5.7 DICHLORVOS (025)

TOXICOLOGY

Dichlorvos is the International Organization for Standardization (ISO)–approved common name for 2,2-dichlorovinyl dimethyl phosphate (International Union of Pure and Applied Chemistry) or 2,2-dichloroethenyl dimethyl phosphate (Chemical Abstracts Service No. 62-73-7). It is a broad-spectrum organophosphorus insecticide, and, like other organophosphorus compounds, its mode of action is via the inhibition of cholinesterase (ChE) activity. The toxicity of dichlorvos was evaluated by the Joint FAO/WHO Meeting on Pesticide Residues in 1965, 1966, 1967, 1970, 1977 and 1993. An acceptable daily intake (ADI) of 0–0.004 mg/kg body weight (bw) was established by the 1966 Meeting and maintained by all subsequent Meetings. Dichlorvos was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues.

All pivotal studies contained certificates of compliance with principles of good laboratory practice or good clinical practice and the Declaration of Helsinki, as appropriate.

Biochemical aspects

Following oral dosing with ^{14}C-labelled dichlorvos, similar patterns of excretion of radioactivity were observed in mice, rats, hamsters and humans. Excretion was also similar in male and female rats and following both oral and intravenous dosing. Recovery of radioactivity was greater than 90%, with the majority excreted within 24 hours of dosing. The main excretion pathways of radioactivity were via carbon dioxide (30% in mice and humans, 50% in hamsters, up to 60% in rats) and urine (30% in mice, up to 17% in rats, 20% in hamsters and 8% in humans). Relatively low levels of radioactivity were detected in faeces (3% in mice, 5% in hamsters and 13% in rats). The detection of radioactivity in the carcass of mice (30%), rats (26%) and hamsters (15%) is likely due to the incorporation of ^{14}C into protein. Based on the level of radioactivity in carbon dioxide, urine, the carcass and tissues following oral dosing, absorption was estimated to be 92–95% in rats. Analysis of urine identified similar levels of hippuric acid in mice, hamsters and humans (< 1% of the administered dose); desmethyl dichlorvos was detected in mouse and human urine at ~19% and 2%, respectively; and urea was detected at concentrations below 1% in both mouse and human urine. In rats, the levels of hippuric acid and urea were less than 6% and 3–30% of total faecal radioactivity and 4–24% and 19–33% of total urinary radioactivity, respectively; no other metabolites were identified in excreta, which may be due to their volatility or degradation. In rats, approximately 6–13% of urinary metabolites were glucuronidated.

The in vitro half-life of dichlorvos in human blood was less than 15 minutes at 37 ºC.

The level of dermal absorption in rats was 22–30%, which occurred within 10 hours of exposure.

Toxicological data

As with other organophosphorus insecticides, inhibition of ChE activity is the most sensitive toxicological end-point following acute or repeated exposures to dichlorvos.

Dichlorvos has marked acute oral toxicity. In acute oral dosing studies, clinical signs and deaths occurred rapidly in rats and rabbits. Consistent with the cholinergic effects observed with other organophosphorus compounds, signs of acute intoxication with dichlorvos included salivation, lacrimation, dyspnoea and tachypnoea (muscarinic effects), muscle tremors, clonic–tonic spasms, lethargy, paresis, splayed gait, prostration/lateral positioning (nicotinic effects), and restlessness, ataxia and coma (central nervous system effects).

The results of acute toxicity studies evaluated by the current Meeting were consistent with the acute toxicity profile of dichlorvos established by previous Meetings. The oral median lethal dose (LD_{50}) in rats was 57–108 mg/kg bw, whereas the oral LD_{50} in rabbits was 74 mg/kg bw. The dermal LD_{50} in rats was 210 mg/kg bw. In rats and mice, median lethal concentration (LC_{50}) values were 0.23 and greater than 0.22 mg/L, respectively, for head-only exposure to dichlorvos aerosols. It was not possible to determine the skin and eye irritancy potential of dichlorvos because of high levels of toxicity in the study animals. In a non-guideline study, dichlorvos was classifiable as a skin sensitizer in guinea-pigs (maximization test).

