at week 13. Activities at weeks 6 and 13 were comparable in both sexes. Activities at week 13 in males at 10 mg/kg bw per day and

Table 7. Body weights and body weight gains in rats exposed to chlorpyrifos-methyl in the diet for 13 weeks

Parameter	Day	Body weight or body weight gain (g)									
		Males					Females				
		Dose (mg/kg bw per day)									
		0	0.1	1	10	250	0	0.1	1	10	250
Body weight	14	194.7	194.7	193.8	189.2	186.2*	132.0	129.7	131.1	132.3	125.0*
	42	266.1	263.5	260.5	257.4	246.3*	169.7	167.2	168.7	166.3	151.0*
	91	321.0	312.2	313.1	308.3	289.4*	193.0	190.6	190.7	186.8	166.3*
Body weight gain	0–91	194.5	185.5	187.1	183.5	161.1*	95.4	94.5	94.0	89.5	70.3*
Body weight	112	327.0				315.2	193.7				179.9*

From Barna-Lloyd, Szabo & Davis (1990)

* $P \leq 0.05$

higher and in females at 10 mg/kg bw per day were reduced, but did not attain statistical significance. Some evidence of recovery in erythrocyte acetylcholinesterase activity was seen at week 17, although values for treated animals remained significantly lower than those of controls. Brain cholinesterase activity was significantly decreased in both sexes at 10 mg/kg bw per day and higher (Table 8). Evidence of significant recovery was seen 4 weeks after cessation of treatment.

Red blood cell count, haemoglobin content and packed cell volume were significantly decreased in both sexes at 250 mg/kg bw per day. Evidence of recovery was seen; however, some parameters remained significantly lower than controls. Platelet count was significantly higher in both sexes at 250 mg/kg bw per day; changes in females at lower doses lacked a clear dose–response relationship and are not considered to be of toxicological significance. Some evidence of recovery was seen at week 17. Significantly lower alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and alkaline phosphatase (AP) activities at the top dose level are not considered to be of toxicological significance in their own right and are possibly secondary to reduced body weight gain. No notable changes were evident at 10 mg/kg bw per day. Urinalysis did not reveal any treatment-related findings.

For the FOB investigations, decreased condition of hair, increased urine staining and lacrimation were observed at the top dose level. Tremor was noted in one top-dose female at the day 30 observation. No behavioural effects were noted in any animals. Mean absolute and relative adrenal, kidney and liver weights were significantly increased in both sexes at the top dose level. Mean relative adrenal weights were also increased at 10 mg/kg bw per day in both sexes. With the exception of kidney weights in males, organ weights in treated animals were considerably lower following the recovery period.

No treatment-related macroscopic findings were noted at necropsy. Microscopically, bilateral diffuse hypertrophy and vacuolation of the adrenal cortex zona fasciculata were noted in all animals at 10 and 250 mg/kg bw per day (Table 9). The severity of these findings was decreased following the recovery period. Adrenal findings were stated by the report authors to be consistent with lipid accumulation. Adrenal necrosis with accompanying inflammation was also noted in females at week 13 only. Degeneration/regeneration of the renal tubules was noted with increased severity in males at 250 mg/kg bw per day. Very slight hepatic centrilobular hypertrophy was also noted in this group. Microscopic renal and hepatic findings were not apparent following the recovery period.

Administration of Reldan to rats for 90 days resulted in marked inhibition of cholinesterase activities. Evidence of the reversibility of signs of toxicity, cholinesterase inhibition, organ weight

Table 8. Cholinesterase activity in rats exposed to chlorpyrifos-methyl in the diet for 13 weeks

Cholinesterase sample	Week	Cholinesterase activity									
		Males					Females				
		Dose (mg/kg bw per day)									
		0	0.1	1	10	250	0	0.1	1	10	250
Plasma, U/ml (% of control)	6	0.649	0.649	0.568*	0.478*	0.333*	2.82	2.93	1.86*	0.803*	0.359*
		(—)	(100)	(88)	(74)	(51)	(—)	(104)	(66)	(28)	(13)
	13	0.631	0.585*	0.502*	0.418*	0.292*	2.66	3.01	1.76*	0.721*	0.358*
		(—)	(93)	(80)	(66)	(46)	(—)	(113)	(66)	(27)	(13)
	17	0.590				0.549*	3.193				3.132
		(—)				(93)	(—)				(98)
Red blood cells, U/ml (% of control)	6	2.18	2.04	1.84	1.62*	1.52*	2.43	2.24	2.14	1.66*	1.81*
		(—)	(94)	(84)	(74)	(70)	(—)	(92)	(88)	(68)	(74)
	13	2.25	2.52	2.07	1.91	1.87	1.52	1.50	1.58	1.36	1.10*
		(—)	(112)	(92)	(85)	(83)	(—)	(99)	(104)	(89)	(72)
	17	2.18				1.92*	2.04				1.68*
		(—)				(88)	(—)				(82)
Brain, U/g (% of control)	13	10.55	10.31	10.15	8.77*	3.95*	10.35	10.32	10.19	8.84*	4.26*
		(—)	(98)	(96)	(83)	(37)	(—)	(100)	(98)	(85)	(41)
	17	10.38				8.38*	10.55				8.73*
		(—)				(81)	(—)				(83)

From Barna-Lloyd, Szabo & Davis (1990)

* $P \leq 0.05$

effects and microscopic findings was seen after a 4-week recovery period. A NOAEL of 1 mg/kg bw per day can be determined for this study, based on decreased brain cholinesterase activity and adrenal microscopic findings at 10 mg/kg bw per day and higher (Barna-Lloyd, Szabo & Davis, 1990).

Dogs

Beagle dogs (four of each sex per group) were administered Reldan (purity 95.2%) in the diet at dose levels calculated to be equivalent to 0, 0.1, 10 or 50 mg/kg bw per day for 13 weeks. Blood samples were taken prior to the study and on days 36 and 85 for the assessment of haematological and clinical chemistry parameters and blood and plasma cholinesterase activities. Urinalysis was performed at necropsy. The adrenals, brain, kidneys, liver, pituitary, gonads and thyroid were weighed at necropsy. A comprehensive list of tissues from all animals were subjected to histopathology. Brain cholinesterase activity was measured at necropsy. Cholinesterase activities were measured using an automated procedure based on the method of Ellman et al. (1961).

Dietary analyses revealed that concentration, stability and homogeneity of the test material were within acceptable limits. No animals died during the study period. Weakness and severe muscle wasting were noted in one top-dose female; this animal was killed on day 85. Moderate muscle wasting was noted in an additional animal in this group. Decreased weight gain was noted in top-dose males. Mean body weights of top-dose males were lower from day 28; one animal lost weight over the study period, and intermittent weight loss was noted in another animal. Mean body weights of top-dose females were lower than those of controls from day 14. Weight gain over the study period was noted in only one animal in this group; other animals lost weight over the study period. No effects on body weight or body weight gain were noted at lower dose levels. Food consumption at 50 mg/kg bw per day was lower in both sexes (15–30%).

Table 9. Microscopic findings in rats (*n* = 10) exposed to chlorpyrifos-methyl in the diet for 13 weeks

Finding	Severity	Week	Incidence									
			Males					Females				
			Dose (mg/kg bw per day)									
			0	0.1	1	10	250	0	0.1	1	10	250
Adrenal hypertrophy	Slight	13	0	0	0	10	0	0	0	0	9	0
		17	0				10	0				0
	Moderate	13	0	0	0	0	10	0	0	0	1	10
		17	0				0	0				0
Adrenal vacuolation	Very slight	13	0	0	0	0	0	0	0	0	0	0
		17	0				0	0				10
	Slight	13	0	0	0	0	0	0	0	0	10	0
		17	0				10	0				0
	Moderate	13	0	0	0	0	10	0	0	0	0	10
		17	0				0	0				0
	Adrenal necrosis	13	0	0	0	0	0	0	0	0	2	8
Kidney tubule degeneration	Very slight	13	10	10	10	9	1	1	1	0	1	0
	Slight	13	0	0	0	0	9	0	0	0	0	0
Centrilobular hypertrophy	Very slight	13	0	0	0	0	7	0	0	0	0	0

From Barna-Lloyd, Szabo & Davis (1990)

Red blood cell count, packed cell volume and haemoglobin concentration were significantly decreased in both sexes at the top dose level at termination. Platelet numbers were significantly increased in both sexes at this dose level. AP, ASAT and creatine kinase activities were increased at the top dose level. Significant decreases in blood urea nitrogen, plasma creatinine, glucose, total protein, albumin, globulin, cholesterol and triglyceride levels seen in this group are considered to be secondary to weight loss and decreased food consumption in these animals. There were no notable effects at 10 mg/kg bw per day or below.

A dose-related decrease in plasma cholinesterase activity was seen at all dose levels in both sexes. Erythrocyte cholinesterase activities were reduced at all dose levels in both sexes; however, effects at 0.1 mg/kg bw per day were marginal. Plasma and erythrocyte cholinesterase activities at weeks 6 and 13 were comparable. Brain cholinesterase activity was significantly reduced at 50 mg/kg bw per day only (Table 10).

Mean relative liver and kidney weights were significantly increased in both sexes at the top dose level. Effects on kidney weight may reflect the lower body weights of animals in these groups. Gross findings at necropsy were limited to decreased mesenteric fat and skeletal muscle atrophy in two top-dose females. Microscopically, slight vacuolation of the renal proximal tubules was noted in top-dose females. Slight, diffuse centrilobular hepatocyte hypertrophy was noted in both sexes at the top dose level, skeletal muscle degeneration was noted in females at the top dose and thymic atrophy was observed in all top-dose males. No changes in histopathology of the adrenals were noted. No treatment-related findings were noted at lower dose levels.

A NOAEL of 10 mg/kg bw per day can be determined for this study, based on clinical signs, body weight effects, haematological and clinical chemistry findings, histopathological effects and

Table 10. Cholinesterase activities in dogs exposed to chlorpyrifos-methyl in the diet for 13 weeks

Cholinesterase sample	Time	Cholinesterase activity							
		Males				Females			
		Dose (mg/kg bw per day)							
		0	0.1	10	50	0	0.1	10	50
Plasma, U/ml (% of control)	Week -1	2.244	2.313	2.298	2.300	2.424	2.409	2.260	2.253
	Week 6	1.984	1.764	0.967	0.460	2.169	1.873	0.844	0.501
		(—)	(89)	(49)	(23)	(—)	(86)	(39)	(23)
	Week 13	1.781	1.773	0.798	0.427	1.924	1.653	0.822	0.478
		(—)	(100)	(45)	(24)	(—)	(86)	(43)	(25)
Red blood cells, U/ml (% of control)	Week -1	3.035	3.025	2.725	2.900	3.000	2.675	2.935	2.875
	Week 6	2.360	2.235	1.690	1.630	2.440	2.415	1.725	1.640
		(—)	(95)	(72)	(69)	(—)	(99)	(71)	(67)
	Week 13	1.805	2.040	1.350	1.485	1.890	1.775	1.520	1.395
		(—)	(113)	(75)	(82)	(—)	(94)	(70)	(74)
Brain, U/g (% of control)	Week 13	4.40	4.81	4.48	1.48**	4.95	4.77	4.39	1.48**
		(—)	(109)	(102)	(34)	(—)	(96)	(89)	(30)

From Szabo & Davis (1990)

U, units; ** □ = 0.05 (analysis of variance)

inhibition of brain cholinesterase activity at 50 mg/kg bw per day. Animals were fasted overnight prior to termination; it is therefore possible that cholinesterase inhibition was underestimated in this study (Szabo & Davis, 1990).

Monkeys

In a 1975 (non-GLP) study, Rhesus monkeys (three of each sex per group) were gavaged with Dowco 214 (batch and purity not specified), suspended in gum tragacanth, at 0, 0.1, 0.2, 1 or 5 mg/kg bw per day, 6 days/week, for 26 weeks. Weekly blood samples were taken for the assessment of plasma and erythrocyte cholinesterase activities from 6 weeks pre-exposure according to the method of Nabb & Whitfield (1967). Samples were also taken for the assessment of routine haematological and clinical chemistry parameters at intervals throughout the study. Liver samples were taken at necropsy for the determination of mixed-function oxidase (*N*-demethylase and biphenyl hydroxylase) activity. Samples of mid-brain were taken for the determination of cholinesterase activity. The heart, liver, brain and kidneys were weighed at necropsy, and a comprehensive list of tissues were evaluated microscopically, including sciatic nerve, cervical spinal cord, medulla, pons, optic chiasm, cerebellum and cerebrum using haematoxylin and eosin (H&E) staining.

One male at 1 mg/kg bw per day was terminated early during the study owing to a persistent infection. There were no further deaths. No clinical signs of toxicity were reported during the study period, and no effects were seen on body weight. No treatment-related effects were seen on haematological or clinical chemistry parameters. No effects were seen on mixed-function oxidase activity. Plasma cholinesterase activities showed considerable intraindividual and interindividual variation, but were decreased in all groups throughout the study period. Consistent decreases in activity were seen at 2 mg/kg bw per day and higher in males and at 1 mg/kg bw per day and higher in females. Erythrocyte cholinesterase activities were less variable; decreased activity (~60% inhibition) was seen in both sexes at the top dose level (Table 11). No consistent time-related change was seen in plasma or erythrocyte cholinesterase activity. Brain cholinesterase activity was not consistently

Table 11. Cholinesterase activity in monkeys administered chlorpyrifos-methyl for 26 weeks

Cholinesterase sample	Time	Cholinesterase activity, $\mu\text{mol/min}$ per milligram protein (% of control)									
		Dose (mg/kg bw per day)									
		0		0.1		1		2		5	
		M	F	M	F	M	F	M	F	M	F
Plasma	Pretest ^a	11.40	11.50	7.57	12.53	10.30	7.80	6.80	9.13	7.17	11.00
	Week 6	9.07	8.90	6.57	9.67	8.27	6.30	4.10	4.73	2.17	3.00
		(—)	(—)	(72)	(109)	(91)	(71)	(45)	(53)	(24)	(34)
	Week 13	10.40	9.50	8.47	11.60	10.23	6.63	5.30	5.77	3.40	3.10
		(—)	(—)	(81)	(122)	(98)	(70)	(51)	(61)	(33)	(33)
	Week 27	8.97	8.93	6.90	10.47	8.70	5.63	3.95	5.20	2.53	2.50
		(—)	(—)	(77)	(117)	(97)	(63)	(76)	(58)	(28)	(28)
Red blood cells	Pretest ^a	11.57	12.60	12.73	12.17	12.57	12.80	11.53	12.97	10.33	12.43
	Week 6	11.67	12.40	12.83	12.13	12.80	12.83	10.63	11.60	3.17	4.17
		(—)	(—)	(110)	(98)	(110)	(103)	(91)	(94)	(27)	(34)
	Week 13	11.50	12.87	13.13	11.90	11.70	12.43	12.33	11.17	2.57	3.20
		(—)	(—)	(114)	(92)	(102)	(97)	(107)	(87)	(22)	(25)
	Week 27	12.83	11.37	12.40	11.40	11.50	11.23	9.50	11.37	3.20	3.00
		(—)	(—)	(97)	(100)	(90)	(99)	(74)	(100)	(25)	(26)
Brain	Week 27	0.106	0.099	0.106	0.114	0.119	0.101	0.115	0.107	0.090	0.110
		(—)	(—)	(100)	(108)	(112)	(95)	(109)	(101)	(85)	(104)

From Griffin & Coulston (1975)

^a Mean of six pretest time points.

affected at any dose level. No effects on organ weights were noted at necropsy. No treatment-related gross or microscopic findings were noted.

A NOAEL of 5 mg/kg bw per day can be determined for this study in the absence of any treatment-related adverse effects at the top dose level (Griffin & Coulston, 1975).

