

**COMMITTEE ON CARCINOGENICITY OF CHEMICALS IN FOOD,
CONSUMER PRODUCTS AND THE ENVIRONMENT**

DICHLORVOS

Introduction

1. Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is an insecticide acting by acetylcholinesterase inhibition. The genotoxicity and carcinogenicity of dichlorvos were evaluated by the CoM in 2002. Since then evaluations of dichlorvos have been performed by the US Environmental Protection Agency (EPA) and the Scientific Panel on Plant Health, Plant Protection Products and their Residues (PPR) of the European Food Safety Authority (EFSA). There are some differences between the three groups in the conclusions on carcinogenicity. The core data available to all three groups are broadly consistent with no new data of any significance having been made available since the CoM discussions in 2002.

2. HSE is seeking the views of CoC on whether a threshold can be assumed for the carcinogenicity of dichlorvos. This advice will be used in formulating a position for the ongoing EU discussions on the use of dichlorvos as a biocide.

CoM consideration

3. Following an evaluation of dichlorvos by the Advisory Committee on Pesticides in 2001, the views of CoM were sought. The opinion of CoM covered both genotoxicity and carcinogenicity, the latter being considered by some members of CoC. The full CoM opinion is presented at Annex 1, the overall conclusion was:

“The Committee concluded that dichlorvos should be regarded as an in-vivo mutagen at the site-of-contact (ie at the initial sites of exposure. The COM felt there was no evidence for systemic mutagenic effects.) High doses of dichlorvos induced mutagenic effects in the skin following topical application and in the liver following intraperitoneal dosing. The Committee noted the limited evidence for a carcinogenic effect of dichlorvos. This related to tumours of the forestomach in mice after gavage dosing and also the oesophageal tumours seen after dietary administration. There was no satisfactory explanation proven for the mechanisms of these tumours and the Committee felt, given the available mutagenicity data on dichlorvos, that it would be prudent to assume a genotoxic mechanism. The Committee agreed that in the absence of appropriate mechanistic data a precautionary approach should be adopted and no threshold could be assumed for the mutagenic and carcinogenic effects of dichlorvos.”

4. The Advisory Committee on Pesticides (ACP) agreed that, in the absence of appropriate mechanistic data, a precautionary approach should be adopted and no threshold could be assumed for the mutagenic and carcinogenic effects of dichlorvos.

EFSA PPR opinion

5. Following the consideration of dichlorvos at an EU expert meeting in 2005, the SCP was asked to review the mutagenic and carcinogenic properties of dichlorvos. The full PPR opinion is at Annex 2. A note from Prof. Boobis giving some background to the remit and discussions is at Annex 3. The overall conclusion was:

The PPR Panel concluded that the available data clearly demonstrate that dichlorvos is an *in vitro* mutagen, and that there is some limited evidence that dichlorvos is a site-of-contact *in vivo* mutagen but that the mechanism of this effect is unclear; the evidence for alkylation of DNA *in vivo*, a possible mechanism, is very weak.

The Panel concluded that there was insufficient evidence to identify a mode of action for the forestomach tumours produced by dichlorvos in the mouse. However, the Panel concluded that irrespective of the mode of action, the response appeared to be a consequence of the high sustained local concentrations of dichlorvos that could be achieved in this specific exposure situation and was therefore limited to this site. The Panel further concluded that there was a threshold dose for this response. The Panel was of the opinion that the weight of evidence suggests that this would not occur at the levels of exposure that would be encountered by the proposed use of the compound. In addition severe systemic toxicity would occur before any concentration in tissues other than in the forestomach is reached that would induce the tumourigenic effect. This is because the forestomach is a unique structure that retains material appreciably longer than the glandular stomach and oesophagus.

The full PPR opinion contains a detailed overview of the toxicity of dichlorvos, particularly the genotoxicity and carcinogenicity.

6. The ACP reviewed the PPR opinion in 2006 and concluded :

Dichlorvos [ACP 14 (320/2006)]

- The Committee considered the recently published 'Opinion of the Scientific Panel on Plant health, Plant protection products and their Residues (PPR Panel) on dichlorvos in the context of Council Directive 91/414/EEC'.
- Members agreed that there was no scientific basis yet for them to change their previous advice on dichlorvos. It was noted that the PPR opinion presented a rather novel way of thinking about genotoxicity and Members considered that there was no scientific support presented to justify this approach.
- Members advised that the UK approvals should remain suspended pending the submission and evaluation of the further data requested as a result of the UK review.