The main toxicological findings in repeated-dose studies in rats and dogs were inhibition of ChE activity and, at higher doses, reduced body weight gain and signs of neurotoxicity. In short-term studies of toxicity of less than 12 months' duration, the no-observed-adverse-effect level (NOAEL) for inhibition of erythrocyte acetylcholinesterase (AChE) activity was 0.1 mg/kg bw per day in rats and 0.05 mg/kg bw per day in dogs. The NOAEL for inhibition of brain ChE activity was 1.5 mg/kg bw per day in rats and 0.05 mg/kg bw per day in dogs. Toxicity observed in rats and dogs was limited to the characteristic muscarinic signs (salivation or vomiting) and reduced body weight gain. The effect doses for these clinical signs in short-term studies correlated with moderate levels of inhibition of brain ChE activity (up to ~50%).

Previous Meetings have evaluated more than 10 carcinogenicity studies conducted in mice and rats that received dichlorvos orally (diet, drinking-water or gavage) or by inhalation. The majority of the oral dosing studies and all of the inhalation studies found no evidence of carcinogenicity. The 1993 Meeting concluded that the occurrence of a small number of forestomach lesions in B6C3F1 mice (papillomas) in a United States National Toxicology Program study was attributable to the localized effect of dichlorvos administered by corn oil gavage. The 1993 Meeting concluded that dichlorvos would not result in chronic human health hazards at doses below those that result in AChE inhibition.

No new long-term studies of toxicity or carcinogenicity were considered by the current Meeting. Two drinking-water studies conducted in mice and rats were resubmitted, as the studies now had an improved English translation and had been statistically reanalysed by the authors. Dichlorvos was not carcinogenic under the conditions of either study. In the mouse study, observations of squamous cell hyperplasia, with apparent progression to papillomas in males, suggested treatment-related proliferative changes in the glandular region of the stomach. However, the same findings were not observed in females and were not corroborated by findings from a gavage study using the same mouse strain in which papillomas were observed in the forestomach of females.

Numerous in vitro and in vivo experiments have tested the genotoxic potential of dichlorvos. The 1993 Meeting concluded that dichlorvos and its major metabolite, dichloro-acetaldehyde, had been adequately tested in in vitro and in vivo genotoxicity assays. Unpublished genotoxicity studies evaluated by the current Meeting indicated that dichlorvos was mutagenic to mouse lymphoma cells in vitro (the mutation frequency higher in the absence of exogenous metabolic activation), whereas five unpublished in vivo assays detected no evidence of genotoxicity (mouse dominant lethal assay, mouse chromosomal aberration assay in bone marrow and spermatocytes, sister chromatid exchanges in mice and mouse micronucleus test). Published studies reported a genotoxic response for a number of in vitro end-points, including mutations, chromosomal aberrations, micronuclei, sister chromatid exchanges and deoxyribonucleic acid (DNA) damage. In those published in vivo studies considered suitable for regulatory purposes, dichlorvos was not genotoxic. The consistently negative in vivo genotoxicity response can be attributed to the rapid metabolism of dichlorvos, which limits systemic exposure to intact dichlorvos at concentrations likely to lead to direct interactions with DNA. The occurrence of mutations in the liver of transgenic mice administered repeated intraperitoneal doses is consistent with a mechanism of genotoxicity resulting from high localized tissue concentrations of unmetabolized dichlorvos; in humans, scenarios of prolonged systemic exposure to unmetabolized dichlorvos are highly unlikely.

The Meeting noted the weight of evidence from previously considered carcinogenicity studies, which indicated that dichlorvos possesses no systemic genotoxic potential. Further, the 1993 Meeting noted that dichlorvos methylated DNA in vitro at a rate that is 8–9 orders of magnitude lower than the rate of phosphorylation. Therefore, DNA alkylation is unlikely to occur at doses of dichlorvos that are not inhibitory to erythrocyte/brain ChE activities.