(b) *Dermal route*

Rats

Groups of Fischer 344 rats (10 of each sex) were exposed dermally to Reldan F (purity 96.8%; lot No. NB05272036) at levels of 0, 10, 100 or 300 mg/kg bw per day, 6 h/day, 7 days/week, for 28 days, to evaluate the potential for systemic toxicity. Reldan F was applied in 0.5% methyl cellulose and covered with a semiocclusive dressing. An additional 10 male and 10 female rats in the control and high dose groups were untreated for an additional 2 weeks following the treatment period to assess recovery. Regular observations (including dermal), ophthalmology examinations, detailed clinical observations, body weight, food consumption, haematology (including prothrombin time), clinical chemistry (including cholinesterase activity), urinalysis and organ weights were evaluated. In addition, a gross necropsy was conducted, with extensive histopathological examination of tissues.

There were no deaths during the study. Cage-side observations of the dosing-phase animals revealed no abnormal findings. The only treatment-related alteration was periocular soiling at days 22 and 28 in four females given 300 mg/kg bw per day. Local irritant effects were seen from day 8 onwards at 100 and 300 mg/kg bw per day; histopathology identified the responses as hyperplasia, hyperkeratosis and inflammation, which were present at all dose levels. There were no statistically

Table 12. Inhibition of cholinesterase activity in rats administered chlorpyrifos-methyl dermally for 28 days

Cholinesterase sample	Time	Cholinesterase activity (% inhibition)							
		Dose (mg/kg bw per day)							
		0		10		100		300	
		M	F	M	F	M	F	M	F
Red blood cells	Week 4	0	0	3*	7*	30*	29*	53*	43*
	Week 6	0	0	—	—	—	—	30*	27*
Brain	Week 4	0	0	1	6*	7*	14*	26	27*
	Week 6	0	0	—	—	—	—	10*	12*
Heart	Week 4	0	0	17*	19*	43*	58*	72*	72*
	Week 6	0	0	—	—	—	—	0	2

From Stebbins, Yano & Baker (2000)

* $P < 0.05$

identified body weight differences between any treated groups compared with their respective controls. At the end of 4 weeks, males given 100 or 300 mg/kg bw per day had a 7% and 13% lower body weight gain, respectively, compared with controls. Females given 100 mg/kg bw per day had a 29% lower body weight gain compared with controls, but body weight gain in 300 mg/kg bw per day females was comparable with that of controls. These minimal/inconsistent alterations in body weight were considered not to be adverse. All dose levels had food consumption comparable with their respective controls throughout the study. Dose-related decreases in plasma, red blood cells, and brain and heart cholinesterase activities occurred in males and females at all dose levels. These decreases were statistically significant at each dose level, with the exception of brain cholinesterase of males given 10 mg/kg bw per day (Table 12). There was evidence of recovery, almost complete for heart cholinesterase activity. There were no notable effects on haematology, clinical chemistry or urinalysis parameters.

Increased adrenal weight (20–40%) and vacuolation of the adrenal cortex were evident at necropsy in both sexes dosed at 300 mg/kg bw per day and in males dosed at 100 mg/kg bw per day. Increased vacuolation of the adrenal zona fasciculata and reticulata was seen in all animals at all dose levels, but not in controls; at 10 mg/kg bw per day, the grading was very slight, in mid-dose males, it was slight, and in mid-dose females and all top-dose animals, it was graded moderate. In recovery-phase animals, the grading had reduced to very slight. Taking account of the reversibility and severity, the adrenal findings at the low dose level are not considered to be adverse.

The NOAEL was 10 mg/kg bw per day, based on marked inhibition of heart cholinesterase activity and vacuolation of the adrenals at 100 mg/kg bw per day; the magnitude of these effects at 10 mg/kg bw per day is not considered adverse. There was no NOAEL for local effects (Stebbins, Yano & Baker, 2000).

(c) Inhalation route

Rats

Groups of Fischer 344 rats (10 of each sex) were exposed nose only to Reldan F (purity 96.8%; lot No. NB05272036) at levels of 0, 0.49, 3.7 or 18 parts per billion (ppb) for 6 h/day, 5 days/week, for 2 weeks. The atmosphere was generated by passing air over molten (~50 °C) Reldan F; the mass median aerodynamic diameter was 0.68 µm. Five rats of each sex per group were terminated after

2 weeks; the remainder formed a 2-week recovery group. Clinical observations, body weights, food consumption and gross pathology were evaluated for all animals. In addition, haematology, clinical chemistry, urinalysis, organ weights and an extensive histopathological examination of tissues were evaluated in the exposure group rats only. There were no clinical signs other than those associated with confinement in nose-only exposure chambers. There were no notable effects on any measured parameter, including cholinesterase activities.

The no-observed-adverse-effect concentration (NOAEC) is 18 ppb (approximately 100 µg/m³, equivalent to 0.027 mg/kg bw per day) (Stebbins & McGuirk, 2000).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Crj:CD-1 mice (96 of each sex per group; 52 scheduled for 78-week termination) were administered chlorpyrifos-methyl (purity 97.4%; batch No. AGR 219561) in the diet at concentrations of 0, 1, 5, 50 or 500 ppm for up to 18 months. Ten animals of each sex per group were terminated at weeks 26 and 52; urinalysis and measurement of haematological and clinical chemistry parameters were performed on these animals. An additional 10 animals of each sex per group were killed at weeks 26 and 52 for the measurement of non-fasted plasma, erythrocyte and brain cholinesterase activities using an automated procedure based on the method of Ellman et al. (1961). Groups of 10 animals of each sex were similarly investigated prior to necropsy at week 78. Weights of the brain, liver, adrenals, heart, kidneys and testes were recorded at necropsy (10 of each sex per group). A comprehensive list of tissues from animals killed at week 52 (0 and 500 ppm) and week 78 (all groups) and from all unscheduled deaths were subjected to histopathological examination. Histopathology was also performed on the lungs, liver, kidneys, adrenals and gross lesions from animals in intermediate dose groups killed at week 52.

Dietary analyses showed that the concentration, stability and homogeneity of the test material were within acceptable limits. Achieved mean intakes were 0, 0.08, 0.4, 4.4 and 44 mg/kg bw per day in males and 0, 0.08, 0.4, 3.9 and 41 mg/kg bw per day in females. No treatment-related effect on survival was seen. Survival of animals is considered to be adequate for the assessment of tumorigenicity. No clinical signs of toxicity were noted. Mean body weights of top-dose males were consistently lower than those of controls throughout the study period; values occasionally attained statistical significance. Mean body weights of top-dose females were lower than those of controls during the first months of the study and occasionally attained statistical significance. Urinalysis did not reveal any treatment-related findings. No treatment-related haematological changes were noted. Total plasma cholesterol concentrations were increased in top-dose animals of both sexes; values attained statistical significance in females at all time points and in males at week 26 only. Plasma ASAT and ALAT activities were significantly increased in top-dose females at week 52 (Table 13).

Plasma and erythrocyte cholinesterase activities were significantly reduced at 50 ppm and above at all time points. Brain cholinesterase activities were significantly decreased at the top dose level at all time points and at 50 ppm in week 52 females; the latter finding is not considered treatment related (Table 14), as it was not reproduced at 78 weeks, unlike the effect on red blood cells and on brain at 500 ppm. The high variation of control cholinesterase activity values is noted.

No treatment-related effects were seen on organ weight. Macroscopically, the incidence of pale liver was increased at the top dose level from week 26. Microscopically, the incidence of hepatic centrilobular fatty change was increased in both sexes at the top dose level; findings were apparent from week 52. The total incidence of diffuse hepatic fatty change was increased in females at all dose levels. This finding is of unclear toxicological significance in the absence of a dose-response relationship or similar findings in males. Renal tubular atrophy was noted with greater frequency in

Table 13. Clinical chemistry results in mice exposed to chlorpyrifos-methyl for 78 weeks

Parameter	Week	Males					Females				
		Dietary concentration (ppm)									
		0	1	5	50	500	0	1	5	50	500
Cholesterol (mg/dl)	26	114	115	166	103	159*	85	85	69	95	123*
	52	123	122	110	100	173	75	87	98	85	134*
	78	134	115	113	94	128	76	80	99	108	116**
ASAT (U/l)	52	42	53	47	60	63	38	52	59*	54	88**
	78	66	74	87	70	127	85	103	64	65	76
ALAT (U/l)	52	29	34	36	47	48	14	22	25	22	54**
	78	42	39	68	46	83	20	37	24	23	25

From Yoshida et al. (1988)

U, units; * $P < 0.05$; ** $P < 0.01$ **Table 14. Cholinesterase activities in mice exposed to chlorpyrifos-methyl for 78 weeks**

Cholinesterase sample	Week	Cholinesterase activity, U/ml (% of controls)									
		Males					Females				
		Dietary concentration (ppm)									
		0	1	5	50	500	0	1	5	50	500
Red blood cells	26	0.17	0.18	0.16	0.09**	0.01**	0.09	0.09	0.08	0.06**	0.01**
		(—)	(106)	(94)	(53)	(6)	(—)	(100)	(89)	(67)	(11)
	52	0.22	0.23	0.22	0.11**	0.01**	0.15	0.12	0.13	0.10**	0.01**
		(—)	(105)	(96)	(48)	(4)	(—)	(80)	(87)	(67)	(7)
	78	0.15	0.16	0.06	0.08*	0.01**	0.16	0.15	0.14	0.11**	0.02**
		(—)	(107)	(107)	(53)	(7)	(—)	(94)	(88)	(69)	(13)
Brain	26	0.32	0.33	0.35	0.32	0.15**	0.20	0.20	0.20	0.18	0.11**
		(—)	(103)	(109)	(100)	(47)	(—)	(100)	(100)	(90)	(55)
	52	0.60	0.61	0.62	0.54	0.27**	0.36	0.32	0.30	0.27**	0.18**
		(—)	(102)	(103)	(90)	(45)	(—)	(89)	(83)	(75)	(50)
	78	0.36	0.34	0.34	0.31*	0.13**	0.40	0.39	0.38	0.38	0.20**
		(—)	(94)	(94)	(86)	(36)	(—)	(98)	(95)	(95)	(50)

From Yoshida et al. (1988)

U, units; * $P < 0.05$; ** $P < 0.01$

males at 50 ppm and higher; the incidence of renal cortical cysts was increased in top-dose males. Both of these lesions are reported to be common findings in ageing CD-1 mice. Histopathological examination revealed cytoplasmic swelling of cells in the zona fasciculata of the adrenal cortex in both sexes; findings were more marked in males (Table 15). Amyloid change in a number of organs was apparent in a small number of animals of both sexes at 50 ppm and higher. The incidence of hepatocellular adenoma was marginally increased at the top dose level in both sexes; however, no clear dose-response relationship was apparent, and survival, particularly in males, was lower in controls. The incidence of hepatocellular carcinoma was comparable in all groups. No treatment-related effect on total tumour incidence was seen.

Table 15. Findings at necropsy in mice (*n* = 20 at week 52; *n* = 52 at week 78) exposed to chlorpyrifos-methyl for 78 weeks

Finding		Week	Incidence									
			Males					Females				
			Dietary concentration (ppm)									
	0	1	5	50	500	0	1	5	50	500		
Survival (%)		78	62	67	67	62	73	71	64	73	71	75
Pale liver		52	1	1	0	2	3	0	0	0	1	3
		78	1	1	0	0	6	0	0	0	1	1
		Total	7	6	3	4	12	3	2	2	4	6
Centrilobular fatty change		52	5	4	5	6	18**	1	2	0	3	17*
		78	9	17	12	11	17	3	3	2	5	20**
		Total	15	21	17	18	39**	4	6	2	9	38**
Diffuse fatty change		Total	0	1	1	0	0	2	8*	9*	9*	8*
Renal tubular atrophy		78	16	23	19	23	29*	13	10	16	14	15
		Total	25	35	26	36**	40**	23	18	19	20	20
Renal cortical cyst		78	1	3	3	6	7*	5	6	5	8	2
		Total	3	5	5	8	11*	8	9	6	14	8
Adrenal cortex cell swelling		52	0	0	0	0	6*	0	0	0	0	0
		78	0	0	0	0	21*	0	0	0	0	3
		Total	0	0	0	0	28**	0	0	0	0	3
Hepatocellular adenoma		78	15	12	14	18	21	3	0	5	2	5
		Total	21	26	25	28	28	3	3	5	4	7
Total tumour incidence	Benign		32	36	38	41	39	21	15	27	23	24
	Malignant		24	19	31	24	24	21	23	21	27	20
	Neoplastic		45	49	55	53	52	38	34	42	40	38

From Yoshida et al. (1988)

* $P < 0.05$; ** $P < 0.01$

No evidence of carcinogenicity was seen in this study. A NOAEL of 50 ppm, equal to 3.9 mg/kg bw per day, can be determined for this study, based on decreased brain cholinesterase activity (>20%) in both sexes at 500 ppm (Yoshida et al., 1988).

Rats

In a 1974 (non-GLP) chronic toxicity study, CD rats (55 of each sex per group) were administered chlorpyrifos-methyl (purity 99.68%) at variable levels equivalent to 0, 0.03, 0.1, 1 or 3 mg/kg bw per day in the diet for up to 2 years. Sequential interim kills reduced the number of rats to 30 of each sex at week 52. A range of observations and investigations were performed during the study. Brain cholinesterase activity was measured in all rats at scheduled necropsy. Cholinesterase activities were assessed using the titrimetric method of Casterline & Williams (1967). Weights of nine organs were recorded at necropsy. Twenty-five tissues from the control and top dose groups were subjected to histopathological investigation.

Dietary analyses revealed acceptable concentration and stability of the test material. No treatment-related deaths or clinical signs were reported. No effects were seen on survival, food consumption, body weight or food conversion efficiency. Overall survival was 67–76% for males and 62–73% for females. Urinalysis did not reveal any treatment-related effects. Packed cell volume and haemoglobin concentration were significantly decreased in females at weeks 25 and 52; however, these effects are not considered to be clearly treatment related in the absence of similar findings at later time points. No treatment-related effects on clinical chemistry parameters were noted. Erythrocyte cholinesterase and plasma cholinesterase activities are noted to be variable but were consistently reduced at 3 mg/kg bw per day. No significant effects were seen on brain cholinesterase activity. Ophthalmoscopy did not reveal any treatment-related findings. Absolute and relative thyroid weights were significantly lower in 26-week interim kill females at 1 and 3 mg/kg bw per day and in 3 mg/kg bw per day females at week 52. The toxicological significance of this finding is unclear in the absence of microscopic correlates or similar findings at week 104. No treatment-related macroscopic or microscopic findings attributable to the test material were noted at necropsy. Tumour incidence was comparable in all treatment groups.

A NOAEL of 3 mg/kg bw per day can be determined for this study in the absence of any consistent, treatment-related effects. This study does not comply with modern standards owing to the small group size and the limited nature of the histopathological examinations. The high purity of the test material is also noted (Hunter et al., 1974).

In a 1991 combined chronic toxicity and carcinogenicity study, Fischer 344 rats (50 of each sex per group) were administered chlorpyrifos-methyl (purity ~95%; batch No. AGR 219561) in the diet at concentrations calculated to be equivalent to 0, 0.05, 0.1, 1 or 50 mg/kg bw per day for up to 104 weeks. An additional 10 rats of each sex per group were assigned for interim kill at week 52. Haematological and clinical chemistry parameters (10 of each sex per group) were measured in blood samples taken from the orbital sinus at weeks 26 and 52 (interim kill animals) and at weeks 78 and 104 (terminal kill animals). Plasma and erythrocyte cholinesterase activities were also measured, using an automated procedure based on the method of Ellman et al. (1961). Samples for urinalysis were collected at weeks 26 and 52 (interim kill animals) and weeks 78 and 104 (terminal kill). Brain cholinesterase activity was determined in half-brain homogenates at interim and terminal necropsy (10 of each sex per group). Weights of the adrenals, brain, heart, liver, kidneys and gonads were recorded at necropsy. A comprehensive list of organs were examined histopathologically.

Dietary analyses revealed satisfactory stability, homogeneity and achieved concentration of the test material. Terminal survival (60–72% in males and 64–86% in females) is considered adequate for the assessment of tumorigenicity. Group mean body weights were slightly (but significantly) lower in top-dose males from week 10 to termination. No consistent effects on body weight were seen at lower dose levels or in females. Food consumption in top-dose males was consistently lower than that of controls; however, values did not attain statistical significance.