US EPA review

7. As part of a routine review of organophosphorus compounds, the US EPA finalised their evaluation of dichlorvos in 2006. The conclusion of carcinogenicity is given below; the full report can be accessed at http://www.epa.gov/oppsrrd1/REDS/ddvp_ired.pdf.

Dichlorvos has been classified as a category C carcinogen based primarily on increased incidences of forestomach tumors in female mice and mononuclear cell leukemia (MCL) in male Fischer 344 rats. Both tumor types have been used at various times to derive q_1^* s for quantitation of cancer risk. After lengthy deliberations and consultations with EPA's Scientific Advisory Panel (SAP) and cancer experts with the National Toxicology Program, HED's Cancer Assessment Review Committee has classified dichlorvos as "suggestive" and not requiring quantitation of cancer risks based on the following rationale:

- 1) MCL in the male Fischer rat has certain properties in terms of variability and reliability which limit its usefulness for human risk assessment.
- 2) The forestomach tumors in mice observed at gavage doses causing inhibition of plasma and red blood cell cholinesterase and cholinergic signs, are also limited in their use for human risk assessment.
- 3) The fact that dichlorvos is only positive by the gavage route and negative by the inhalation route, which is the major route of human exposure, indicates that any classification by the oral route may be limited since localized effects in the forestomach may not be applicable to human risk assessment.

Tumours in rats

8. Dichlorvos has been tested in 6 carcinogenicity studies in rats; 2 gavage, 2 dietary, one via drinking water and one via inhalation. Increased incidences of tumours (pancreatic adenoma, mononuclear cell leukaemia, mammary gland adenoma & carcinoma) were seen in one gavage study (Chan, 1989 & Chan et al, 1991). Interpretation of the rat data is possibly confounded by the variety of strains used.

Tumours in mice

9. Dichlorvos has been tested in 4 carcinogenicity studies in mice: 2 gavage, 1 dietary and one via drinking water. Increased incidences of tumours (forestomach papilloma & carcinoma) were seen in one gavage study (Chan, 1989 & Chan et al, 1991). The CoM mentions oesophageal tumours seen at low incidence in a mouse dietary study (carcinoma in one low dose male and one high dose female; papilloma in one high dose female) (NCI, 1977).

10. A tabular summary of the carcinogenicity studies, taken from the EFSA PPR opinion (pp 15 to 17), is presented below.

Table 1 Studies on Carcinogenicity of Dichlorvos

Strain	Experimental Conditions	Observations	Statistical significance	Reference
Mice				
C57Bl/6Bln	Oral (gavage) 0.2 mg/mouse (equivalent to 10 mg/kg b.w.), either two or three times/week for 50 weeks surviving animals killed at 110 weeks	Not carcinogenic		Horn et al., 1987
B6C3F1	Oral (gavage) M: 0, 10 or 20 mg/kg b.w./day F: 0, 20 or 40 mg/kg b.w./day 5 days/week, 103 weeks	<u>Forestomach papillomas</u> : M: 1/50, 1/50 and 5/50 F: 5/49, 6/49 and 18/50* (hist. control in corn oil vehicle NTP studies: M: 1% ± 2%, F: 0.9% ± 2%) <u>Forestomach carcinomas</u> : F: 0/49, 0/49 and 2/50 (hist. control: NTP: 0%)	trend * p<0.01 trend	Chan, 1989; Chan et al., 1991
B6C3F1	Oral (diet) 1000 or 2000 mg/kg for 2 weeks 300 or 600 mg/kg (actual: 318 or 635 mg/kg, equivalent to 48 or 95 mg/kg b.w./day) for 78 weeks surviving animals killed at 92-94 weeks	Not carcinogenic		NCI, 1977; Weisburger, 1982
B6C3F1	Oral (drinking water) 0, 400 or 800 mg/l water (equal to 0, 58 and 95 mg/kg b.w./day in males and 0, 56 and 102 mg/kg b.w./day in females) surviving animals killed at week 102	Not carcinogenic		Konishi et al., 1981; 1989
Rats				
BD IX/Bln	Oral (gavage) 0.1 mg/rat (equivalent to 0.25 mg/kg b.w./day), two or three times/week for 60 weeks surviving animals killed at 111 weeks	Not carcinogenic		Horn et al., 1988