The Meeting concluded that dichlorvos is unlikely to be genotoxic in vivo.

In the absence of an in vivo genotoxic response and any carcinogenic response relevant to humans, the Meeting concluded that dichlorvos is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, in which exposure was via the drinking-water, treatment-related effects included the inhibition of ChE activity and reduced body weight gain. The inhibition of brain ChE activity in parental males and females (up to 50% at the highest dose) was not associated with cholinergic signs. The NOAEL for parental toxicity was 0.5 mg/kg bw per day, based on the inhibition of brain ChE activity at 2 mg/kg bw per day. The NOAEL for offspring toxicity was 2 mg/kg bw per day, based on lower pup weights in both generations at 8 mg/kg bw per day. The NOAEL for reproductive toxicity was 2 mg/kg bw per day, based on reduced fertility and pregnancy indices, increased stillbirths in the F_2 generation and abnormal cycling in F_1 maternal rats at 8 mg/kg bw per day.

Non-guideline studies reported effects on rat sperm following repeated gavage doses of 2–10 mg/kg bw per day, but these were considered to be of questionable biological relevance due to methodological limitations.

In an in vitro assay, dichlorvos did not bind to the human or mouse estrogen receptor and bound with only very low affinity to the human and mouse androgen receptors.

In studies of developmental toxicity with dichlorvos following gavage dosing, teratogenicity was not observed at doses up to 21 and 7 mg/kg bw per day in rats and rabbits, respectively. Maternal toxicity, including cholinergic signs and deaths, was observed at lower doses. In rats, the NOAEL for maternal toxicity was 3 mg/kg bw per day, based on the occurrence of clinical signs (tremors and prone positioning) and reduced body weight gain at 21 mg/kg bw per day. In rabbits, the NOAEL for maternal toxicity was 0.1 mg/kg bw per day, based on the occurrence of deaths at 2.5 mg/kg bw per day and above. It was noted that these deaths occurred at doses lower than the oral LD_{50} for rabbits.

The Meeting concluded that dichlorvos did not cause developmental toxicity and that it was not teratogenic.

In studies of delayed neurotoxicity, dichlorvos was administered by gavage to hens either as a single dose of 16.5 mg/kg bw or as repeated doses of up to 3 mg/kg bw per day for 28 days; there was no evidence of delayed neuropathy. The previous Meeting noted that dichlorvos caused delayed polyneuropathy in hens at doses much higher than the LD_{50}, and cases of delayed polyneuropathy were reported in humans following severe, life-threatening intoxications. A supplementary in vitro study confirmed that dichlorvos is a more potent inhibitor of AChE activity than of neuropathy target esterase (NTE) activity. The Meeting concluded that dichlorvos can cause delayed polyneuropathy in humans, but only after acute poisoning causing a severe cholinergic syndrome that would be lethal if not properly treated.

In studies of neurotoxicity in rats, dichlorvos was administered as a single dose of up to 70 mg/kg bw or as repeated doses of up to 15 mg/kg bw per day. The NOAEL following a single gavage dose was 0.5 mg/kg bw, based on clinical signs of neurotoxicity at 35 mg/kg bw observed during the functional observational battery 15 minutes after dosing; no signs of neurotoxicity were observed 7 or 14 days after dosing. Following repeated gavage doses of up to 15 mg/kg bw per day for 13 weeks, clinical signs of neurotoxicity were observed within 15 minutes of dosing throughout the study, at and above 7.5 mg/kg bw per day. These signs coincided with the inhibition of ChE activity in erythrocytes and brain.