Significant differences in red blood cell parameters and platelet counts were noted at 26 and 52 weeks in both sexes at the top dose level. With the exception of the red blood cell count in females, effects were not apparent after treatment for 78 weeks. No significant haematological effects were seen after 104 weeks (Table 16). Plasma cholesterol levels were consistently increased in top-dose females; values attained statistical significance at 52 and 104 weeks. Decreased serum enzyme (AP, ALAT, ASAT and creatine kinase) activities noted at the top dose level are not considered to be of toxicological significance.

Plasma cholinesterase activities were significantly decreased at 1 mg/kg bw per day and higher at all time points. Erythrocyte cholinesterase activities were decreased consistently at the top dose level; the findings at 0.1 and 1 mg/kg bw per day were inconsistent over time and without a clear

Table 16. Haematology and clinical chemistry findings in rats exposed to chlorpyrifos-methyl for 104 weeks

Parameter	Week	Males					Females				
		Dose (mg/kg bw per day)									
		0	0.05	0.1	1	50	0	0.05	0.1	1	50
Red blood cells (10 ⁶ /ml)	26	9.12	9.21	9.02	9.00	8.64*	8.04	8.16	8.14	8.09	7.78*
	52	8.90	8.90	8.85	8.85	8.60*	8.30	8.10	8.19	8.37	7.83*
	78	8.92	8.85	8.68	8.92	9.11	8.26	8.46	8.46	8.38	7.66*
	104	8.01	8.52	8.60	7.21	7.91	7.78	7.49	7.52	7.69	7.32
Haemoglobin (g/dl)	26	15.5	15.8	15.1	15.12	14.6*	14.8	15.0	14.9	14.7	14.3*
	52	14.3	14.2	14.2	14.1	13.8*	14.9	14.6	14.6	14.9	14.2*
	78	15.4	15.1	14.9	15.2	15.6	14.9	15.2	15.0	15.1	14.1
	104	14.7	15.5	15.3	13.1	14.0	14.7	14.3	14.3	14.5	14.0
Packed cell volume (%)	26	44.5	44.5	43.0	43.1*	41.7*	41.9	42.2	41.5	40.7*	39.8*
	52	44.3	43.5	43.4	43.4	42.4*	45.0	44.0	44.1	44.6	42.4*
	78	44.6	44.2	43.6	44.9	46.0	44.0	45.1	44.8	44.5	42.2
	104	42.5	44.8	44.4	38.0	40.8	40.9	39.5	39.7	40.8	40.0
Platelets (10 ³ /ml)	26	546	584	595	558	619*	556	589	589	572	606
	52	607	584	598	578	646*	505	513	505	517	530
	78	612	580	603	603	617	522	485	452	497	519
	104	513	527	554	489	491	476	446	457	477	502
Cholesterol (mg/dl)	26	74.8	71.0	71.7	76.7	78.4	115.4	114.9	106.3	109.5	127.8
	52	102.1	95.9	97.4	101.0	105.7	143.0	144.8	144.0	148.2	175.1*
	78	76.3	74.8	76.0	70.2	73.8	77.2	74.7	79.8	75.8	101.1
	104	135.9	189.9	179.3	195.3	167.5	128.4	124.1	125.2	130.5	222.4*

From Barna-Lloyd, Szabo & Davis (1991)

* $P < 0.05$

dose–response. Brain cholinesterase activities were significantly decreased at the top dose level only (Table 17).

Mean absolute and relative adrenal weights were significantly increased in top-dose animals of both sexes at interim and final kills. Mean relative liver weights were slightly (but significantly) increased at the top dose level in both sexes at the interim kill and in females only at the terminal kill. The toxicological significance of this finding is unclear in the absence of histological correlates. Mean testes and kidney weights were slightly increased at the terminal kill; kidney weights attained statistical significance in females only.

Treatment-related findings at interim necropsy were limited to slightly increased incidences of altered foci in the adrenal cortex zona fasciculata and Leydig cell hyperplasia in top-dose males. At terminal necropsy, the incidence of splenomegaly was increased in top-dose females; this finding is considered to be secondary to the higher incidence of large granular cell leukaemia (“Fischer rat leukaemia”) in this group. Vacuolation of the adrenal cortex zona fasciculata was significantly increased in males at 1 mg/kg bw per day and higher (Table 18) and in females at the top dose level

Table 17. Cholinesterase activities and organ weights in rats exposed to chlorpyrifos-methyl for 104 weeks

Parameter	Week	Males					Females					
		Dose (mg/kg bw per day)										
		0	0.05	0.1	1	50	0	0.05	0.1	1	50	
Erythrocyte cholinesterase, U/ml (% of control)	26	2.09	2.22	2.19	1.78	1.47	2.69	2.42	2.10*	2.12*	1.74*	
		(—)	(106)	(105)	(85)	(70)	(—)	(90)	(78)	(79)	(65)	
	52	2.03	1.95	1.97	1.82	1.25*	2.11	1.77	1.99	1.89	1.85	
		(—)	(96)	(97)	(90)	(62)	(—)	(84)	(94)	(90)	(88)	
	78	2.92	2.32	2.57	2.77	2.09	1.86	1.52	1.69	1.88	1.67	
		(—)	(79)	(88)	(95)	(72)	(—)	(82)	(91)	(101)	(90)	
	104	2.24	2.12	2.23	2.09	1.78*	2.15	2.19	2.21	2.10	1.74*	
		(—)	(95)	(100)	(93)	(79)	(—)	(102)	(103)	(98)	(81)	
	Brain cholinesterase, U/g (% of control)	52	11.55	10.26	11.30	11.44	6.65*	11.82	11.93	12.19	12.08	7.59*
			(—)	(89)	(98)	(99)	(58)	(—)	(101)	(103)	(102)	(64)
104		10.44	9.46	9.40	10.22	5.53*	10.25	10.7	10.99*	10.66	6.43*	
		(—)	(91)	(90)	(98)	(53)	(—)	(105)	(107)	(104)	(63)	
Organ weights at interim kill (% of body weight)												
Adrenal		0.010	0.011	0.011	0.011	0.019*	0.021	0.023	0.024	0.024	0.0938* 0.0383 ^a	
Liver		2.542	2.443	2.453	2.478	2.678*	2.637	2.636	2.610	2.634	2.830*	
Organ weights at final kill (% of body weight)												
Adrenal		0.017	0.017	0.019	0.111	0.035*	0.018	0.020	0.021	0.022	0.026*	
					0.016 ^a							
Liver		3.136	3.130	3.000	3.034	3.432	2.862	2.713	2.769	2.678	3.231*	
Testes		1.670	1.561	1.553	1.293*	2.055*						
Kidney		0.829	0.965	0.874	0.810	0.870	0.719	0.740	0.741	0.724	0.794*	

From Barna-Lloyd, Szabo & Davis (1991)

U, units; * $P < 0.05$ ^a Excluding one animal with pheochromocytoma.

only; the findings were not graded for severity. Findings were reported to be consistent with lipid accumulation. The incidence in 1 mg/kg bw per day males is within the historical control range for the laboratory (0–42%). Increased incidences of lung microgranuloma and nasal foreign body reaction are thought to be secondary to the inhalation of food material and are not of toxicological significance. The significantly increased incidence of pituitary pars distalis adenoma in top-dose females was not clearly dose related and was stated to be within the historical control range for the rat strain (0–70%). The incidence of pituitary hyperplasia was also slightly increased in this group; however, no treatment-related effect was apparent for pituitary adenocarcinoma or for total pituitary tumours. The incidences of thyroid parafollicular cell hyperplasia and adenoma were slightly increased in top-dose animals of both sexes. The increased incidence of uterine endometrial polyps in top-dose females is within the historical control range for the rat strain (0–37%). The greater tumour burden in top-dose females is largely attributable to increased incidences of benign tumours, specifically pituitary adenoma and uterine endometrial polyp, as discussed above.

Table 18. Findings at necropsy in rats exposed to chlorpyrifos-methyl in the diet for 104 weeks

Finding		Incidence (no. of animals)									
		Males					Females				
		Dose (mg/kg bw per day)									
0	0.05	0.1	1	50	0	0.05	0.1	1	50		
Interim sacrifice (/10)											
Adrenal vacuolation		0	0	0	0	10	0	0	0	0	10
Altered adrenal foci		0	1	1	1	2	1	1	0	1	1
Multifocal Leydig cell hyperplasia		3	0	1	0	7					
Decedents and terminal sacrifice (/50)											
Splenomegaly		15	12	17	20	11	7	5	6	5	14
Spleen: large granular cell leukaemia		14	12	17	20	14	8	5	5	5	14
Adrenal vacuolation		3	5	9	11*	50*	2	2	1	0	49*
Lung microgranuloma		10	9	4	6	20*	4	2	1	1	1
Nasal foreign body reaction		9	1	2	3	18*	2	0	0	0	0
Pituitary	Cystic	6	2	1	5	4	16	19	13	11	13
	Hyperplastic	12	9	14	11	8	10	7	8	11	16
	Adenoma	7	17	12	17	8	13	14	27*	27*	23*
	Adenocarcinoma	1	1	1	0	1	6	6	2	3	3
	Total tumours	8	18	13	17	9	19	20	29	30	26
Thyroid parafollicular	Hyperplasia	6	4	1	1	10	6	0	0	3	12
	Adenoma	2	2	2	0	3	3	0	1	2	6
Endometrial polyp							9	7	11	8	18
Animals with tumours	Benign	49	49	48	50	50	25	23	34	35	36
	Malignant	20	22	22	24	21	19	12	10	10	20
	Total	50	50	50	50	50	37	30	39	40	44

From Barna-Lloyd, Szabo & Davis (1991)

* □ = 0.05

No evidence of tumorigenicity was seen in the rat following administration of chlorpyrifos-methyl for up to 2 years. A NOAEL of 1 mg/kg bw per day can be determined for this study, based on decreased brain cholinesterase activity, increased adrenal weights and associated histopathology at 50 mg/kg bw per day. Animals were fasted prior to termination; it is therefore possible that terminal cholinesterase inhibition was underestimated in this study. However, reassurance is gained from cholinesterase results in a previous 2-year rat study (Barna-Lloyd, Szabo & Davis, 1991).

A histopathology review panel performed a “blind” reading of the adrenal slides from the study of Barna-Lloyd, Szabo & Davis (1991). The review included a scoring for severity of vacuolation that was absent from the original study. The review panel concluded that the findings of adrenal vacuolation at 1 mg/kg bw per day and below were consistent with background findings and that the only dose producing clear effects was the top dose of 50 mg/kg bw per day (Table 19) (Bruner & Gopinath, 2000).

Table 19. Pathology review of adrenal slides in rats exposed to chlorpyrifos-methyl in the diet for 104 weeks

	Males					Females				
	Dose (mg/kg bw per day)									
	0	0.05	0.1	1	50	0	0.05	0.1	1	50
No. examined	50	50	50	50	50	50	50	50	50	50
Incidence (original)	3	5	9	11	50	2	2	1	0	49
Incidence (histopathology review panel)	13	10	14	22	50	10	10	6	7	50
- Minimal	6	5	9	14	0	5	8	4	7	0
- Mild	7	5	5	8	4	5	2	2	0	1
- Moderate	0	0	0	0	46*	0	0	0	0	49*

From Bruner & Gopinath (2000)

* $P < 0.05$

Dogs

In a poorly reported 1974 (non-GLP) study, Beagle dogs (seven of each sex per group) were administered Dowco 214 (purity 99.68%; batch No. 238-12-19) in the diet at concentrations equivalent to 0, 0.03, 0.1, 1 or 3 mg/kg bw per day for up to 104 weeks. Three dogs of each sex per group were killed after 6 months for histopathological examination and determination of brain cholinesterase activity. Plasma and erythrocyte cholinesterase activities were determined prior to treatment and at various points during the study according to the method of Casterline & Williams (1967). Limited haematological and clinical chemistry parameters were measured in dogs receiving 0, 1 and 3 mg/kg bw per day after 1, 3, 6, 12, 18 and 24 months. Urinalysis was also performed on these animals. Ophthalmoscopy and veterinary examinations were performed pretest and following treatment for 6, 12, 18 and 24 months. Weights of 15 organs were recorded at necropsy. Histopathology was performed on a comprehensive list of tissues from animals at 0, 1 and 3 mg/kg bw per day. Many results are presented as overall means, not split by sex.

Dietary analyses showed satisfactory achieved concentration and stability of test material. Weight gain was comparable in all groups from week 1 to week 26. Weight gain from week 0 to week 104 was lower in treated groups; slight mean weight loss was seen in top-dose males from weeks 26 to 40. Absolute body weight (males and females combined) was reduced by about 10% at the top dose level. Food and water consumption were not affected by treatment. The study report states that no treatment-related ophthalmological findings were found; however, observations are not reported. Neurological examinations revealed slightly increased abnormal reactions (reaction to sensation and segmental reflex) at the top dose level. No treatment-related effects on haematological, clinical chemistry or urinalysis parameters were noted at any time point.

A clear, dose-related decrease in plasma cholinesterase activity was apparent at 0.1 mg/kg bw per day and higher from week 4 (the first time point). Erythrocyte cholinesterase activity was clearly reduced at 1 mg/kg bw per day and higher from week 4; activity at 0.1 mg/kg bw per day was also reduced intermittently (Table 20). No clear time-related effects on plasma or erythrocyte cholinesterase activity were noted. No treatment-related effect was seen on mean brain cholinesterase activity; however, activities are noted by the present reviewers to be unusually low.

Mean absolute and relative liver weights were slightly increased at the top dose level at the interim and final necropsies. Values did not attain statistical significance and were stated to be within the normal range for the laboratory. Lower thymus weights at 1 and 3 mg/kg bw per day may reflect

Table 20. Plasma, erythrocyte (males and females combined) and brain cholinesterase activities in dogs exposed to chlorpyrifos-methyl in the diet for 2 years

Week	Cholinesterase activity, $\mu\text{mol/ml}$ per minute (% of control)									
	Plasma					Erythrocyte				
	Dose (mg/kg bw per day)									
	0	0.03	0.1	1	3	0	0.03	0.1	1	3
Mean pretest	1.90	1.81	1.89	1.94	1.93	1.76	1.74	1.70	1.64	1.69
4	2.05	2.07	1.87	1.63	1.68	1.88	1.95	1.94	1.74	1.49
	(—)	(101)	(91)	(80)	(82)	(—)	(104)	(103)	(93)	(79)
26	1.50	1.28	1.16	1.00	0.90	1.49	1.32	1.16	0.87	0.75
	(—)	(85)	(77)	(67)	(60)	(—)	(89)	(78)	(58)	(50)
27 ^a	1.62	1.31	1.25	1.06	0.97	1.25	1.10	0.99	0.87	0.74
	(—)	(81)	(77)	(65)	(60)	(—)	(88)	(79)	(70)	(59)
39 ^a	1.41	1.45	1.36	1.07	1.06	1.37	1.28	1.21	1.21	0.94
	(—)	(103)	(96)	(76)	(75)	(—)	(93)	(88)	(88)	(69)
52 ^a	1.58	1.50	1.40	1.24	0.94	1.28	1.36	1.37	1.19	0.95
	(—)	(95)	(89)	(78)	(59)	(—)	(106)	(107)	(93)	(74)
69 ^a	1.62	1.54	1.48	1.23	1.00	1.49	1.54	1.65	1.32	1.29
	(—)	(95)	(91)	(76)	(62)	(—)	(103)	(111)	(89)	(87)
78 ^a	1.74	1.82	1.65	1.21	1.09	1.52	1.41	1.47	1.18	0.70
	(—)	(105)	(95)	(70)	(63)	(—)	(93)	(97)	(78)	(46)
102 ^a	2.15	1.87	2.05	1.36	1.19	2.18	2.15	2.05	1.93	1.51
	(—)	(87)	(95)	(63)	(55)	(—)	(99)	(94)	(89)	(69)
	Brain									
	Males					Females				
	Dose (mg/kg bw per day)									
	0	0.03	0.1	1	3	0	0.03	0.1	1	3
26 ^b	0.025	0.023	0.017	0.020	0.023	0.022	0.020	0.017	0.021	0.019
	(—)	(92)	(68)	(80)	(92)	(—)	(91)	(77)	(95)	(86)
104 ^a	0.018	0.019	0.024	0.018	0.023	0.020	0.021	0.025	0.017	0.019
	(—)	(106)	(133)	(100)	(128)	(—)	(105)	(125)	(85)	(95)

From Rivett et al. (1974)

^a Four of each sex per dose level.