F344/N	Oral (gavage) 0, 4 or 8 mg/kg b.w./day (actual doses: 0, 4.14 or 7.82 mg/kg b.w./d) 5 days/week, 103 weeks	<u>Pancreatic adenomas:</u> M: 25/50, 30/49* and 33/50* F: 2/50, 3/48 and 6/50 (hist. controls: laboratory: 9%, NTP: 6% (37% ^a)) <u>Mononuclear cell leukaemia:</u> M: 11/50, 20/50* and 21/50* F: 17/50, 21/50 and 23/50 (hist. controls: laboratory: 9% ± 7%, NTP: 15% ± 9%) <u>Mammary gland fibroadenoma/adenomas:</u> F: 9/50, 19/48* and 17/50* <u>Mammary gland carcinoma:</u> F: 2/50, 2/48 and 0/50 <u>Total mammary neoplasms:</u> F: 11/50, 20/48* and 17/50 (hist. controls: laboratory: 31%)	* p<0.05 * p<0.02 trend = 0.08 * p<0.05 trend * p<0.02 trend = 0.07	Chan 1989; Chan <i>et al.</i> , 1991
CD	Oral (diet) 0, 0.1, 1, 10, 100 or 500 mg/kg (0, 0.05, 0.5, 4.7, 47 or 230 mg/kg equivalent to 0, 0.0025, 0.025, 0.235, 2.35 or 11.5 mg/kg b.w./day) 2 years	Not carcinogenic		Witherup <i>et al.</i> , 1967
Osborne-Mendel	Oral (diet) 150 mg/kg (equivalent to 7.5 mg/kg b.w./day): 80 weeks 1000 mg/kg for 3 weeks + 300 mg/kg (actual 326 mg/kg) (equivalent to 16.3 mg/kg b.w./day) for 77 weeks Surviving animals killed at 110-111 weeks	Not carcinogenic		NCI, 1977; Weisburger, 1982
F344	Oral (drinking water) 0, 140 or 280 mg/l water (equal to M: 0, 8.3 or 18 mg/kg b.w./day, F: 0, 10 or 22 mg/kg b.w./day) exposure 104 weeks surviving animals killed at 108 weeks	Not carcinogenic		Enomoto <i>et al.</i> , 1981; Enomoto <i>et al.</i> , 1989
CFE	Inhalation (whole body) 23 hours/day, 2	Not carcinogenic		Blair <i>et al.</i> ,

	years 0, 0.05, 0.5 or 5 mg/m ³ (actual: 0, 0.05, 0.48 or 4.7 mg/m ³) (equivalent to 0.05, 0.5 or 4.9 mg/kg b.w./day)			1974; 1976; Stevenson and Blair, 1977
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^a: retrospective evaluation NTP studies, corn oil gavage (Eustis and Boorman, 1985)

Genotoxicity

11. There is consistency between CoM and PPR regarding the site of contact genotoxic potential of dichlorvos.

Issues for the CoC

12. Are the tumours seen in studies with dichlorvos likely to be produced by a thresholded mechanism? If there is uncertainty, is it still prudent to assume no threshold?

Chemicals Regulation Directorate
HSE

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Chan et al (1991). Carcinogenesis studies of dichlorvos in Fischer rats and B6C3F1 mice. Jpn J Cancer Res 82: 157-164.