Dichlorvos did not cause developmental neurotoxicity following repeated gavage doses of up to 7.5 mg/kg bw per day. In the range-finding study, inhibition of brain ChE activity occurred in dams (~60%) and pups (~20%) during gestation only, in the absence of clinical signs. In the main and supplementary studies, in which no analysis of ChE activity was undertaken, the NOAEL for maternal and offspring toxicity was 7.5 mg/kg bw per day, the highest tested dose.

In studies investigating the inhibition of ChE activity in rats following an acute gavage dose up to 35 mg/kg bw, the NOAEL for the inhibition of erythrocyte and brain AChE activities was 1 mg/kg bw. At the next highest dose of 5 mg/kg bw, inhibition of erythrocyte and brain AChE activities co-occurred (~30%), whereas clinical signs were not observed until the level of inhibition reached approximately 50% (at and above 15 mg/kg bw). There was no difference in erythrocyte and brain ChE inhibition between rat pups of different ages or between rat pups and adults. Following an acute gavage dose of dichlorvos of 15 mg/kg bw, maximum inhibition of erythrocyte and brain ChE activities was measured at 1–3 hours after dosing, with recovery apparent from 8 hours post-dosing. This observation is consistent with the half-life of spontaneous reactivation of erythrocyte AChE activity reported in previous monographs of approximately 2 hours; in comparison, the half-life of reactivation of human erythrocyte AChE activity is approximately15 days. Following 7 consecutive gavage doses of up to 15 mg/kg bw per day, inhibition of erythrocyte and brain AChE activities occurred at and above 5 mg/kg bw per day; the NOAEL was 0.1 mg/kg bw per day.

The Meeting considered new studies in male volunteers in which dichlorvos was ingested in gelatine capsules either as an acute dose or as short-term repeated doses. No inhibition of erythrocyte AChE activity occurred in six volunteers following a single dose of 0.5 mg/kg bw. These same six volunteers then ingested 0.3 mg/kg bw per day for 12 or 15 days. However, dosing was stopped because inhibition of erythrocyte AChE activity exceeded 20% in four subjects (the mean maximum level of inhibition was ~30%); the NOAEL was less than 0.3 mg/kg bw per day. The recovery of erythrocyte AChE activity to near pre-treatment levels occurred approximately 40 days after the cessation of treatment. In a second acute dose study conducted in a different group of six volunteers, the NOAEL was 1 mg/kg bw, based on the absence of erythrocyte AChE inhibition, adverse events or effects on body temperature at this dose. In a 21-day study conducted in a different six volunteers at a dose of 0.1 mg/kg bw per day, there was a time-related decrease in erythrocyte AChE activity, which reached a mean of 16% on day 18. Although dosing continued for a further 3 days, AChE activity was not analysed again until 4–9 days after the final dose, and therefore there is some uncertainty about whether steady state had been fully reached. Further, three of the six volunteers had a greater than 20% level of erythrocyte AChE inhibition on day 18 or during the post-treatment period, and on this basis, the Meeting concluded that a clear NOAEL had not been demonstrated.

In an occupational study, 15% of flower workers (males and females) tested positive for dichlorvos in a skin patch test.

In workers who were exposed to dichlorvos for short periods of time during the manufacture of vaporization units, recovery of plasma and erythrocyte ChE activities took approximately 50 and 82 days, respectively.

Epidemiological studies provided no evidence for the association of parental pesticide exposure with the development of childhood cancer (cohort of 1218) or the lifetime risk of cancer in pesticide applicators (cohort of 4613).

Case reports provided additional clinical observations in humans following acute cholinergic crisis. These observations included four cases of delayed extrapyramidal syndrome, isolated bilateral vocal cord paralysis with intermediate syndrome and two cases of pancreatitis, one with the possible development of a pseudocyst. In a study involving 41 severely poisoned patients, dichlorvos caused reversible myocardial dysfunction.