^b Three of each sex per dose level.

the relatively small group size and the high normal variation for this parameter. No treatment-related histopathological findings were noted.

A NOAEL of 1 mg/kg bw per day can be determined for this study, based on decreased body weights in top-dose males. This study is deficient in a number of areas, specifically the limited nature of the investigations, low brain cholinesterase activity and limited reporting. The high purity of the test material used in this study is noted (Rivett et al., 1974).

2.4 Genotoxicity

Testing of the genotoxicity of chlorpyrifos-methyl has been performed in a wide range of assays. Many of the studies were performed in the 1980s and were not conducted according to GLP

or in compliance with current test guidelines. However, the overall database is considered adequate to conclude that chlorpyrifos-methyl is unlikely to be genotoxic.

Data on the genotoxicity of chlorpyrifos-methyl are summarized in [Table 21](#).

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a 1973 (non-GLP) study, groups of 10 male and 20 female Sprague-Dawley rats were fed Dowco 214 (batch No. 238-1219) in the diet for three generations at concentrations equivalent to 0, 1 or 3 mg/kg bw per day. The purity of the test material was not reported; however, other studies using the same batch have reported a purity of 99.68%. Exposure to the test material was continuous through the 9-week pre-mating, mating, gestation and lactation periods. Following at least 9 weeks of exposure, the P_1 , P_2 and P_3 animals were mated (one male to two females) to produce the first of two litters per generation (F_{1A} , F_{2A} and F_{3A}). Rats were mated 2 weeks after the completion of weaning to produce second litters (F_{1B} , F_{2B} and F_{3B}). Successive generations were derived from second litters. Litters were culled to 10 pups on day 4 postpartum. Plasma, erythrocyte and brain cholinesterase activities were measured in P_3 animals only (five of each sex) according to an automated procedure based on the method of Ellman et al. (1961). Comprehensive histopathological examination of P_3 animals (five of each sex from the control and 3 mg/kg bw per day groups) was performed.

Dietary homogeneity and stability of the test material were not reported. No clinical signs of toxicity or treatment-related deaths were noted during the study period. No treatment-related effects on body weight or food consumption were seen in parental animals of any generation. The fertility index of top-dose dams was slightly but consistently lower in each generation. No treatment-related effects on gestation length or pup survival indices were seen. Survival (days 4–21) of F_{3B} pups was low in all groups; this was attributed to the effects of diarrhoea in this generation (actual incidences were not reported). Body weights of treated F_{3B} pups were significantly lower than those of controls; however, there was no clear dose–response, and these findings are not clearly attributable to the effects of treatment and may be secondary to the diarrhoea reported in this generation. Comparable effects were not seen in F_{3A} pups ([Table 22](#)).

No treatment-related macroscopic or microscopic findings were noted. Plasma cholinesterase activity was significantly decreased in all treated P_3 groups. Erythrocyte cholinesterase activity was decreased in top-dose males and in both groups of treated females. No treatment-related effect on brain cholinesterase activity was seen ([Table 23](#)).

A NOAEL of 1 mg/kg bw per day can be determined for this study, based on slightly (but consistently) decreased fertility at the top dose level. This study is considered to have serious deficiencies, including the lack of purity and stability data, the use of only two dose levels, the lack of maternal toxicity at the high dose level and the limited nature of the histopathological and cholinesterase investigations (Thompson, Dyke & Warner, 1975).

Groups of 30 male and 30 female CD rats were fed diets containing variable levels of Reldan F (purity 96.8%; batch No. NB05272036) to give intakes of 0, 1, 3 or 10 mg/kg bw per day for approximately 10 weeks prior to mating and continuing through gestation (3 weeks) and lactation (3 weeks) for each of two generations. A comprehensive evaluation of male and female reproductive systems was conducted, including an evaluation of estrous cyclicity, gonadal function, mating performance, conception, gestation, parturition, lactation and weaning, as well as survival, growth and development of the offspring. Litters were culled to eight pups on day 4. Cholinesterase activity in

Table 21. Results of studies of genotoxicity with chlorpyrifos-methyl

Test substance (vehicle)	End-point	Test object	Concentration	Purity (%); batch	Result	Reference
In vitro						
Dowco 214 (DMSO)	Reverse mutation	<i>Salmonella typhimurium</i> strains TA98, TA100, TA1535, TA1537, TA1538	1, 10, 500, 500, 100, 2500, 5000, 10 000 µg/plate, ±S9	99.9; AGR 95563	Negative	DeGraff (1983)
Dowco 214 (DMSO)	Gene mutation (<i>HPRT</i>)	Chinese hamster ovary cells	5, 20, 40, 60, ^a 80, 100 µmol/l, ±S9	95.2; AGR 219561	Negative	Mendrala (1985)
Dowco 214 (DMSO)	Chromosomal aberration	Chinese hamster ovary cells	4, 12, 40 ^a µg/ml, -S9 5, ^a 15, 50 µg/ml, +S9	95.2; AGR 219561	Negative -S9 Positive +S9	Gollapudi, Linscombe & Sinha (1985)
Dowco 214 (DMSO)	Unscheduled DNA synthesis	Rat primary hepatocytes	1, 3, 10, 30, ^a 100 µmol/l	95.2; AGR 219561	Negative	Mendrala & Dryzga (1985)
In vivo						
Dowco 214 (corn oil)	Micronucleus assay	CD-1 mouse bone marrow (gavage)	0, 146, 460, 1460 mg/kg bw	95.2; AGR 219561	Negative	Bruce, Gollapudi & Hinze (1985)
Reldan F (corn oil)	Unscheduled DNA synthesis	Sprague-Dawley rats (gavage)	0, 600, 2000 mg/kg bw	98.3; RMM 1710	Negative	Proudlock (1994)

DMSO, dimethyl sulfoxide; S9, 9000 × g supernatant from livers of rats

^a Cytotoxicity.

Table 22. Fertility and litter parameters in a reproduction study in rats with chlorpyrifos-methyl

Parameter	Generation	Litter A			Litter B			
		Dose (mg/kg bw per day)						
		0	1	3	0	1	3	
Fertility index (%)	F ₁	95	100	85	85	95	79	
	F ₂	90	100	85	90	100	70	
	F ₃	100	100	80	100	95	80	
Survival, days 4–21 (%)	F ₁	93	90	95	77	81	75	
	F ₂	97	96	98	79	87	97	
	F ₃	79	76	80	47	65	35	
Pup weight (g)	Day 0	6.1	6.0	6.3*	6.3	6.1*	6.2*	
	Day 4	F ₃	7.7	7.8	8.0	8.0	7.3*	6.4*
	Day 21		35.7	34.9	35.5	36.7	31.8*	33.5*

From Thompson, Dyke & Warner (1975)

* $P < 0.05$ **Table 23. Cholinesterase activities in P₃ rats in a reproduction study with chlorpyrifos-methyl**

Cholinesterase sample	Cholinesterase activity, $\mu\text{mol/ml}$ per 3 min (% of controls)					
	Males			Females		
	Dose (mg/kg bw per day)					
	0	1	3	0	1	3
Plasma	2.3 (—)	1.5* (65)	1.1* (48)	7.4 (—)	3.0* (41)	2.0* (27)
Red blood cells	4.1 (—)	3.3 (80)	1.2* (29)	4.2 (—)	3.2* (76)	1.7* (40)
Brain	5.4 $\pm$ 0.5 (—)	8.0 (148)	7.5 (139)	7.1 $\pm$ 0.8 (—)	8.0 (113)	8.5 (120)

From Thompson, Dyke & Warner (1975)

* $P < 0.05$

plasma, erythrocytes, brain and heart was determined in five P₁ and five P₂ adults of each sex per group. Cholinesterase activities were also determined in 10 F₁ and 10 F₂ pups of each sex per group (taken from five litters) on postnatal day 1 and 5 F₁ and 5 F₂ pups of each sex per group (taken from the same litters as on postnatal day 1) on postnatal days 4 and 22. In-life observations, body weights, food consumption and litter data were evaluated. A gross necropsy of the P₁ adults and day 22 weanlings (three of each sex per litter) was conducted, with extensive histopathological examination of tissues. These postmortem evaluations included oocyte quantification and assessment of sperm count, motility and morphology in adults and gross pathology and organ weights in weanlings.

Adrenal weights and vacuolation of the zona fasciculata were increased in parental animals given 10 mg/kg bw per day and in females given 3 mg/kg bw per day. Red blood cell acetylcholinesterase activity was inhibited in a dose-dependent manner in adult rats of all sexes, generations and dose levels. The only other significant effect on adult acetylcholinesterase activity was a moderate inhibition (<20%) in brain (P₁ and P₂ males and females) and in heart (P₁ males only) at the highest dose level (Table 24). In general, pup acetylcholinesterase activity was unaffected by treatment, with the exception of effects on red blood cell acetylcholinesterase activity in the high-dose pups on postnatal day 1. There were no adverse effects of chlorpyrifos-methyl on any parameter of reproductive performance, pup survival, growth or development (Table 25).

Table 24. Cholinesterase results from a rat multigeneration study with chlorpyrifos-methyl

Age	Dose (mg/kg bw per day)	Cholinesterase activity (% of control)					
		Males			Females		
		Red blood cells	Brain	Heart	Red blood cells	Brain	Heart
P ₁ adults	1	79.7	104.3	97.7	82.7*	97.1	102.4
	3	39.5*	100.1	84.7	32.7*	96.4	97.8
	10	19.3*	86.7*	75.4*	6.0*	81.4*	78.1
P ₁ pups day 1	1	78.9	97.4	88.2	101.9	99.8	91.4
	3	91.6	93.5	91.4	110.0	92.7	100.3
	10	63.5*	93.5	88.5	76.6	91.3	93.2
P ₁ pups day 4	1	102.9	103.3	94.7	106.3	96.2	107.5
	3	82.2	90.0	94.4	102.8	95.2	111.7
	10	70.5	101.7	94.0	78.5	96.0	110.7
P ₁ pups day 22	1	112.0	104.8	98.1	105.8	91.5	99.6
	3	108.6	100.3	99.4	95.9	96.3	97.8
	10	128.6*	102.5	95.7	120.5	90.8	86.7
P ₂ adults	1	82.9	97.3	100.3	83.5*	101.3	91.9
	3	30.7*	94.9	95.0	37.8*	100.3	85.9
	10	9.3*	88.1*	86.4	6.8*	90.4*	77.1*
P ₂ pups day 1	1	103.6	91.8	95.7	113.6	91.9	104.1
	3	111.9	92.3	100.4	113.9	90.5	101.0
	10	74.2*	93.5	102.0	84.7	97.8	100.7
P ₂ pups day 4	1	126.1	101.8	92.7	112.7	96.7	100.2
	3	100.4	105.2	99.9	89.9	108.3	100.9
	10	94.7	106.4	109.0	74.7	107.4	97.2
P ₂ pups day 22	1	124.8	104.9	91.3	102.7	100.3	105.7
	3	104.7	101.9	101.1	93.8	100.8	106.4
	10	119.6	103.7	92.1	103.7	100.9	109.2

From Carney et al. (2002)

* $P < 0.05$

The NOAEL for reproduction is 10 mg/kg bw per day, the highest dose tested. The NOAEL for parental toxicity is 1 mg/kg bw per day, based on adrenal changes at 3 and 10 mg/kg bw per day. The NOAEL for pup development is 10 mg/kg bw per day, the highest dose tested (Carney et al., 2002).

(b) *Developmental toxicity*

Rats

Mated female Sprague-Dawley rats (≥ 20 per group) were gavaged with Dowco 214 (purity not specified, in corn oil) at 0, 50, 100 or 200 mg/kg bw per day on days 6–15 of gestation. Blood samples were taken on day 16 from control and 200 mg/kg bw per day animals for the analysis of cholinesterase activity using an automated method based on Ellman et al. (1961). Animals were terminated on day 21 of gestation. Approximately half of the fetuses were examined by serial sectioning, and half were examined for skeletal variations following staining with Alizarin Red. Cholinesterase activity was measured in control and top-dose fetal homogenates ($n = 5$).

Table 25. Reproductive parameters in rats exposed to chlorpyrifos-methyl

Parameter	F ₁ mating				F ₂ mating			
	Dose (mg/kg bw per day)							
	0	1	3	10	0	1	3	10
Number of males	30	30	30	30	29	30	30	30
Number of females	30	30	30	30	30	30	30	30
Time to mating (days)	2.4 ± 1.6	2.2 ± 1.8	2.5 ± 2.5	2.3 ± 0.9	2.7 ± 1.7	2.2 ± 1.1	2.3 ± 1.3	2.4 ± 1.8
Male mating index (%)	100	100	100	96.7	100	96.7	100	100
Female mating index (%)	100	100	100	96.7	100	96.7	100	100
Male fertility index (%)	83.3	83.3	83.3	86.7	82.8	66.7	86.7	80.0
Female fertility index (%)	83.3	83.3	83.3	86.7	80.0	66.7	86.7	80.0
Gestation length (days)	21.6 ± 0.6	21.5 ± 0.5	21.6 ± 0.5	21.8 ± 0.5	21.6 ± 0.6	21.5 ± 0.5	21.5 ± 0.5	21.9 ± 0.3
Pregnant dams delivering (%)	100	100	100	100	100	100	100	100
Gestation survival index (%)	99.0	98.5	97.7	99.7	99.4	99.0	98.6	99.0
Day 1 survival (%)	96.5	98.7	99.1	99.4	98.7	98.9	99.7	98.7
Days 1–4 survival (%)	89.7	91.9	93.1	93.1	91.3	89.8	94.1	90.9
Days 4–21 survival (%)	95.3	97.4	98.5	97.1	98.4	98.7	98.5	97.9
Litter size day 21	7.2 ± 1.3	7.6 ± 1.0	7.9 ± 0.3	7.7 ± 0.8	7.5 ± 1.7	7.8 ± 0.7	7.6 ± 1.1	7.6 ± 0.9
Mean pup weight day 21 (g)	53.4	52.2	53.9	54.6	50.1	46.6	49.9	51.6

From Carney et al. (2002)

No clinical signs of toxicity were reported in this study, although erythrocyte and plasma cholinesterase activities were essentially zero on day 16 (Table 26). Maternal body weight was slightly (but significantly) decreased at 200 mg/kg bw per day. Small increases in the incidence of omphalocele, renal pelvis dilatation, subcutaneous oedema, lumbar ribs or spurs and delayed sternebral ossification were noted in the fetuses of treated groups. Fetal cholinesterase activity was significantly reduced at 200 mg/kg bw per day (Table 26).

No evidence of teratogenicity was noted in this study. In the absence of any measurements of brain or erythrocyte cholinesterase activities in intermediate dose groups, a NOAEL cannot be determined for this study (Schwetz et al., 1973).

In a range-finding study, mated female CD rats (10 per dose level) were gavaged with Reldan (purity 96.9%; batch No. EK900512002/RMM1710) in corn oil at 0, 12.5, 50 or 200 mg/kg bw per day from days 6 to 15 of gestation. Salivation was observed in most rats on day 7 and subsequently at 200 mg/kg bw per day. At 50 mg/kg bw per day, about half the animals salivated after dosing on

Table 26. Maternal and fetal findings in pregnant rats exposed to chlorpyrifos-methyl by gavage

Finding	Dose (mg/kg bw per day)			
	0	50	100	200
Maternal findings				
Maternal body weight, day 16 (g)	320	319	328	307*
Cholinesterase activity ($\mu\text{mol/ml}$ per minute)	Plasma	2.846 ^a		0.012* ^b
	Red blood cells	1.226 ^a		0.000* ^b
Fetal findings				
Omphalocele, % fetal incidence (% litter incidence)	—	—	—	0.7 (4)
Renal pelvis dilatation, % fetal incidence (% litter incidence)	2 (10)	—	1 (8)	7 (20)
Subcutaneous oedema, % fetal incidence (% litter incidence)	19 (45)	—	29 (67)	32 (68)
Lumbar ribs/spurs, % fetal incidence (% litter incidence)	0.6 (3)	0 (0)	2 (8)	5 (20)
Delayed sternebral ossification, % fetal incidence (% litter incidence)	0.6 (3)	5 (26)	11 (38)	8 (28)
Brain cholinesterase activity ($\mu\text{mol/ml}$ per minute)	1.038			0.670**

From Schwetz et al. (1973)

* $P \leq 0.05$; ** $P < 0.01$

^a $n = 2$.