2002 COM Statement on Dichlorvos

Mutagenicity of dichlorvos

COM/02/S2 - January 2002

Introduction

Background to COM review

1. Dichlorvos (O-(2,2-dichlorovinyl)-O,O-dimethylphosphate, DDVP) was first introduced into the UK as an agricultural pesticide in 1962. Non-agricultural uses were first assessed under the voluntary Pesticides Safety Precaution Scheme in 1975-78. A review by the ACP of approvals issued under the Control of Pesticides Regulatory (COPR 1986) was undertaken in 1994. Currently dichlorvos is widely used by amateur and professional users as a public hygiene insecticide (e.g. use of hand held aerosols for surface/space spray and slow release products e.g. strips, cassettes). A relatively small number of products are approved for use in animal husbandry and in agriculture and horticulture on edible crops (e.g. cucumbers) and on non-edible crops (e.g. chrysanthemums). Dichlorvos is currently used in a veterinary medicinal product for control of fleas in cats and dogs. The use of dichlorvos in pesticide products and in veterinary medicines is currently being reviewed.

2. The COM considered generic aspects arising from the paper by Sasaki YF et al(1) on the performance of the *in-vivo* COMET assay with respect to the newly published strategy in the COM guidance at its 8 February 2001 meeting. The Committee agreed that the positive results reported in the COMET assay using dichlorvos suggested that a full review of all the mutagenicity data was required.

COM reviews

3. The toxicokinetics, mutagenicity and carcinogenicity data sections from the draft evaluation prepared by HSE for the Advisory Committee on Pesticides meeting on 5 April 2001 were made available to the COM. The Committee evaluated the data from all of the mutagenicity studies cited in the HSE review.(1-102) The COM also considered additional information and mutagenicity data submitted by industry to HSE prior to the COM meeting on 26 April 2001.(103-107) A further meeting was held on 23 July 2001 to consider additional submitted information and to hear a presentation. from industry. The COM advice was forwarded to the regulatory authorities (the Biocides and Pesticides Authorisation Unit (BPAU) at HSE and the Pesticides Safety Directorate (PSD)) at the end of July 2001 and was subsequently published in December 2001 after a judicial review of the regulatory decisions regarding dichlorvos. An extraordinary meeting of COM was held on 9 January 2002 to consider the new information submitted to the High Court during a judicial review of the regulatory decision on the pesticide products containing dichlorvos and data provided to regulatory authorities up to 4

January 2002 and to decide whether this warranted any revision of the COM statement on dichlorvos.

Overall Assessment of In-vitro mutagenicity studies

4. Members agreed that dichlorvos is a weak methylating agent (compared to methyl methanesulphonate; MMS). The Committee concurred with the following assessment of the *in-vitro* mutagenicity studies.

i) Dichlorvos is mutagenic, both in the presence and absence of exogenous metabolism, to bacteria, yeast cells and in mammalian cell gene mutation assays, chromosome aberrations assay, the *in-vitro* micronucleus test and sister chromatid exchange assays.

ii) Positive results have been reported in *in-vitro* UDS assays using human lymphocytes and human epithelial-like cells.

iii). Dichlorvos has been shown to methylate nucleophiles and to induce strand breaks in isolated DNA.

5. Members agreed that DNA methylation induced by dichlorvos contributed towards the mutagenicity reported in *in-vitro* test systems but noted that other mechanisms might also be involved. Members considered that the positive results obtained in *in-vitro* mutagenicity tests with dichlorvos in the presence of an exogenous metabolising fraction and in the assay for single strand breakage of DNA also suggested that dichlorvos and/or its metabolites were genotoxic. This might include dichloroacetaldehyde although the available evidence was insufficient to identify all potential mutagenic metabolites of dichlorvos.

6. The Committee concluded that dichlorvos is an *in-vitro* mutagen

Assessment of *in-vivo* mutagenicity studies

7. The Committee noted that there were a large number of *in-vivo* studies available. Dichlorvos was negative in most published *in-vivo* mutagenicity assays where it was administered as a single dose. These included mouse bone-marrow micronucleus (using i.p route)(70,90,94) and bone-marrow chromosome aberration studies in mice(23,60) and hamsters(22) using oral and, in two studies (mice/hamster) inhalation exposure. Negative results were also reported in SCE in mice(44,53,95) and UDS assays [liver (rats)/forestomach (mice)](9,55,99,101). A negative result was also reported in an adequately conducted bone-marrow chromosome aberration study where mice were given daily oral doses of dichlorvos by gavage for five days.(100)

8. Members also noted that there were a number of positive studies and these are discussed below.

9. The Committee agreed that dichlorvos has been reported to induce micronuclei in keratinocytes in mice following the topical application to skin.(81) Members agreed that the approach used in this study had not been fully validated but agreed the authors had used an appropriate positive control chemical and that the results with dichlorvos were indicative of an *in-vivo* site-

of-contact mutagenic effect. Members also noted a positive response in a nuclear anomaly assay in hair follicles of mice following topical application.⁷⁵ Although the latter is not considered to be a definitive genotoxicity assay, the results might be indicative of a biological effect in the skin.