In three separately reported cases of fatal ingestion, dichlorvos was detected in various human tissues. However, a meaningful comparison between the cases was difficult because of differences in the ingested dose and sampling interval. A relatively high concentration of dichlorvos was uniquely

detected in the spleen or heart in separate cases, whereas the majority of dichlorvos was detected in the stomach contents. Relatively low concentrations were detected in the liver, brain, blood and urine.

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

Toxicological evaluation

The Meeting confirmed the current ADI of 0–0.004 mg/kg bw based on the NOAEL of 0.04 mg/kg bw per day for the inhibition of erythrocyte AChE activity in a 21-day study in male volunteers (Annex 5, reference *70*). The ADI was previously based on the NOAEL of 0.033 mg/kg bw per day in a 28-day study in male volunteers for the same end-point (Annex 5, reference *9*) and before that on the NOAEL of 0.37 mg/kg bw per day in a 90-day study in dogs for the inhibition of brain ChE activity (Annex 5, reference *7*).

The Meeting considered two new studies conducted in male volunteers at doses higher than those tested in the two pivotal human studies underpinning the current ADI. Neither study was considered a suitable basis for an ADI, because clear NOAELs had not been demonstrated. The Meeting considered the ADI to be protective for other, non-neurotoxic effects of dichlorvos observed in short- and long-term studies with repeated doses and in studies of reproductive and developmental toxicity, where the use of an interspecies safety factor of 10 would be appropriate. The absence of any age- or sex-specific differences in ChE inhibition in rats confirmed the current ADI to be protective of the entire population.

The Meeting established an acute reference dose (ARfD) of 0.1 mg/kg bw, based on the NOAEL of 1 mg/kg bw for erythrocyte AChE inhibition in the acute oral study in male volunteers and using a 10-fold intraspecies safety factor. The NOAEL is supported by observations in two other volunteer studies in which no erythrocyte AChE inhibition occurred 1 day after dosing at 0.5 and 0.1 mg/kg bw, respectively.

An addendum to the toxicological monograph was prepared.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute toxicity study[a]	Toxicity (inhibition of brain ChE activity)	1 mg/kg bw	5 mg/kg bw
	Acute neurotoxicity study[a]	Toxicity (clinical signs)	0.5 mg/kg bw	35 mg/kg bw
	Developmental toxicity study[a]	Maternal toxicity	3 mg/kg bw per day	21 mg/kg bw per day
		Embryo and fetal toxicity	21 mg/kg bw per day[b]	—
	Developmental neurotoxicity study[a]	Developmental neurotoxicity, maternal toxicity and offspring toxicity	7.5 mg/kg bw per day[b]	—
	Thirteen-week toxicity study[c]	Toxicity (inhibition of brain ChE activity and clinical signs)	1.5 mg/kg bw per day	15 mg/kg bw per day
	Thirteen-week neurotoxicity study[a]	Toxicity (inhibition of brain ChE activity and clinical signs)	0.1 mg/kg bw per day	7.5 mg/kg bw per day
	Two-generation	Reproductive toxicity	2 mg/kg bw per day	8 mg/kg bw per day

Species	Study	Effect	NOAEL	LOAEL
	reproduction study[d]	Parental toxicity	0.5 mg/kg bw per day	2 mg/kg bw per day
		Offspring toxicity	2 mg/kg bw per day	8 mg/kg bw per day
Rabbit	Developmental toxicity study[a]	Maternal toxicity	0.1 mg/kg bw per day	2.5 mg/kg bw per day
		Embryo and fetal toxicity	7 mg/kg bw per day[b]	—
Dog	One-year toxicity study[e]	Toxicity (inhibition of brain ChE activity and clinical signs)	0.05 mg/kg bw per day	1 mg/kg bw per day
Human	Acute toxicity study[e]	Toxicity (inhibition of erythrocyte AChE activity)	1 mg/kg bw[b]	—
	Twenty-one-day toxicity study[e]	Toxicity (inhibition of erythrocyte AChE activity)	—	0.1 mg/kg bw per day[e]
	Twenty-one-day toxicity study[e,f]	Toxicity (inhibition of erythrocyte AChE activity)	0.04 mg/kg bw per day[b]	—
	Twenty-eight-day toxicity study[e,g]	Toxicity (inhibition of erythrocyte AChE activity)	0.033 mg/kg bw per day[b]	—

[a] Gavage administration.