^b $n = 5$.

occasional days after three or more doses; there was no indication of increased frequency as the dosing period progressed, and there was no salivation reported on the last day of dosing. Blood samples for the measurement of cholinesterase activity were taken 2 h following the final dose; brains were removed 1 day after the final dose. Fetuses were examined for gross external malformations. Details of the cholinesterase results are in Table 27 below. No fetal abnormalities were reported.

In the main study, which was designed to meet United States Environmental Protection Agency guidelines, mated female CD rats (30 per dose level) were gavaged with Reldan (purity 96.9%; batch No. EK900512002/RMM1710) in corn oil at 0, 1, 12.5 or 50 mg/kg bw per day from days 6 to 15 of gestation. Blood samples were taken immediately prior to termination on day 20 for the measurement of cholinesterase activity according to the method of Ellman et al. (1961). Brain cholinesterase activity was determined from half-brain homogenates. Approximately half of the fetuses were examined for visceral abnormality by freehand serial sectioning and half for skeletal abnormalities by Alizarin Red staining. In this study, blood samples for the measurement of cholinesterase activity were taken at termination on day 16, 2 h following the final dose; brains were removed 4 days after the final dose.

No effects on body weight or food consumption were seen in dams. Salivation immediately post-dosing was noted in a small number of animals at 50 mg/kg bw per day; no further signs of toxicity were observed. Plasma cholinesterase activity was significantly decreased in dams at 50 mg/kg bw per day, and erythrocyte cholinesterase activity was significantly decreased at 12.5 mg/kg bw per day and higher. Brain cholinesterase activity was slightly (but significantly) decreased at 50 mg/kg bw per day (Table 27). No treatment-related findings were noted at gross necropsy of dams.

The combined incidence of microphthalmia/anophthalmia and the incidences of interventricular septal defect, renal pelvis dilatation and reduced ossification of the cervical vertebral arches were increased in fetuses at 50 mg/kg bw per day (Table 28); however, the increases are marginal and are not clearly a direct result of treatment. The number of normal sternebrae was significantly higher in the top dose group.

Table 27. Cholinesterase activity in pregnant rats exposed to chlorpyrifos-methyl by gavage

Cholinesterase sample	Cholinesterase activity, $\mu\text{mol/ml}$ per minute (% of control)							
	Dose (mg/kg bw per day)							
	0	1	12.5	50	200			
	Main	Range-finding	Main	Main	Range-finding	Main	Range-finding	Range-finding
Plasma	1.60 (—)	1.46 (—)	1.47 (92)	1.48 (93)	0.33 (23)	1.40** (88)	0.18 (12)	0.10 (7)
Red blood cells	1.59 (—)	1.37 (—)	1.60 (101)	1.06** (67)	0.46 (34)	0.85** (53)	0.48 (35)	0.17 (12)
Brain	8.01 (—)	4.67 (—)	8.67 (108)	7.93 (99)	4.18* (90)	7.09** (89)	3.54** (76)	2.11** (45)

From Bryson (1992)

* $P < 0.05$; ** $P < 0.01$

Samples for the measurement of cholinesterase activity in this study were taken at termination (i.e. 5 days following the final administration of test material). Considerable underestimation of cholinesterase inhibition is therefore likely. In the range-finding study, samples were taken 2 h following termination at day 16. Cholinesterase values from the range-finding study are therefore considered to be more representative and are noted by the Meeting to be lower than those from the main study.

Owing to the marginal nature of the brain cholinesterase effects at 12.5 mg/kg bw per day in the range-finding study, an overall maternal NOAEL of 1 mg/kg bw per day can be determined. There was no evidence of teratogenicity, and the NOAEL for teratogenicity is 50 mg/kg bw per day, the highest dose tested. Indications of fetotoxicity (developmental delay) were seen at the top dose level, and a developmental NOAEL of 12.5 mg/kg bw per day can therefore be determined for this study (Bryson, 1992).

Rabbits

In a 1976 (non-GLP) study, mated female Japan White rabbits (10 per dose level) were gavaged with chlorpyrifos-methyl (dissolved in acetone and mixed with corn oil) at 4, 8 or 16 mg/kg bw per day from days 6 to 18 of gestation. Rabbits were terminated on day 29 of gestation, and pups were examined for visceral and skeletal abnormalities. The dose levels used in this study were based on the results of a range-finding study in which decreased plasma cholinesterase activities of 23.3% and 32.3% were seen at 16 and 32 mg/kg bw per day, respectively, following 14 days of administration of chlorpyrifos-methyl.

Mean body weight, mean body weight gain and mean food consumption were lower at 8 and 16 mg/kg bw per day. The numbers of fetuses were also lower at these dose levels. However, values reflected lower corpora lutea and implantation numbers (Table 29) and therefore, taken with the absence of a dose-response, are not considered to be treatment related. No increase in preimplantation or postimplantation loss was seen at these dose levels. No gross external or visceral malformations were noted in any fetus. No treatment-related effect on the incidence of skeletal findings was reported.

Table 28. Fetal findings in rats exposed to chlorpyrifos-methyl during pregnancy

Fetal observation	Dose (mg/kg bw per day)			
	0	1	12.5	50
Anophthalmia/microphthalmia, fetal (litter) incidence	—	—	—	1 (1)
Interventricular septal defect, fetal (litter) incidence	—	1 (1)	—	2 (2)
Renal pelvis dilatation, fetal (litter) incidence	1 (1)	—	—	3 (2)
Reduced ossification – cervical vertebral arches, fetal (litter) incidence	—	—	—	2 (2)
Normal sternebrae (% of total)	40.8	34.3	45.1	58.9*
Abnormal sternebrae (% of total)	59.2	65.7	54.9	41.1*

From Bryson (2002)

* $P < 0.05$

Only 10 pregnant animals per dose level were used in this study, whereas the current OECD guideline recommends the use of 20 animals, with a minimum of 16. Even so, the study is considered adequate to assess the teratogenic potential of chlorpyrifos-methyl in rabbits.

No evidence of teratogenicity was noted in this study. A maternal NOAEL of 4 mg/kg bw per day can be determined for this study, based on decreased body weight, body weight gain and food consumption. No assessment of cholinesterase activity was carried out in the main study; inhibition of plasma cholinesterase activity was noted at 16 mg/kg bw per day in the range-finding study. A developmental NOAEL of 16 mg/kg bw per day, the highest dose tested, can be determined for this study (Asai et al., 1976).

2.6 Special studies

(a) Data on metabolites

Data on the primary plant metabolite of chlorpyrifos-methyl, TCP, were considered by the 1999 JMPR during the review of chlorpyrifos (Annex 1, reference 86). The acute oral toxicity of TCP is moderate, with LD₅₀s in the range of 380–1000 mg/kg bw. In repeated-dose toxicity studies, the liver was the main target organ, with the lowest NOAEL of 12 mg/kg bw per day in the dog. TCP was not genotoxic in vitro or in vivo. There were no developmental effects in rats at dose levels up to 150 mg/kg bw per day, but in rabbits, there were increased incidences of abnormalities, primarily dilatation of the cerebral ventricles and hydrocephaly at 100 mg/kg bw per day and above. The NOAEL was 25 mg/kg bw per day.

(b) Effects on the central nervous system

No acute or repeated-dose neurotoxicity studies or developmental neurotoxicity studies have been performed with chlorpyrifos-methyl.

Acute delayed neuropathy

Domestic hens (six per group) were gavaged with a single dose of Reldan (purity and batch details not given) in corn oil at 0, 3240, 3645, 4050, 4455 or 4860 mg/kg bw and observed for 21 days. A positive control group was dosed with tri-*ortho*-cresyl phosphate (TOCP) at 500 mg/kg bw. Survivors from the 4455 mg/kg bw group and two additional animals were redosed on day 21 following intramuscular injection of atropine (10 mg/kg bw) and pralidoxime methiodide (50 mg/kg bw).

Table 29. Maternal and fetal findings in rabbits dosed with chlorpyrifos-methyl

Observation	Time	Dose (mg/kg bw per day)			
		0	4	8	16
Body weight (kg)	Day 18	3.33 ± 0.08	3.54 ± 0.09	3.11 ± 0.07	3.04 ± 0.06
Body weight gain (kg)	Days 6–18	0.23	0.22	0.16	0.16
Food consumption (g/day)	Days 6–18	141.4	134.7	112.4	113.3
Number of corpora lutea		8.5 ± 0.34	9.0 ± 0.39	6.7 ± 0.42	7.2 ± 0.78
Number of implantations		8.3 ± 0.39	8.5 ± 0.42	6.3 ± 0.39	6.9 ± 0.90
Litter size		7.6 ± 0.47	8.4 ± 0.42	6.1 ± 0.45	6.5 ± 0.87

From Asai et al. (1976)

Deaths occurred in TOCP- and Reldan-treated groups. Lethargy was observed in hens dosed with Reldan and also in the control group, suggesting a vehicle effect. Ataxia, ranging from slight loss of coordination to difficulty in walking, was noted in all birds dosed with TOCP. No consistent effect was seen on body weight. Significant neuropathological changes, including spinal axonal degeneration and mid-brain neuronal and perineuronal vacuolation, were observed in the three TOCP-dosed decedents. Hens dosed with Reldan were not examined histopathologically owing to the absence of clinical signs.

The acute oral LD₅₀ of Reldan in the hen was found to be 4455–4860 mg/kg bw per day. No evidence of neurotoxicity was seen in hens administered chlorpyrifos-methyl; however, histopathological investigations were not performed, there was no forced activity and NTE activity was not measured (Ross, Burroughs & Roberts, 1975).

In a 1979 (non-GLP) study, “adult” White Leghorn hens (10 per group) were administered a single dose of chlorpyrifos-methyl (purity 95.9%; batch No. AGR-142688) by capsule at 0, 2500 or 5000 mg/kg bw. All hens had been administered atropine sulfate at 30 mg/kg bw by capsule 30 min previously. A positive control group was similarly treated using tri-*ortho*-tolyl phosphate (TOTP) at 250 mg/kg bw. Hens were observed for 21 days, and body weights were recorded weekly. Sciatic nerve and cervical, thoracic and lumbar sections of the spinal cord were examined histopathologically following staining with H&E and Luxol Blue. The sciatic nerve was additionally investigated using Bodian’s silver stain.

No deaths occurred during the study period. No clinical signs were noted in controls or at 2500 mg/kg bw. Ataxia and unsteady gait were noted in four hens of the high dose group from day 2. All hens appeared normal by day 7, suggesting incomplete protection by atropine. Hens administered TOTP exhibited progressive signs of neurotoxicity (altered locomotion and loss of coordination) from day 11. Weights of hens dosed with chlorpyrifos-methyl were significantly less than those of controls at day 7, but were comparable at day 14. Body weights of TOTP-dosed hens were significantly lower than those of controls at day 21. Histopathological examination revealed effects in all positive control birds. Spinal findings consisted of vacuolation and demyelination of the cord, with swollen and fragmented axons. Observations in the sciatic nerve consisted of vacuolation and distension of the neurolemma and axonal fragmentation. A number of findings were associated with local inflammatory responses. Similar histopathological findings were noted in six birds dosed with chlorpyrifos-methyl at 5000 mg/kg bw (Table 30). One bird showed evidence of neurotoxicity in both the spinal cord and sciatic nerve, and one additional bird showed evidence of spinal change only. Findings in other birds in this dose group were apparent in either the sciatic nerve or the spinal cord and were described as equivocal. One hen from the 2500 mg/kg bw group had focal alterations of the

Table 30. Neuropathy findings in hen given a single dose of chlorpyrifos-methyl

Test material	Dose level (mg/kg bw)	Deaths	Clinical signs		Histopathology	
			Number	Duration	Spine	Nerve
Chlorpyrifos-methyl	0	—	—	—	—	—
	2500	—	—	—	—	1+
	5000	—	4/10	Days 2–6	2+/2±	1+/2±
TOTP	250	—	10/10	Days 11–21	10+	10+

From Clarke, Warner & Johnstone (1975)

+, positive findings; ±, equivocal findings

sciatic nerve, vacuolation/distension of the neurolemma sheath, granular/fragmentation of axons and increased sarcolemma cells or mononuclear inflammatory cells.

Equivocal histological evidence of neurotoxicity was seen in this study following a supralethal dose of chlorpyrifos-methyl, with only a few test animals showing effects. This study does not comply fully with modern guidelines, as forced motor activity was not assessed, no histopathology was performed on the brain or tibial nerve and NTE activity was not measured (Clarke, Warner & Johnstone, 1975).

Repeated-dose delayed neuropathy

In a 1983 range-finding study, 30-week-old White Leghorn hens (five per group) were gavaged with chlorpyrifos-methyl (purity 96.2%; batch No. AGR 209075) in corn oil at 0, 50, 100, 250, 500, 750 or 1000 mg/kg bw per day, 5 times weekly, for 4 weeks. At necropsy, brain, spinal cord, sciatic nerve and/or tibial nerve were examined histopathologically. Two top-dose birds were found dead on days 15 and 24. Egg production ceased after 1 week at 500 mg/kg bw per day and above. Slight to severe ataxia was noted in two birds at 750 mg/kg bw per day (days 16–17, days 16–22). Signs of mild central nervous system depression described as a “tendency to sleep when undisturbed” were noted in three top-dose birds. Slight ataxia was noted in all top-dose survivors on day 17, with apparent recovery by the following day. “Severe ataxia” was noted in one bird on day 22 prior to its death on day 24. “Early paralysis/inability to walk” was noted in an additional top-dose bird on day 23. Body weights of top-dose birds were significantly lower than those of controls from day 14. Loss of body fat was noted in this group at necropsy. No adverse findings were noted in nervous tissue (Barna-Lloyd et al., 1984).

In a 1984 (non-GLP) study, groups of 10 White Leghorn hens were gavaged with chlorpyrifos-methyl (purity 96.2%; batch No. AGR 209075) in corn oil at 0, 5, 50 or 500 mg/kg bw per day, 5 days/week, for 13 weeks. Additional groups were similarly administered TOCP at 10 or 30 mg/kg bw per day. Routine observations included forced activity on a 30-degree ramp. High-dose positive controls were killed after treatment for 6 weeks as a result of marked signs of neurotoxicity. Selected nervous system tissues were removed at necropsy and examined histopathologically, as detailed in Table 31.

Mean body weights of the top-dose chlorpyrifos-methyl group (from day 3) and high-dose TOCP group (from day 31) were significantly lower than those of controls. Egg production was significantly reduced in the top-dose TOCP and top-dose chlorpyrifos-methyl groups. No clinical signs of neurotoxicity were noted in hens dosed with chlorpyrifos-methyl. Slight ataxia was noted in two hens dosed with TOCP at 10 mg/kg bw per day following forced exercise. Clinical signs in high-dose TOCP hens ranged from severe ataxia to severe paralysis.

Table 31. Histopathology techniques for the repeated-dose delayed neuropathy study with chlorpyrifos

Tissue	Staining procedure ^a		
	1	2	3
Cerebellum, brainstem and optic lobes (TS at middle of optic lobe)	✓	✓	✓
Cerebellum and brainstem (TS at cerebellar peduncles)	✓	—	—
Caudal medulla (TS)	✓	—	—
Mid-cervical, thoracic and lumbosacral spinal cord (TS and LS)	✓	✓	✓
Sciatic and tibial nerve (TS and LS)	✓	✓	✓

From Barna-Lloyd et al. (1984)

LS, longitudinal section; TS, transverse section

^a 1, H&E; 2, Luxol Fast Blue/Periodic acid-Schiff (myelin specific); 3, Sevier-Munger silver (axon specific).