10. Members agreed that the positive results reported in an abstract by Majeeth et al in a mouse bone-marrow micronucleus assay could not be interpreted, as insufficient information on the methods and results were available.⁽⁵²⁾ The Committee considered that equivocal evidence of chromosomal aberrations in bone-marrow smears had been reported in a study where hamsters were given a single oral dose of up to half the LD₅₀ of the formulation.⁽³⁰⁾

11. The Committee agreed that evidence for the induction of changes in chromosome number had been documented in the bone-marrow of rats following repeated oral dosing with dichlorvos for 6 weeks (5 days/week).⁽⁶³⁾ Members considered that the methods used were satisfactory and noted that, although the adequacy of reporting was limited, the results indicated a positive effect for the induction of numerical chromosome aberrations. It was noted that a clear dose-response would not be expected in this study as the dose range selected was relatively narrow.

12. Regarding the recently published COMET assay⁽¹⁾, Members considered that the approach adopted by Sasaki and colleagues to the mutagenicity testing of several hundreds of chemicals had a number of drawbacks, for example, limited reporting of signs of toxicity seen in animals. Members considered that the appropriateness of the isolated nuclei method used by Sasaki and colleagues had not been established and noted that there was no cellular measure of cytotoxicity or apoptosis in this study. In respect of the study on dichlorvos, members agreed that the dose level chosen (ca 80% of the LD₅₀) was too high. Members agreed that in view of these limitations, little weight could be placed on this study. The positive data in all tissues examined was unexpected given all the available mutagenicity data on dichlorvos. Members considered that it was not possible to conclude that dichlorvos had mutagenic effects in a wide range of tissues on the basis of these data. Thus, although the authors suggested that dichlorvos had an in-vivo genotoxic effect, the data were uninterpretable.

13. Members considered the in-vivo mutagenicity study in I lacZ transgenic (Muta™ Mouse) undertaken by Plesta and colleagues.⁽¹⁰²⁾ The authors had reported a statistically significant (3-fold) increase in mutant frequency in the liver and a slight non-statistically significant increase in mutant frequency in the bone-marrow following repeated dosing with dichlorvos (5 x 11 mg/kg) given intraperitoneally. Members noted that the dose levels used in this study were high and did induce severe toxicity in the animals. They agreed that although the methods used and standards of reporting used in this study had limitations, the data were indicative of a mutagenic effect of dichlorvos in-vivo at the site-of-contact i.e. the liver. The Committee noted that the authors had failed to identify any O(6) and N-7 methylguanine adducts in tissue DNA from transgenic mice given a single intraperitoneal dose of either 4.4 mg/kg bw or 11 mg/kg dichlorvos bw but agreed that the methods used by the authors were of inadequate sensitivity and it was unlikely that any alkyl adducts could have been detected. In support of this conclusion Members commented that the levels of DNA adducts (O(6) and N-7 methylguanine) in transgenic mice

(Muta™ Mouse) following repeated dosing with dimethyl sulphate (10 x 6 mg/kg bw i.p) were only approximately 4-fold higher than the limit of detection. Members considered that evaluation of DNA adducts in dichlorvos treated animals after the repeat dosing regime might have provided valuable information but these analyses had not been undertaken.

14. The Committee concluded that a consistent pattern of mutagenic effects had been documented in the in-vivo studies in which dichlorvos induced mutagenic effects at high doses in the skin following topical application(81), and in the liver following repeated intraperitoneal dosing(102), suggesting a potential site-of-contact effects (i.e. at initial sites of exposure).