[b] Highest dose tested.

[c] Dietary administration.

[d] Administration in drinking-water.

[e] Administration in capsules.

[f] Evaluated previously (Annex 5, reference *70*).

[g] Evaluated previously (Annex 5, reference *9*).

Estimate of acceptable daily intake for humans

0–0.004 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 exposures

Critical end-points for setting guidance values for exposure to dichlorvos

Absorption, distribution, excretion and metabolism in mammals

Rate and extent of oral absorption	Rapid (T_{max} < 0.5 h) and essentially complete (92–95% in rats)

Dermal absorption	22–30% within 10 h (rats)
Distribution	Distributes to most tissues; highest levels of radiolabel detected in the carcass and liver, with lower levels in the blood and kidneys
Potential for accumulation	Low; no evidence of accumulation
Rate and extent of excretion	Rapid (within 24 h) and extensive excretion of radiolabel (mainly via carbon dioxide and urine)
Metabolism in animals	Extensive by hydrolysis and demethylation (in vitro half-life in human blood < 15 min)
Toxicologically significant compounds (animals, plants and the environment)	Dichlorvos, dichloro-acetaldehyde
Acute toxicity	
Rat, LD_{50}, oral	57–108 mg/kg bw
Rat, LD_{50}, dermal	210 mg/kg bw
Rat, LC_{50}, inhalation	0.23 mg/L (4 h, head-only exposure)
Rabbit, dermal irritation	Not assessed due to high toxicity
Rabbit, ocular irritation	Not assessed due to high toxicity
Human, skin sensitization (skin patch test)	Skin sensitizer
Short-term studies of toxicity	
Target/critical effect	Cholinesterase inhibition
Lowest relevant oral NOAEL	0.05 mg/kg bw per day (dogs)
Lowest relevant dermal NOAEL	No new data
Lowest relevant inhalation NOAEC	No new data
Genotoxicity	
	Not genotoxic in vivo following oral dosing
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Cholinesterase inhibition
Lowest relevant oral NOAEL	No new data
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproduction target/critical effect	Reduced fertility and pregnancy indices, increased stillbirths and abnormal cycling in maternal rats
Lowest relevant reproductive NOAEL	2 mg/kg bw per day (rats)
Developmental target/critical effect	No developmental toxicity, including teratogenicity (rats, rabbits)
Lowest relevant developmental NOAEL	21 mg/kg bw per day (rats), 7 mg/kg bw per day (rabbits); highest doses tested

Neurotoxicity/delayed neurotoxicity	
Neurotoxicity	Neurotoxic due to cholinesterase inhibition
	No evidence of delayed neuropathy up to 16.5 mg/kg bw (hens) or 70 mg/kg bw (rats), the highest doses tested
	Very weak inhibitor of NTE activity in vitro
Lowest relevant oral NOAEL	0.1 mg/kg bw per day (13-week rat study)
Developmental neurotoxicity	No evidence of developmental neurotoxicity up to 7.5 mg/kg bw per day (rats), highest dose tested
Medical data	
	No epidemiological evidence of increased cancer risk in agricultural workers or their children
	Poisoning case reports suggest extrapyramidal syndrome, isolated bilateral vocal cord paralysis, pancreatitis and myocardial dysfunction following acute cholinergic crisis
	Many human volunteer data available with the critical effect of ChE inhibition
	Evidence of polyneuropathy following severe, life-threatening intoxications

Summary

	Value	**Study**	**Safety factor**
ADI	0–0.004 mg/kg bw	Human, 21-day oral dosing study	10
ARfD	0.1 mg/kg bw	Human, study of acute oral toxicity	10