Necropsy revealed macroscopic findings in high-dose chlorpyrifos-methyl and high-dose TOCP groups as detailed in Table 32. Microscopic examination of peripheral and central nervous system tissues revealed findings characteristic of delayed neurotoxicity in TOCP groups; findings were more marked at the high dose level. Histopathological findings were apparently increased in the chlorpyrifos-methyl dose groups; however, these findings were of low grade, showed no significant dose-response, were stated to be similar to background changes reported in the literature and were not considered by the examining pathologist to represent evidence of delayed neurotoxicity (Table 32). NTE inhibition was not assessed in this study (Barna-Lloyd et al., 1984).

NTE inhibition

An in vitro investigation of the potency of chlorpyrifos-methyl oxon to inhibit NTE was performed in 2001. Brain microsomal homogenates were prepared from White Leghorn hens. Assays for NTE and acetylcholinesterase activities were performed to determine the inhibition rate constant (k_i) and the concentration producing 50% inhibition of activity (IC_{50}) for test compounds. Test compounds were chlorpyrifos oxon, chlorpyrifos-methyl oxon and mipafox (a compound reported to produce organophosphate-induced delayed neuropathy in humans).

The results showed that chlorpyrifos-oxon and chlorpyrifos-methyl oxon were very similar in their inhibitory properties, showing a far higher potency against acetylcholinesterase than NTE, whereas mipafox was approximately equipotent (Table 33). The results indicate that chlorpyrifos-methyl is unlikely to induce delayed neurotoxicity at sublethal doses (Richardson & Kropp, 2001).

3. Observations in humans

3.1 Studies in volunteers

Dowco 214 was applied daily to the non-abraded dorsal and abdominal skin of three human volunteers at different dose levels (10, 25 or 50 mg/kg bw per day). A number of applications were made under occlusive dressings for 12 h. Plasma and erythrocyte cholinesterase activities were measured according to the method of Nabb & Whitfield (1967). Rabbits were similarly exposed for comparison purposes. No clinical signs of toxicity were noted in any subject. Plasma cholinesterase activity was slightly depressed in the volunteer administered daily dermal exposures of 10 mg/kg bw per day from day 10. Effects were maximal at 12 days; further applications did not depress values further. Cholinesterase activity had returned to normal by day 25. Following daily exposure to 25 mg/kg bw, plasma cholinesterase activity had decreased by 47.5% at day 4. Recovery was

Table 32. Findings at necropsy in the repeated-dose delayed neuropathy study with chlorpyrifos-methyl

Findings	Incidence (no. of animals out of 10 examined)					
	Control	Chlorpyrifos-methyl			TOCP	
		Dose (mg/kg bw per day)				
		5	50	500	10	30
Shrunken comb	0	0	0	6	0	3
Muscle atrophy	0	0	0	4	0	2
Loss of body fat	0	0	0	2	0	7
Cervical spinal cord						
- Axonal degeneration (dorsal funiculi)	—	—	—	—	1a, 3b, 2c	4b, 2c, 4d
- Axonal degeneration (lateral funiculi)	—	—	—	—	—	4b, 3c, 3d
- Focal gliosis	2a	5a	3a	4a	2a, 4b	6b, 2c, 2d
Thoracic spinal cord						
- Axonal degeneration	—	—	—	2a	1a, 4b, 1c	2b, 2c, 4d, 2e
- Focal gliosis	1a	5a	7a, 1b	6a	4a, 3b	5b, 1c, 2d, 2e
Lumbosacral spinal cord						
- Axonal degeneration	—	—	1a	—	1a, 5b, 2d	5d, 5e
- Focal gliosis	—	1a, 1b	2a, 1b	3a	5b	4b, 3c, 3e
Peripheral nerve						
- Fibre degeneration	—	—	—	1a	2a	1a, 2b, 2c, 2d
Cerebellar peduncle						
- Axonal degeneration	—	1a	—	1a	4a, 1c, 1d	1b, 1c, 1d, 7e
- Focal gliosis	—	—	2a	2a	1a, 1b	1b, 2c, 7d
Caudal medulla						
- Axonal degeneration	—	—	—	—	3a, 5b	5d, 5e
- Focal gliosis	—	—	—	—	2a, 1b	5b, 5c

From Barna-Lloyd et al. (1984)

a, very slight; b, slight; c, slight to moderate; d, moderate; e, moderate to severe

seen by day 28. No effect on plasma cholinesterase activity was seen following a single application of 50 mg/kg bw. Erythrocyte cholinesterase activity was not affected in any volunteer. Effects on rabbits were more severe, with almost complete inhibition of plasma cholinesterase activity at 10 and 25 mg/kg bw per day. Significant inhibition was also noted following a single application of 50 mg/kg bw. Inhibition of erythrocyte cholinesterase activity (90–95%) was seen at 10 and 25 mg/kg bw in the rabbit; marked inhibition was also noted at 50 mg/kg bw. The results of this study suggest that humans are relatively insensitive to the chlorpyrifos-methyl-induced inhibition of plasma and erythrocyte cholinesterase activity following dermal exposure. It is unclear whether this is due to lower penetration through the skin or a lower susceptibility to cholinesterase inhibition, or a combination of both (Killian, Edwards & Pennington, 1971).

In a 1975 (non-GLP) study, healthy male volunteers (4–5 per dose level) were administered capsules containing chlorpyrifos-methyl of unspecified purity at 0 (placebo control), 0.03 or 0.1 mg/

Table 33. Inhibition data for acetylcholinesterase and NTE from hen brain preparations incubated with three organophosphorus compounds

Inhibitor	k_i (per $\mu\text{mol/l}$ per minute)		20-min IC_{50} ($\mu\text{mol/l}$)		RIP
	AChE	NTE	AChE	NTE	
Chlorpyrifos oxon	17.8 ± 0.3	0.0993 ± 0.0049	$0.001\ 95 \pm 0.000\ 03$	0.349 ± 0.017	179 ± 9
Chlorpyrifos-methyl oxon	10.9 ± 0.1	0.0582 ± 0.0013	$0.003\ 18 \pm 0.000\ 03$	0.595 ± 0.013	187 ± 4
Mipafox	$0.004\ 29 \pm 0.000\ 01$	$0.004\ 98 \pm 0.000\ 06$	8.08 ± 0.02	6.96 ± 0.08	0.861 ± 0.011

From Richardson & Kropp (2001)

AChE, acetylcholinesterase; RIP, relative inhibitory potency = $[k_i(\text{AChE})/k_i(\text{NTE})] = [\text{IC}_{50}(\text{NTE})/\text{IC}_{50}(\text{AChE})]$

kg bw per day for 28 days. Urinalysis, haematology and clinical chemistry investigations were performed pretest and weekly during the study. Erythrocyte and plasma cholinesterase activities were measured pretest and twice weekly during the study period according to the method of Nabb & Whitfield (1967). Volunteers signed informed consent forms and were monitored and interviewed twice weekly to assess signs and symptoms of toxicity; particular attention was given to cholinergic symptoms.

The purity and stability of the test material were not reported. A number of volunteers occasionally failed to take their doses; however, this is not considered to have affected the overall integrity of the study. Urinalysis, haematology and clinical chemistry investigations did not reveal any treatment-related effects. No effects were seen on the pulse rate or blood pressure of volunteers. Erythrocyte and plasma cholinesterase activities were not affected by administration of chlorpyrifos-methyl. The study report states that no signs or symptoms attributable to chlorpyrifos-methyl toxicity were noted. A NOAEL of 0.1 mg/kg bw per day can be determined for this study in the absence of any effects on cholinesterase activity (Coulston, Rosenblum & Griffin, 1975).

In a 1975 study, three male and three female volunteers were administered capsules of chlorpyrifos-methyl (purity 99.2%) in corn oil at 0.1 mg/kg bw per day for 21 days and at 0.3 mg/kg bw per day for the following 14 days. Following a 28-day recovery period, the same volunteers were dosed at 0.2 mg/kg bw per day for 21 days. Four control subjects (two of each sex) were administered placebo capsules. Erythrocyte and plasma cholinesterase activities were determined pretest and at intervals throughout the study period according to the method of Levine, Scheidt & Nelson (1965), a DTNB-based procedure based on that of Ellman et al. (1961). Selected haematological, urinalysis and clinical chemistry parameters were also assessed. No reference to informed consent was made in the study report.

No adverse effects on blood pressure, pulse rate, pupil size, light reflex, eye accommodation, chest sound, muscle tone, knee jerk or tongue tremor were reported. No treatment-related effects on haematological, clinical chemistry or urinalysis parameters were noted.

Significant interindividual and intraindividual variations in plasma and erythrocyte cholinesterase activities were noted. Treatment-related decreases in plasma cholinesterase activity were reported in volunteers following administration of chlorpyrifos-methyl at 0.2 and 0.3 mg/kg bw per day; no effects were seen at 0.1 mg/kg bw per day (Table 34). No consistent effects were seen on erythrocyte cholinesterase activity at any dose level in this study. Increased cholinesterase activity in volunteer CS was attributed to “withdrawal from birth-control medication”.

Table 34. Cholinesterase activities in human volunteers receiving chlorpyrifos-methyl

Group	Subject (sex)	Cholinesterase activity, μmol/ml per 3 min (% of control)									
		Pretest mean	0.1 mg/kg bw per day		0.3 mg/kg bw per day		Re- covery	0.2 mg/kg bw per day		Recovery	
			Low ^a	Term ^b	Low	Term		Term	Low		Term
Plasma cholinesterase											
Control	PW (F)	10.65	9.88	10.56	9.93	9.93	10.12 ^c	9.66	9.66		
		(—)	(93)	(99)	(94)	(94)	(95)	(91)	(91)		
	CM (F)	8.79	7.59	8.50	8.01	9.20	9.18	8.02	8.59		
		(—)	(86)	(97)	(91)	(105)	(104)	(91)	(98)		
	CB (M)	13.69	11.80	13.05	12.63	13.71	12.78	12.77	13.84		
		(—)	(86)	(95)	(92)	(100)	(93)	(93)	(101)		
	PS (M)	16.18	15.35	16.23	14.00	16.83	17.20	16.51	17.03		
		(—)	(95)	(100)	(87)	(104)	(106)	(102)	(105)		
	Test	CS ^d (F)	7.88	7.38	9.03	8.28	8.28	8.06	7.34	7.34	—
			(—)	(94)	(115)	(105)	(105)	(102)	(93)	(93)	
	JC (F)	10.55	9.75	9.75	8.40	8.40	10.35	9.38	9.38	9.18	
		(—)	(92)	(92)	(80)	(80)	(98)	(89)	(89)	(87)	
	BW (F)	11.38	10.56	10.56	9.63	9.95	11.02	7.77	7.77	11.82	
		(—)	(93)	(93)	(85)	(87)	(97)	(68)	(68)	(104)	
	RP (M)	14.16	13.36	14.45	13.01	13.17	14.80	14.34	14.66	—	
		(—)	(94)	(102)	(92)	(93)	(105)	(101)	(104)		
MG (M)	15.11	13.97	14.45	12.43	12.91	14.80	14.37	14.37	—		
	(—)	(92)	(96)	(82)	(85)	(98)	(95)	(95)			
KH (M)	12.35	11.42	12.80	9.34	9.20	12.14	8.00	8.00	9.04		
	(—)	(92)	(104)	(76)	(74)	(98)	(65)	(65)	(73)		
Erythrocyte cholinesterase											
Control	PW (F)	31.05	30.26	31.70	24.62	24.62	26.37 ^a	30.05	30.05		
		(—)	(97)	(102)	(79)	(79)	(85)	(97)	(97)		
	CM (F)	29.48	28.45	29.75	26.15	28.58	29.52	29.87	30.51		
		(—)	(97)	(101)	(88)	(97)	(100)	(101)	(103)		
	CB (M)	35.74	33.34	35.66	32.76	32.76	32.53	27.50	35.28		
		(—)	(93)	(98)	(92)	(92)	(91)	(77)	(99)		
	PS (M)	28.94	26.39	29.75	24.46	26.55	27.74	27.90	27.90		
		(—)	(91)	(100)	(85)	(92)	(96)	(96)	(96)		

Table 34 (contd)

Group	Subject (sex)	Cholinesterase activity, $\mu\text{mol/ml}$ per 3 min (% of control)								
		Pretest mean	0.1 mg/kg bw per day		0.3 mg/kg bw per day		Re- covery	0.2 mg/kg bw per day		Recovery
			Low ^a	Term ^b	Low	Term		Term	Low	
Test	CS ^b (F)	35.89	30.13	39.07	30.16	30.55	35.45	34.46	34.70	—
		(—)	(84)	(109)	(84)	(85)	(99)	(96)	(97)	
	JC (F)	39.36	37.66	40.63	33.43	34.63	38.89	38.40	42.30	—
		(—)	(96)	(103)	(85)	(88)	(99)	(98)	(107)	
	BW (F)	37.55	34.48	39.34	31.72	35.29	36.09	37.15	37.15	29.29
		(—)	(92)	(105)	(84)	(94)	(96)	(99)	(99)	(78)
	RP (M)	29.24	26.78	31.70	25.99	25.99	27.74	28.70	28.70	30.74
		(—)	(92)	(108)	(89)	(89)	(95)	(98)	(98)	(105)
	MG (M)	33.10	29.79	33.86	28.58	28.58	31.47	31.48	31.48	—
		(—)	(90)	(102)	(86)	(86)	(95)	(95)	(95)	
	KH (M)	32.31	30.26	35.04	27.67	27.67	30.96	26.77	32.50	31.65
		(—)	(94)	(108)	(86)	(86)	(96)	(83)	(101)	(98)

From Chmiel (1975)

F, female; M, male

^a Lowest value for treatment period.^b Value at termination of treatment period.^c Results for previous time point.^d See text.

A NOAEL of 0.3 mg/kg bw per day can be determined for this study in the absence of any biologically significant effects on erythrocyte cholinesterase inhibition (Chmiel, 1975).

In a double-blind, randomized, placebo-controlled study of the effects of single oral doses of the closely related and more potent cholinesterase inhibitor, chlorpyrifos (purity 99.8%), groups of six fasted men and six fasted women aged 18–55 received doses of 0, 0.5, 1 or 2 mg/kg bw in lactose powder. The study was conducted in two phases separated by 14 days. The volunteers were dosed with 0, 0.5 or 1 mg/kg bw in the first phase, and the results were assessed before administration of 0 or 2 mg/kg bw in the second phase. Blood samples were collected 10 and 0 h before treatment and 2, 4, 8, 12, 24, 36, 48, 72, 96, 120, 144 and 168 h after treatment and analysed for erythrocyte cholinesterase activity and chlorpyrifos and its metabolites. In addition, all urine voided from 48 h before dosing to 168 h after dosing was collected at 12 or 6 h intervals and analysed for chlorpyrifos and its metabolites. Haematology, clinical chemistry, urinalysis and a brief physical examination were performed at completion of the study. Blood and urine were collected to determine each volunteer's paraoxonase status and the concentrations of chlorpyrifos and its metabolites, but these data were not reported. The volunteers were screened for general health according to set criteria and instructed to refrain from alcohol, strenuous exercise and prescription medications before and during the study. The doses were taken by capsule after an overnight fast. The health status of subjects was monitored closely; vital signs (blood pressure, pulse, respiration and temperature) were assessed before dosing and 1, 2, 4, 8, 12, 24, 48 and 168 h after treatment. The subjects were questioned about their well-being at each sampling time, and the symptoms were evaluated clinically. The subjects were aware of the signs and symptoms of cholinergic toxicity and were instructed to inform the study physician of any adverse

Table 35. Mean erythrocyte cholinesterase activity in volunteers given single oral doses of chlorpyrifos

Dose (mg/kg bw)	Mean erythrocyte cholinesterase activity as % of mean baseline value ($\pm$ SD)							
	Time in relation to dosing (h)							
	-10	0	4	8	12	24	36	48
Women								
0.0 ^a	99.5 (2.1)	100.5 (2.1)	98.3 (2.0)	102.1 (2.4)	100.7 (1.8)	99.7 (3.9)	98.4 (1.7)	100.2 (2.3)
0.0 ^b	99.2 (4.4)	100.8 (4.4)	102.5 (5.9)	103.1 (5.4)	98.4 (3.2)	99.4 (3.7)	94.6 (5.3)	97.7 (5.1)
0.5	101.2 (6.7)	98.8 (6.7)	96.3 (6.5)	98.1 (2.1)	95.8 (3.9)	97.0 (3.6)	94.7 (4.7)	97.1 (4.1)
1	98.2 (0.9)	101.8 (0.9)	104.6 (4.2)	100.8 (3.5)	98.9 (3.7)	100.9 (5.6)	96.0 (4.1)	98.9 (2.1)
2	99.8 (1.8)	100.2 (1.8)	100.3 (3.2)	96.6 (6.81)	91.1 (9.7)	95.2 (10.6)	95.9 (7.4)	94.5 (7.7)
Men								
0.0 ^a	100.6 (1.8)	99.4 (1.8)	99.1 (2.2)	99.6 (4.6)	99.8 (4.3)	98.4 (4.3)	97.6 (2.8)	98.2 (3.1)
0.0 ^b	96.1 (5.1)	103.9 (5.1)	101.6 (4.0)	102.5 (5.2)	102.9 (5.8)	102.6 (2.3)	98.2 (3.8)	95.9 (6.8)
0.5	98.7 (1.1)	101.3 (1.1)	102.0 (3.0)	103.4 (4.9)	99.7 (3.0)	101.9 (3.3)	98.1 (3.0)	92.8 (4.7)
1	98.6 (1.9)	101.4 (1.9)	104.5 (7.8)	101.1 (4.2)	101.1 (4.7)	101.7 (4.7)	98.5 (4.6)	92.1 (2.0)
2	101.2 (2.3)	98.8 (2.3)	99.2 (3.1)	98.9 (3.5)	98.7 (4.0)	99.2 (2.6)	99.5 (5.1)	98.4 (3.1)

From Kisicki et al. (1999)

^a Phase 2 control.