Additional data submitted by industry: AMVAC (for 26 April 2001)

15. A number of papers had been submitted just prior to the COM meeting. Members agreed that no substantive new mutagenicity data had been submitted.(103-105) The additional data from the mouse lymphoma test undertaken as part of the US NTP assessment of dichlorvos was consistent with other assays and indicated a positive result in this assay. Regarding the specific comments on the most recent in-vivo mutagenicity assays, members agreed with the reservations proposed by industry regarding the interpretation of COMET assay(106) but did not agree with the views expressed regarding the conduct of the mutagenicity study in transgenic animals(107). Members considered that the mutagenicity study in transgenic mice indicated a potential mutagenic hazard at the site-of -contact.

16. The Committee commented on the "Blue-Ribbon" evaluation of dichlorvos completed in July 1998(105) and noted that differences in the rate of methylation compared to the rate of phosphorylation could not be used to discount a potential in-vivo mutagenic hazard of dichlorvos. Additionally the role of phosphorylation in the induction of genotoxic effects could not be discounted.

COM review of submission from industry (23 July 2001)

17. A further meeting of the Committee was held on the 23 July 2001 to consider a presentation from industry on the mutagenicity of dichlorvos. This consisted mainly of a critique of the four positive in-vivo studies underpinning the COM statement (referred to in paragraphs 9-13 above), and of the Committee's approach to weight-of-evidence considerations. No additional information was provided to support the reference submitted by industry to the Committee on the possibility of oxidative damage (108) as a mechanism for the induction of mutations seen in the Muta™ mouse study.

COM consideration of additional information (9 January 2002)

18. The COM considered the new information submitted to the High Court during a judicial review of the regulatory decision on the pesticide products containing dichlorvos (held between 5-9 November 2001) and additional data provided to regulatory authorities up to 4 January 2002 to see if any revision of the COM statement on dichlorvos was warranted.

19. The COM considered the documents listed under reference 111 of this statement. Members only considered the information relating to the scientific

assessment of dichlorvos and did not consider information of a legal nature. There were a number of topics raised in the documents.

20. Members agreed that mutagenicity studies that had not been subject to full international validation could be used to inform hazard assessment and regulatory decision making. This was particularly relevant when considering in-vivo activity at sites of initial contact for compounds shown to be direct acting mutagens in-vitro. The COM guidance (published in December 2000; See <http://www.doh.gov.uk/com/guidance.pdf>) recognised that such situations needed to be approached on a case-by-case basis. The current COM guidance listed a number of non-standard test methods which could be used including the use of transgenic animal models. Members concluded that the information available to the Committee to assess the potential for site-of-contact mutagenicity for dichlorvos was very limited, namely in-vivo skin micronucleus test, the intraperitoneal Muta™ Mouse study and forestomach UDS assay. The design of all these studies had limitations which had been noted by the COM. However in the absence of more definitive data, the results of these tests can be used to provide a provisional hazard assessment and could not be dismissed. Members considered the comments put forward on the conduct of the Muta™ Mouse study undertaken by Plesta and colleagues and reaffirmed that this study was acceptable for hazard identification and had given a positive result.

21. A number of the documents commented on the possibility that a threshold existed for the mutagenic effects of dichlorvos. Members reaffirmed their view (see COM statement COM/01/S3 published June 2001 <http://www.doh.gov.uk/comivm.htm>) that in the absence of specific investigations concerning mechanisms and possible thresholds, the prudent assumption was that there was no threshold for *in-vivo* mutagens. Members recalled that sufficient data had been provided to determine the existence of a threshold for aneugens that acted by inhibition of the mitotic spindle and also in the case of rapid detoxification of hydroquinone after oral administration; however this was not the case with dichlorvos.