^b Phase 1 control.

effects. The subjects were unaware of their treatment, and the signs and symptoms were assessed and treated by a physician who was also unaware of the treatment status of the subject.

There were no significant deviations from the study protocol. One male control in phase 1 and one woman receiving 2 mg/kg bw did not provide a complete series of blood and urine samples. Treatment had no effect on general health or on clinical chemical parameters measured 7 days after dosing. The only treatment-related effect was found in the woman who withdrew from the study, who had decreased erythrocyte cholinesterase activity when compared with her pretreatment values at most sampling times, with 98.4% of the pretreatment value at 4 h after dosing, 77% at 8 h, 72% at 12 h, 74% at 24 h, 81% at 36 h and 80% at 48 h (Table 35). When the data for this subject are removed from the analysis, the mean for women receiving 2 mg/kg bw is indistinguishable from the value for concurrent controls (Table 35). The NOAEL for clinical signs or symptoms was thus the highest dose tested, and the NOAEL for inhibition of erythrocyte cholinesterase activity was 1 mg/kg bw on the basis of significant inhibition in 1 of 12 subjects (Kisicki et al., 1999).

3.2 Workplace monitoring

Chlorpyrifos-methyl is produced in a single plant alongside other materials, including chlorpyrifos. A specific occupational hygiene monitoring and health surveillance programme was designed with regard to the manufacture and packaging of chlorpyrifos-methyl (Reldan) in the United Kingdom. Baseline examinations, involving physical, haematological and clinical chemistry investigations, were undertaken in 1980 prior to production. Plasma and erythrocyte cholinesterase activities were measured on three separate occasions before potential exposure. The mean of these estimations was used as each employee's baseline pre-exposure cholinesterase level. Exposure to chlorpyrifos-methyl (and intermediates) during manufacture was designed to be minimized by the use of engineering controls and personal protective equipment. Measured airborne concentrations of chlorpyrifos-methyl were typically less than 10% of those recommended in the production plant's internal occupational hygiene guide.

Plasma and erythrocyte cholinesterase activities were subsequently measured in all employees potentially exposed to chlorpyrifos-methyl. Results were recorded and compared with individual baseline values. Data were further analysed by shift and job type. Significant drops in cholinesterase activity of either individuals or groups resulted in modified work practices and investigation by the occupational health team. In accordance with company guidelines, employees were to be suspended from work if plasma cholinesterase activities were reduced by 80%. A depression of this extent was not encountered during the 15-year manufacturing period.

In 1994, a review and analysis of the health records of all employees currently (104) or previously engaged (114) in the manufacturing plant since 1980 were undertaken. The total data represented approximately 716 person-years. Conclusions from the analysis of these data revealed no significant health problems in the group studied when compared with colleagues on the same site unexposed to organophosphate insecticides. In addition, no documented cases of organophosphate poisoning or severe cholinesterase depression were found.

Data collected from 1991 to 1993 are stated to reveal a small but consistent drop in plasma and erythrocyte cholinesterase activities. The notifier suggests that exposure is probably via skin absorption and reports that this finding is not obvious unless individual data are pooled and compared with a suitable control group. No increases in morbidity, mortality or the incidence of neurological disorders in the study population over a 15-year period were seen when compared with a comparative control population (Dow, 2008).

More recent workplace monitoring data on chlorpyrifos-methyl have not been compiled.

Comments

Biochemical aspects

Chlorpyrifos-methyl is rapidly and extensively absorbed in rats given a single oral dose at 16 or 30 mg/kg bw. Excretion was rapid (largely within 24 h) and primarily in the urine. Urinary metabolites were identified as the glucuronide conjugate of TCP (68.6%), the desmethyl metabolite *O*-methyl-*O*-(3,5,6-trichloropyridyl) phosphorothioate (17.8%) and free TCP (13.8%). Although these results were reported very briefly, they are broadly consistent with data for the closely related compound chlorpyrifos ([Annex 1](#), reference 86). The fate of the phosphorothioate moiety was not investigated.

Toxicological data

Chlorpyrifos-methyl is of low acute toxicity when administered orally, dermally (LD_{50} >2000 mg/kg bw) or by inhalation (LC_{50} >0.67 mg/l). Chlorpyrifos-methyl is a slight, transient irritant to skin and eye and has been found to produce skin sensitization in a Magnusson & Kligman maximization test, but not in a Buehler test.

Short-term studies of toxicity identified decreased cholinesterase activity and adrenal vacuolation as the most sensitive indicators of toxicity caused by chlorpyrifos-methyl. Studies did not show any consistent time-related progression in the inhibition of plasma or erythrocyte cholinesterase activity with repeated or prolonged administration of chlorpyrifos-methyl, suggesting that inhibition reaches a “steady state” relatively rapidly. There was evidence of significant but not complete recovery of cholinesterase activities after 2 or more weeks. In the 28-day study in mice, the NOAEL was 10 ppm, equal to 1.3 mg/kg bw per day, on the basis of reduced brain cholinesterase activity and vacuolation of the zona fasciculata of the adrenals. The same end-points were the basis for the NOAEL of 1 mg/kg bw per day in the 90-day study in rats. Decreased brain cholinesterase activity, decreased body weight gain, clinical chemistry and haematological findings were noted at the highest dose of 50 mg/kg bw per day in a 90-day study in dogs, with a NOAEL of 10 mg/kg bw per day.

No evidence of toxicity, including brain cholinesterase activity, was reported in a 6-month study in Rhesus monkeys given doses of up to 5 mg/kg bw per day.

The potential genotoxicity of chlorpyrifos-methyl has been investigated in an adequate battery of tests in vitro and in vivo. No evidence of mutagenicity was noted; however, chlorpyrifos-methyl was found to be clastogenic in Chinese hamster ovary cells in the presence of metabolic activation. Studies in vivo on micronucleus formation in bone marrow and on unscheduled DNA synthesis gave negative results.

The Meeting concluded that chlorpyrifos-methyl is unlikely to be genotoxic.

No evidence of carcinogenicity was seen in long-term studies of toxicity/carcinogenicity with chlorpyrifos-methyl in rats or mice. Adrenal pathology (vacuolation of the adrenal cortex zona fasciculata consistent with lipid accumulation) was noted in rats and mice. Having considered the outcome of a pathology review by a group that re-examined the slides of adrenal tissues obtained in the study in rats, the Meeting concluded that the findings at 1 mg/kg bw per day were not adverse. Decreased brain cholinesterase activity was found to be a consistent and sensitive indicator of chronic toxicity caused by chlorpyrifos-methyl. The inhibition of cholinesterase activity by chlorpyrifos-methyl seen in the long-term studies did not increase with duration of dosing. The NOAEL was 1 mg/kg bw per day in rats and 3.9 mg/kg bw per day in mice. Toxicity in a limited 2-year study in dogs was limited to reduced body weight gain at the highest dose of 3 mg/kg bw per day, with a NOAEL of 1 mg/kg bw per day.

The Meeting concluded that chlorpyrifos-methyl is not carcinogenic.

Marginal effects on fertility were seen at the highest dose of 3 mg/kg bw per day in an early three-generation study in rats; the NOAEL was 1 mg/kg bw per day. A subsequent, more extensive, two-generation study in rats found no effects on reproduction or pup development at 10 mg/kg bw per day; the NOAEL for parental toxicity was 1 mg/kg bw per day on the basis of findings in the adrenal gland. In an initial study of developmental toxicity in rats, there was no indication of teratogenicity at 200 mg/kg bw per day. Indications of delayed fetal development were seen at all doses (50 mg/kg bw per day and above), but without a clear dose–response relationship. In a range-finding study of developmental toxicity in rats, there was no indication of teratogenicity at 200 mg/kg bw per day, a dose producing salivation immediately after the second and subsequent doses and significant inhibition of cholinesterase activity. At 12.5 mg/kg bw per day, there was slight inhibition (10%) of brain cholinesterase activity 1 day after the final dose. The NOAEL for maternal toxicity was considered to be 1 mg/kg bw per day. The Meeting considered that the salivation was unlikely to be a result of systemic toxicity, as it occurred immediately after dosing, whereas the $C_{\max}$ was at 5 h, and there was evidence that chlorpyrifos-methyl tasted unpleasant at high concentrations. In a full study of developmental toxicity in rats, the NOAEL for maternal toxicity (brain cholinesterase activity 4 days after the final dose) and pup development (overall rate of anomalies) was 12.5 mg/kg bw per day, with a NOAEL for teratogenicity of 50 mg/kg bw per day, the highest dose tested. The only study of developmental toxicity in rabbits given chlorpyrifos-methyl was not performed to modern standards, but was considered adequate to assess the potential for teratogenicity. The NOAEL for maternal toxicity was 4 mg/kg bw per day on the basis of reductions in body weight gain and food consumption. The NOAEL for teratogenicity and fetal developmental toxicity was 16 mg/kg bw per day, the highest dose tested.

The Meeting concluded that chlorpyrifos-methyl caused developmental toxicity only at doses that were maternally toxic, but that it was not teratogenic.

The primary plant and mammalian metabolite of chlorpyrifos-methyl, TCP, was considered by the 1999 JMPR during the review of chlorpyrifos ([Annex 1](#), references 86 and 88). The acute oral toxicity of TCP is moderate, with LD_{50} s in the range of 380–1000 mg/kg bw. In studies of toxicity with repeated doses, the liver was the main target organ, with the lowest NOAEL of 12 mg/kg bw per day being identified in a study in dogs. TCP was not genotoxic in vitro or in vivo. There were no

developmental effects at doses of up to 150 mg/kg bw per day in rats, but rabbits showed increased incidences of abnormalities, primarily dilatation of the cerebral ventricles and hydrocephaly at 100 mg/kg bw per day and above, and the NOAEL was 25 mg/kg bw per day.

Some histopathological evidence of neuropathy was noted in hens given a single potentially lethal dose of chlorpyrifos-methyl at 5000 mg/kg bw. Equivocal histopathological findings noted in a short-term study of delayed neurotoxicity were considered to be similar to background findings and not consistent with delayed neuropathy. No assessment of NTE activity was made in the studies of neurotoxicity, but a study in vitro showed that chlorpyrifos-methyl oxon had a potency for inhibiting cholinesterase activity that was more than 100-fold that of NTE. This study also showed that chlorpyrifos-methyl oxon was less potent than chlorpyrifos oxon as an inhibitor of brain cholinesterase activity in hens.

The Meeting concluded that chlorpyrifos-methyl was unlikely to produce delayed neuropathy in the absence of very severe cholinergic toxicity.

In two studies in human volunteers exposed orally to chlorpyrifos-methyl for 21 or 28 days, there were no adverse findings concerning clinical signs, clinical chemistry or cholinesterase activity. The NOAEL was 0.3 mg/kg bw per day, the highest dose tested, over 21 days. A single oral dose of (the closely related compound) chlorpyrifos of up to 1 mg/kg bw did not significantly inhibit erythrocyte cholinesterase activity in human volunteers. The studies in human volunteers were considered to have been performed according to contemporary ethical standards.

There were no reports of adverse effects in production plant workers.

The Meeting concluded that the existing database on chlorpyrifos-methyl was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.01 mg/kg bw based on the NOAEL of 1 mg/kg bw per day, identified on the basis of inhibition of brain acetylcholinesterase activity and adrenal vacuolation in the 2-year study of toxicity and carcinogenicity in rats and with a safety factor of 100. This value is supported by the NOAEL of 1 mg/kg bw per day for inhibition of parental brain acetylcholinesterase activity in the multigeneration study of reproductive toxicity in rats and by the NOAEL of 1 mg/kg bw per day for inhibition of maternal brain acetylcholinesterase activity in the study of developmental toxicity in rats. The Meeting did not consider changes observed in the adrenals of rats given a dose of 1 mg/kg bw per day in the 2-year study to be treatment related, a conclusion that is consistent with that of the pathology review group. Limited studies in human volunteers, while not of sufficient quality (e.g. too few subjects, limited duration of treatment and the fact that no assessment of the adrenals was possible) to support their use in the derivation of an ADI, provide no basis for concern that the proposed ADI would not be adequately protective. In a number of studies, erythrocyte acetylcholinesterase activity was more sensitive than brain acetylcholinesterase activity to inhibition by chlorpyrifos-methyl. However, the Meeting noted that after oral administration, the sensitivity of heart acetylcholinesterase activity to inhibition by chlorpyrifos-methyl was similar to that of brain acetylcholinesterase. It was further noted that the differential sensitivity of acetylcholinesterase was the same as that observed with the close structural analogue chlorpyrifos (Marable et al., 2007). In vivo, the sensitivity of the enzyme in peripheral neuronal tissue is similar to that in the brain, whereas the enzyme in erythrocytes is more sensitive. The Meeting therefore concluded that inhibition of brain acetylcholinesterase activity, not erythrocyte acetylcholinesterase activity, was the appropriate end-point for use in the risk assessment of chlorpyrifos-methyl.