22. COC Members attending the COM meet of 9 January 2002 reviewed the available information from the carcinogenicity bioassays reviewed in the HSE review (submitted to ACP in April 2001) and in the documents submitted to the court and most recently to the regulatory authorities as part of the data call in up to 4 January 2002. COC members considered that it was extremely difficult to assess the extent of exposure from the available information. However the evidence suggested that some exposure of the skin would have occurred during the inhalation study undertaken by Blair et al in 1974. Regarding the other studies in rats, COC members considered there were limitations in all of the studies (e.g age of study, numbers of animals used, extent of pathology investigations) and that, apart from evidence of mononuclear cell leukaemia in F344 male rats in two studies, there was no evidence for a carcinogenic effect in rats. It was noted that a Pathology Working Group (PWG) had subsequently discounted the finding of mononuclear cell leukaemia in the NTP bioassay in rats, but the report presenting the basis for this decision was not available to COC members. Regarding other studies in mice, COC members considered that there were limitations in the conduct of these studies similar to those undertaken in the rat. Members considered that it was not possible to undertake a comparison of the studies in mice where dichlorvos had been administered in corn oil and

those where dichlorvos had been administered in the drinking water or as an aqueous solution by gavage. COC members reaffirmed, however that when the NCI and NTP bioassays in mice were considered together there was limited evidence for an effect on squamous epithelium of the forestomach and oesophagus in mice. However the latter study should be viewed in terms of its age and small number of oesophageal tumours. On considering the overall package of carcinogenicity bioassays COC members felt that there was no consistent evidence for a genotoxic carcinogenic effect. COC members noted that there was no agreed mechanism for the forestomach tumours.

COM Discussion

23. The Committee agreed that there is clear unequivocal evidence that dichlorvos can induce DNA damage, chromosomal breakage and mutations in mammalian cells from in-vitro studies. The compound has been shown to interact with DNA via methylation, however several other mechanisms are theoretically possible. In-vivo dichlorvos can be rapidly detoxified by hydrolysis before it reaches the systemic circulation. Members noted from the HSE review that retention of ¹⁴C-vinyl-labelled dichlorvos in skin was recorded in a study where radiolabelled dichlorvos was applied to the skin on the backs of male rats. Several non-standard in-vivo mutagenicity assays have indicated that dichlorvos can induce genetic damage when systemic detoxification mechanisms are bypassed, e.g. following exposure to the skin and exposure to the liver following intraperitoneal dosing. The COM agreed that there was a potential risk of mutagenicity at site of contact tissues, i.e. at the initial sites of exposure. The COM felt there was no evidence for systemic mutagenic effects. The Committee agreed that until evidence was provided to the contrary and in the absence of appropriate mechanistic data, a precautionary approach should be adopted and no threshold could be assumed for the mutagenic activity of dichlorvos.

24. Members were aware that there was some limited evidence for a carcinogenic effect in mice from standard bioassays.(109,110) This related to an increase in squamous cell papillomas of the forestomach in mice and carcinomas of the forestomach in female mice given gavage doses of dichlorvos(110) together with the finding of squamous cell papilloma and carcinoma of the oesophagus in a small number of mice.109 Members noted there was no evidence for carcinogenicity from a number of other carcinogenicity bioassays including an inhalation bioassay in the rat, although there were limitations with all of these studies.

25. Members noted that negative results had been obtained with dichlorvos in a single dose UDS assay in the forestomach of mice using gavage dosing.(9,99,101) An increase in replicative DNA synthesis had been reported in this study. Members noted that there were a number of proposals regarding the mechanism of dichlorvos tumourigenicity in the mouse forestomach including localised irritancy of dichlorvos in corn oil. The Committee agreed that this proposal had not been proven and considered that it was not possible to exclude a genotoxic effect from these data given the relative insensitivity of the method used as indicated by the response with the positive control chemical; they felt that repeat dosing would most likely be required to identify any mutagenic effect of dichlorvos in this assay.

Conclusion

26. The Committee concluded that dichlorvos should be regarded as an *in-vivo* mutagen at the site-of-contact (ie at the initial sites of exposure. The COM felt there was no evidence for systemic mutagenic effects.) High doses of dichlorvos induced mutagenic effects in the skin following topical application and in the liver following intraperitoneal dosing. The Committee noted the limited evidence for a carcinogenic effect of dichlorvos. This related to tumours of the forestomach in mice after gavage dosing and also the oesophageal tumours seen after dietary administration. There was no satisfactory explanation proven for the mechanisms of these tumours and the Committee felt, given the available mutagenicity data on dichlorvos, that it would be prudent to assume a genotoxic mechanism. The Committee agreed that in the absence of appropriate mechanistic data a precautionary approach should be adopted and no threshold could be assumed for the mutagenic and carcinogenic effects of dichlorvos.