The Meeting established an ARfD of 0.1 mg/kg bw based on the NOAEL of 1 mg/kg bw, identified on the basis of the absence of inhibition of erythrocyte acetylcholinesterase activity in a single-dose study in human volunteers given the closely related compound chlorpyrifos, and with a safety factor of

10. The Meeting discussed whether an ARfD was necessary for chlorpyrifos-methyl, given the absence of any clear indications of systemic toxicity after single exposures. In the absence of adequate single-dose studies with extensive investigations of cholinesterase activity and clinical signs, the Meeting considered that it was not able to discount the possibility that chlorpyrifos-methyl could produce acute effects. The Meeting considered basing the ARfD on the repeated-dose study in human volunteers given chlorpyrifos-methyl, in which an overall NOAEL of 0.3 mg/kg bw per day was identified. It was noted that this was somewhat inconsistent with the higher NOAEL of 1 mg/kg bw in a single-dose study in humans given the closely related, but more potent, compound chlorpyrifos. Having considered data on the kinetics and acetylcholinesterase inhibition characteristics of chlorpyrifos and chlorpyrifos-methyl, the Meeting concluded that, although likely to be conservative, it was appropriate to use data from the single-dose study in humans given chlorpyrifos to establish the ARfD for chlorpyrifos-methyl. No other potentially acute effect that might serve as the basis for derivation of an ARfD was identified in studies in experimental animals.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year study of toxicity and carcinogenicity ^a	Toxicity	50 ppm, equal to 3.9 mg/kg bw per day	500 ppm, equal to 41 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 41 mg/kg bw per day ^b	—
Rat	Two-year studies of toxicity and carcinogenicity ^a	Toxicity	1 mg/kg bw per day	50 mg/kg bw per day
		Carcinogenicity	50 mg/kg bw per day ^b	—
	Multigeneration study of reproductive toxicity ^a	Reproductive toxicity	10 mg/kg bw per day ^b	—
		Parental toxicity	1 mg/kg bw per day	3 mg/kg bw per day
		Offspring toxicity	10 mg/kg bw per day ^b	—
	Developmental toxicity ^c	Maternal toxicity	1 mg/kg bw per day	12.5 mg/kg bw per day
		Embryo/fetotoxicity	12.5 mg/kg bw per day	50 mg/kg bw per day
Rabbit	Developmental toxicity ^c	Maternal toxicity	4 mg/kg bw per day	12–16 mg/kg bw per day
		Embryo/fetotoxicity	16 mg/kg bw per day ^b	—
Dog	Ninety-day study of toxicity	Toxicity	10 mg/kg bw per day	50 mg/kg bw per day
	Two-year study of toxicity ^a	Toxicity	1 mg/kg bw per day	3 mg/kg bw per day
Rhesus monkey	Twenty-six-week study of toxicity ^c	Toxicity	5 mg/kg bw per day ^b	—
Humans	Twenty-eight-day study of toxicity ^d	Toxicity	0.2 mg/kg bw per day ^b	—
	Twenty-one-day study of toxicity ^d	Toxicity	0.3 mg/kg bw per day ^b	—
	Single-dose study of toxicity with chlorpyrifos ^d	Toxicity	1 mg/kg bw ^b	—

^a Dietary administration.

^b Highest dose tested.

^c Gavage administration.

^d Capsule administration.

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw

Estimate of acute reference dose

0.1 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to chlorpyrifos-methyl

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption	Rats: rapid and extensive, >80%
Dermal absorption	Low: <5%, concentrated and diluted, rat epidermis in vitro
Distribution	Widely distributed
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and almost complete, within 72 h, mainly via urine (83–85%), after a single dose
Metabolism in animals	Extensively metabolized; demethylation, hydrolysis, conjugation, oxidative desulfuration
Toxicologically significant compounds (animals, plants and the environment)	Parent and oxon

Acute toxicity

Rat, LD ₅₀ , oral	2814 mg/kg bw
Rat, LD ₅₀ , dermal	>2000 mg/kg bw
Rat, LC ₅₀ , inhalation	>0.67 mg/l air (nose only)
Rabbit, dermal irritation	Slight, transient irritant
Rabbit, ocular irritation	Slight, transient irritant
Guinea-pig, dermal sensitization	Negative results in Buehler test; positive results in Magnusson & Kligman maximization test

Short-term studies of toxicity

Target/critical effect	Inhibition of acetylcholinesterase activity, adrenal vacuolation
Lowest relevant oral NOAEL	1 mg/kg bw per day (rat)
Lowest relevant dermal NOAEL	10 mg/kg bw per day (systemic)
Lowest relevant inhalation NOAEC	18 ppb (approximately 100 µg/m ³)

Genotoxicity

No genotoxic potential in vivo

Long-term studies of toxicity and carcinogenicity

Target/critical effect	Inhibition of acetylcholinesterase activity, adrenal vacuolation
Lowest relevant NOAEL	1 mg/kg bw per day
Carcinogenicity	Not carcinogenic

Reproductive toxicity

Reproduction target/critical effect	Not toxic to reproduction
Lowest relevant reproductive NOAEL	10 mg/kg bw per day
Developmental target/critical effect	Not teratogenic; delayed fetal development, slight increase in abnormalities at maternally toxic doses
Lowest relevant developmental NOAEL	12.5 mg/kg bw per day (rat)

Neurotoxicity/delayed neurotoxicity

Histopathological indications of neuropathy at 5000 mg/kg bw;
no indications of delayed neuropathy at 500 mg/kg bw per day
for 13 weeks; very weak inhibitor of NTE in vitro

Other toxicological studies

Studies in human volunteers

No adverse effects at doses of up to 0.3 mg/kg bw per day for
21 days

Single-dose study in human volunteers given
chlorpyrifos

No adverse effects at doses of up to 1 mg/kg bw

Medical data

No adverse effects in production plant workers

Summary

	Value	Study	Safety factor
ADI	0–0.01 mg/kg bw	Rat, 2 years, dietary Rat, reproductive toxicity Rat, developmental toxicity Dog, 2 years	100
ARfD	0.1 mg/kg bw	Single-dose study in human volunteers given chlorpyrifos	10

References

- Asai, M. et al. (1976) Study of the effects of chlorpyrifos-methyl on rabbits, embryonal and fetal development. Unpublished report from the Laboratory of Toxicology & Safety, Sankyo, Japan. Dow Report No. GHF-R-4. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Bakke, J.E. & Price, C.E. (1976) Metabolism of *O,O*-dimethyl-*O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate in sheep and rats and of 3,5,6-trichloro-2-pyridinol in sheep. *Journal of Environmental Science and Health. Part B, Pesticides, Food Contaminants, and Agricultural Wastes*, 11(1):9–22.
- Barna-Lloyd, T., Szabo, J.R. & Davis, N.L. (1990) Chlorpyrifos-methyl (Reldan R): rat subchronic dietary toxicity and recovery study. Unpublished report from Dow Chemical Co., TX, USA. Report No. TXT:K-0461-93-026. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Barna-Lloyd, T., Szabo, J.R. & Davis, N.L. (1991) Chlorpyrifos-methyl (Reldan insecticide): chronic dietary toxicity/oncogenicity study in rats. Unpublished report No. from Dow Chemical Co., TX, USA. Report No. TXT:K-0461-93-031. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Barna-Lloyd, T. et al. (1984) Chlorpyrifos-methyl insecticide: sub-chronic (3 month) delayed neurotoxicity study in laying chicken hens. Unpublished report from Dow Chemical Co., TX, USA. Report No. TXT:K-0461-93-018. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Branson, D.R. & Litchfield, N.H. (1970) Comparative absorption, elimination and distribution of DOWCO 179, its methyl analog DOWCO 214 and their major metabolite, 3,5,6-trichloro-2-pyridinol. Unpublished report from Dow Chemical Co., MI, USA. Report No. BC R-603. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Bruce, R.J., Gollapudi, B.B. & Hinze, C.A. (1985) Evaluation of chlorpyrifos-methyl in the mouse bone marrow micronucleus test. Unpublished report from Dow Chemical Co., TX, USA. Report No. TXT:K-0461-93-020. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.

- Bruner, R.H. & Gopinath, C. (2000) Chlorpyrifos-methyl (RELDAN insecticide): pathology peer review—adrenocortical vacuolar change. Unpublished report by Pathology Associates International, OH, USA, and Huntingdon Life Sciences, Cambridgeshire, United Kingdom. Report No. K-046193-031. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Bryson, A.M. (1992) A study of the effect of technical Reldan on pregnancy of the rat. Unpublished report from Huntingdon Research Centre, United Kingdom. Report No. GHE-T-413. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Carney, E.W. et al. (2002) Chlorpyrifos-methyl: two-generation dietary reproduction toxicity study in CD rats. Unpublished report from Dow Chemical Co., MI, USA. Report No. 011132. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Casterline, J.L., Jr & Williams, C.H. (1967) The detection of cholinesterase inhibition in erythrocytes of rats fed low levels of the carbamate Banol. *Journal of Laboratory and Clinical Medicine*, 69(2):325–329.
- Chmiel, J. (1975) A study on the effects of chlorpyrifos-methyl on plasma and erythrocyte cholinesterase activity in human subjects during sub-acute oral administration. Unpublished report from Industrial Bio-Test Labs Inc., IL, USA. Report No. IBT 636-07142. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Clarke, W.E., Warner, S.D. & Johnstone R.U. (1975) Acute delayed neurotoxicity evaluation in chlorpyrifos-methyl in White Leghorn hens. Unpublished report from Dow Chemical Co., TX, USA. Report No. TXT:3280.0. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Clouzeau, J. (1992) Chlorpyrifos-methyl technical: acute oral toxicity in mice. Unpublished report from C.I.T., France. Report No. 8756 TAS. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Coulston, F., Rosenblum, I. & Griffin, T. (1975) Study of chlorpyrifos-methyl in human volunteers. Unpublished report from Albany Medical College, NM, USA. Dow Report No. GH-RC 27. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- DeGraff, W.G. (1983) Evaluation of DOWCO 214 in the Ames *Salmonella*/mammalian microsomal bacterial mutagenicity assay. Unpublished report from Dow Chemical Co., TX, USA. Report No. TXT: HET-K-4619-3-(16). Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Dow (2008) Information provided in section 5.9.1 of Document M-II—Chlorpyrifos-methyl; section 3—Mammalian toxicology, Tier 2 summary (December 2008). Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Ellman, G.L. et al. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7:89–95.
- Gollapudi, B.B., Linscombe, W.A. & Sinha, A.K. (1985) Evaluation of chlorpyrifos-methyl in an in vitro chromosomal aberration assay utilizing Chinese hamster ovary (CHO) cells. Unpublished report from Dow Chemical Co., TX, USA. Report No. TXT-K-0461-93-023. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Griffin, T.B. & Coulston, F. (1975) A safety evaluation of DOWCO 214 in rats and Rhesus monkeys. Unpublished report from Albany Medical College, NM, USA. Dow Report No. GH-RC 15. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Hardy, C.J. & Jackson, G.C. (1984) RELDAN technical: acute inhalation toxicity in rats. Unpublished report from Dow Chemical Co., TX, USA. Report No. DWC 410/84796. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Hunter, B. et al. (1974) DOWCO 214 toxicity following dietary administration to rats for two years and tumorigenicity screening. Unpublished report from Huntingdon Research Centre, United Kingdom. Report No. DWC 89/74319 & 89/7437. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Jones, J.R. (1984) RELDAN F: primary skin irritation and corrosivity study in the rabbit. Unpublished report from Hazleton Laboratories, Harrogate, United Kingdom. Report No. 4177-50/396. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.

- Jones, J.R. (1985a) RELDAN F: acute oral median lethal dose in the rat. Unpublished report from Hazleton Laboratories, Harrogate, United Kingdom. Report No. 4214-50/394. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Jones, J.R. (1985b) RELDAN F: acute dermal median lethal dose in the rat. Unpublished report from Hazleton Laboratories, Harrogate, United Kingdom. Report No. 4214-50/395. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Jones, J.R. (1985c) RELDAN F: eye irritation study in the rabbit. Unpublished study from Hazleton Laboratories, Harrogate, United Kingdom. Report No. 4192-50/397. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Jones, J.R. (1985d) RELDAN F: delayed contact hypersensitivity study in the guinea pig (Buehler test). Unpublished report from Hazleton Laboratories, Harrogate, United Kingdom. Report No. 4312-50/423. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Killian, D.J., Edwards, H.N. & Pennington, J.Y. (1971) Blood cholinesterase depression in humans, rabbits and dogs following inhalation or dermal exposure to DOWCO 214. Unpublished report from Dow Chemical Co., TX, USA. Report No. TMD-72-2. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Lackenby, F. (1985) OP2: acute oral toxicity study in the rat. Unpublished report from Hazleton Laboratories, Harrogate, United Kingdom. Report No. 4745-50/582. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Levine, J.B., Scheidt, R.A. & Nelson, V.A. (1965) An automated micro determination of serum cholinesterase. In: *Automation in analytical chemistry. Technicon Symposia 1965*. New York, NY, Median Inc., pp. 582–585.
- Marable, B.R. et al. (2007) Differential sensitivity of blood, peripheral, and central cholinesterases in Beagle dogs following dietary exposure to chlorpyrifos. *Regulatory Toxicology and Pharmacology*, 47:240–248.
- Mendrala, A.L. (1985) Evaluation of chlorpyrifos-methyl in the Chinese hamster ovary cell–hypoxanthine (guanine) phosphoribosyl transferase (CHO/HGPRT) assay. Unpublished report from Dow Chemical Co., MI, USA. Report No. HET-K-0461-93-022. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Mendrala, A.L. & Dryzga, M.D. (1985) Evaluation of chlorpyrifos-methyl in the rat hepatocyte unscheduled DNA synthesis assay. Unpublished report from Dow Chemical Co., MI, USA. Report No. HET-K-0461-93-021. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Nabb, D.P. & Whitfield, F. (1967) Determination of cholinesterase by an automated pH-stat method. *Archives of Environmental Health*, 15:147–154.
- Perkins, J. (1995) Determination of dermal penetration of a chlorpyrifos-methyl formulation (RELDAN 22, EF1066) and spray dilution through rat skin in an in vitro study. Unpublished report No. G-HE-P-3916 from Agricultural Research & Development Centre, DowElanco Europe, Letcombe, United Kingdom. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Proudlock, R.J. (1994) RELDAN*F in vivo rat liver DNA repair test. Unpublished report by Huntingdon Research Centre, United Kingdom. Report No. DWC 696/931261. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Richardson, R.J. & Kropp, T.J. (2001) Relative inhibitory potencies of chlorpyrifos oxon, chlorpyrifos-methyl oxon and mipatox for acetylcholinesterase versus neuropathy target esterase. Unpublished report from University of Michigan, Ann Arbor, MI, USA. Report No. 01k3. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Rivett, K.F. et al. (1974) DOWCO 214 dietary toxicity studies in Beagle dogs (final report 0–104 weeks). Unpublished report from Huntingdon Research Centre, United Kingdom. Report No. GHE-P-179. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Ross, D.B., Burroughs, S.J. & Roberts, N.L. (1975) Examination of chlorpyrifos-methyl (DOWCO 214) for neurotoxicity in the domestic hen. Unpublished report by Huntingdon Research Centre, United Kingdom. Report No. DWC 255/75401. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.

- Schwetz, B.A. et al. (1973) A study of the prenatal toxicity of DOWCO 214 in rats. Unpublished report from Toxicology Research Laboratory, Health & Environmental Research, Dow Chemicals, Midland, MI; report No. NB T35, 12-46193-9. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Stebbins, K.E. & McGuirk, R.J. (2000) Reldan F insecticide: 2-week repeated exposure nose-only vapor inhalation toxicity and 2-week recovery study in Fischer 344 rats. Unpublished report from Dow Chemical Co., MI, USA. Report No. DECO HET K-046193-111. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Stebbins, K.E., Yano, B.L. & Baker, P.C. (2000) Reldan F: 4-week dermal toxicity study with recovery in Fischer 344 rats. Unpublished report from Dow Chemical Co., MI, USA. Report No. DECO HET K-046193-107 [991220]. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Szabo, J.R. & Davis, N.L. (1990) Chlorpyrifos-methyl (RELDAN): 13-week dietary toxicity study in Beagle dogs. Unpublished report from Dow Chemical Co., MI, USA. Report No. HET-K-0461-93-027. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Thompson, D.J., Dyke, I.L. & Warner, S.P. (1975) Results of a three generation, two litter reproduction study on *O,O*-dimethyl *O*-(3,5,6-trichloro-2-pyridyl) phosphorothioate (DOWCO 214) in the rat. Unpublished report from Dow Chemical Co., IN, USA. Report No. HET-K-0461-93-021. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Wilson, C.W. (2000) Technical grade chlorpyrifos-methyl: dermal sensitization study in guinea pigs—maximisation design. Unpublished report from Springborn Laboratories, OH, USA. Report No. 3504.79. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Yoshida, A. et al. (1985) Chlorpyrifos-methyl: 28 day oral toxicity study in mice. Unpublished report from the Institute of Environmental Toxicology, Tokyo, Japan. Report No. GHF-R-80. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.
- Yoshida, A. et al. (1988) Chlorpyrifos-methyl: 18-month oral chronic toxicity and oncogenicity study in mice. Unpublished report from the Institute of Environmental Toxicology, Tokyo, Japan. Report No. GHF-R-166. Submitted to WHO by Dow AgroSciences, Midland, MI, USA.