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111. Additional information submitted to high court during judicial review (5-9 November) and to BPAU/PSD up to 4 January 2002.

New Documents (AMVAC)

1. Cancer summary - 'Dichlorvos: An Assessment of Carcinogenic Potential'.
2. Letter to Ian Chart from J A MacGregor dated 7 December 2001.
3. Letter from Dr. Ward Richter, Director of Pathology Southern Research Laboratory, dated 4 December 2001

New Documents (Other data holders)

1. Submission from Denka
2. Submission from Product Safety Assessment Ltd

Documents previously submitted during the Judicial Review proceedings

1. Written Comments submitted on 11 May 2001 to COM following the first draft COM Statement (F3 920-942).
2. Written summary submitted on 28 June 2001 prior to presentation to the 23 July 2001 COM Meeting (D1 86-93).

3. Written comments submitted on 26 July 2001 following the second draft COM statement (D1 98 - 104).
4. Expert opinion of David Brusick August 2001 (D1 117-122).
5. Expert opinion of John Ishmael August 2001 (D1 147 - 157).
6. Expert opinion of Karel de Raat August 2001 (D1 167-180).
7. Expert opinion of John Mennear August 2001 (D1 184-197).
8. First witness statement of J. A. MacGregor August 2001 (D1 217-243).
9. Second witness statement of J. A. MacGregor August 2001 (D1 312-326),
10. Third witness statement of J A MacGregor (D2 674-677).
11. Affidavit of Anju Sanehi (D1 1-7 paragraphs 13-18).

EFSA PPR Opinion on dichlorvos



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Scientific Panel Opinic

Note from Prof A Boobis explaining the context of the PPR Opinion

-----Original Message-----

From: Boobis, Alan R [mailto:a.boobis@imperial.ac.uk]

Sent: Friday, May 19, 2006 2:17 PM

To: Wilder, Jayne (PSD)

Subject: RE: EFSA scientific opinion on dichlorvos

Jayne

I believe that the information that formed the basis of the PPR panel opinion was essentially the same as that considered by ACP et al in the UK. The key difference was the interpretation of the studies and the weight of evidence given to them. You may recall that in the UK the COC view was "On considering the overall package of carcinogenicity bioassays COC members felt that there was no consistent evidence for a genotoxic carcinogenic effect. COC members noted that there was no agreed mechanism for the forestomach tumours." COM concluded that there was good evidence that dichlorvos was an in vitro mutagen and also evidence that it was a site of contact mutagen in vivo. Their recommendation, adopted by ACP, was that it would be prudent to assume that the tumours observed with dichlorvos could have arisen by a genotoxic mechanism and hence would not have any threshold. The PPR considered all aspects of the carcinogenic potential of dichlorvos at some length, and like the COC was not convinced by many of the carcinogenicity studies. Its conclusion was that only the forestomach tumours in mice were compound-related and of potential relevance to the risk assessment. The PPR was not convinced that there were sufficient data to support cytotoxicity as the mode of action. This is similar to the ACP position. Further, the PPR was of the view that the forestomach tumours could have arise by a genotoxic mechanism, again like the ACP. However, the PPR was not at all convinced that this was via methylation. In considering all of the information, including the intrinsic reactivity of dichlorvos with esterases and other macromolecules, it was concluded that the tumours were a high dose phenomenon, exacerbated by the mode of administration and the unusual physiology of the mouse forestomach, which would result in sustained high local concentrations. The Panel was of the view that there was a threshold for the effects observed in the mouse forestomach. This is probably the single critical difference between the ACP and the PPR in their evaluations of dichlorvos. The PPR felt that toxicity via esterase inhibition would prevent systemic exposure reaching a level where genotoxicity could occur in other tissues. It is important to note that the PPR was not asked to conduct a risk assessment, only to comment on the significance of the tumourigenic findings. In that respect it should also be noted that dichlorvos has not been shown to cause tumours of the skin (albeit there is no specific study in which this was investigated) and hence it was outside the terms of reference to discuss the possibility of this as a target site.

I hope this helps.

Best wishes,

Alan