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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

TETRACHLORVINPHOS

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 22248-79-9 [also 22350-76-1 for the analogous (*E*)- isomer; and 961-11-5 for the mixed (*Z*)- + (*E*)- isomers]

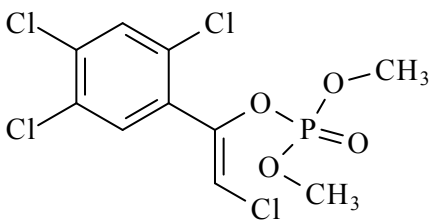
Chem. Abstr. Serv. Name: Phosphoric acid, (1*Z*)-2-chloro-1-(2,4,5-trichlorophenyl) ethenyl dimethyl ester

Preferred IUPAC Name: (1*Z*)-2-chloro-1-(2,4,5-trichlorophenyl)ethenyl dimethyl phosphate

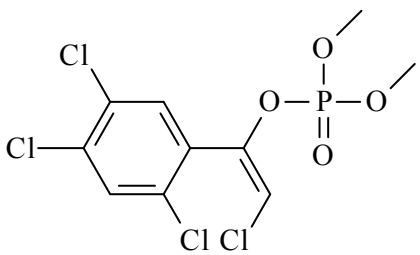
Synonyms: CVMP; stirofos; stirophos; TCVP; tetrachlorvinfos, vinfos

Trade Names: Tetrachlorvinphos products are sold worldwide under several trade names, including Appex, Dust M, Gardcide, Gardona, Rabon, Rabon Oral Larvicide (ROL), and Rabond Ravap (IARC, 1983; ChemIDplus, 2015).

1.1.2 Structural and molecular formulae, and relative molecular mass



(1*Z*)-2-chloro-1-(2,4,5-trichlorophenyl) ethenyl dimethyl phosphate (CAS No., 22248-79-9)



(1*E*)-2-chloro-1-(2,4,5-trichlorophenyl) ethenyl dimethyl ester phosphoric acid (CAS No., 22350-76-1)

Molecular formula: $C_{10}H_9Cl_4O_4P$

Relative molecular mass: 365.96

Additional chemical structure information is available in the PubChem Compound database (NCBI, 2015).

1.1.3 Chemical and physical properties of the pure substance

Description: Off-white, tan to brown crystalline solid with a mild chemical odour (EPA, 1995a; NCBI, 2015)

Solubility: Very slightly soluble at 11 mg/L in water at 20 °C, soluble at < 200 g/kg at 20 °C in acetone, < 150 g/kg at 20 °C in xylene, 400 g/kg in chloroform and dichloromethane (IARC, 1983)

Volatility: Vapour pressure (*Z*-isomer), 4.2×10^{-8} mm Hg (20 °C) (IARC, 1983), not expected to volatilize from dry soil surfaces

Stability: Stable below 100 °C; slowly hydrolysed by water, neutral, and acid environments, and more quickly in an alkaline environment (IARC, 1983; NCBI, 2015)

Octanol/water partition coefficient (P): log P, 3.53 (Hansch et al., 1995)

Henry's law: 1.8×10^{-9} atm m^3 mol^{-1} at 25 °C (NIH, 2015), not expected to volatilize from water

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), 1 mg/m^3 = 15.0 ppm.

1.1.4 Technical products and impurities

The technical product typically contains 98% *Z*-stereoisomer and 2% *E*-stereoisomer (Worthing, 1979).

1.2 Production and use

1.2.1 Production

(a) Manufacturing processes

Tetrachlorvinphos, a phenyl organophosphate insecticide, was introduced and first used commercially in 1966 in the USA (EPA, 2006).

Tetrachlorvinphos is produced by the reaction of trimethyl phosphate with 2,2,2′,4′,5′-pentachloroacetophenone (IARC, 1983; Tomlin, 2000).

Tetrachlorvinphos is formulated as wettable powder (active ingredient, a.i., 50%), dust (a.i., 1–3%), granular (a.i., typically 0.2–7.8%), and emulsifiable concentrate (a.i., 3–24%), impregnated material (a.i., 13–14.5%, as pet collars and cattle ear tags), ready-to-use liquid (a.i., 1–2%, as spray-on/wipe-on/backrub materials for pets, horses, and cattle), and pressurized liquid (a.i., 1%, as flea and tick spray for cats). Tetrachlorvinphos is also available in pelleted/tableted form and as mineral blocks for livestock (EPA, 2006). It is sometimes formulated in conjunction with the insect growth regulator *S*-methoprene (EPA, 2006).

(b) Production volume

In 1978, the total world production volume of tetrachlorvinphos was reported to be 450 tonnes (IARC, 1983). As of 2002, approximately 400 tonnes of tetrachlorvinphos active ingredient were used annually in the USA, of which about 200 tonnes were used for poultry (EPA, 2002a). Tetrachlorvinphos was not listed among the top 25 agricultural pesticides (by mass of a.i. per year) in the USA between 1987 and 1997 (EPA, 1999); however, 47 unique tetrachlorvinphos products were reported to be available in the USA from 12 primary registrant companies (NPIRS, 2015).

No production, import, or use quantities were available for countries other than the USA. Tetrachlorvinphos was not listed on the 2007 list of high production chemicals published by the Organisation for Economic Co-operation and Development (OECD), suggesting that tetrachlorvinphos was not produced or imported at levels greater than 1000 tonnes per year in any member country or region, although products containing tetrachlorvinphos were reportedly available for sale to consumers in several countries (OECD, 2009).

1.2.2 Uses

Tetrachlorvinphos is a selective insecticide and miticide with contact and stomach action (NIH, 2015). It can be used against ectoparasites on poultry, against flies in dairies and livestock barns, as a larvicide in livestock, and to control fleas on pets (EPA, 2002a). It has also been used on crops, where it is effective against various pests of fruits, vegetables, cereals, and cash crops, including fruit flies and moths in cotton, maize, rice, tobacco, vegetables, and fruit (NIH, 2015). Target pests also include fleas, ticks, lice, flies (adults and larvae), chiggers, mites, spiders, wasps, and cattle grubs. In addition, tetrachlorvinphos has been used on agricultural premises, agricultural equipment, and recreational areas (NTP, 1978).

(a) Agriculture

Tetrachlorvinphos can be applied dermally to livestock to control flies and mites; it can be used as an oral larvicide in cattle, pigs, goats, and horses; in cattle ear tags and as a feed additive to control flies; in poultry dust boxes to control poultry mites; and as paint on and sprays in poultry houses (EPA, 2002a).

(b) Residential use

Tetrachlorvinphos is used in pet flea and tick collars, shampoos, and as a dust or powder, aerosol, and pump spray for direct treatment of pets and in pet sleeping areas. In the USA, tetrachlorvinphos is used in an estimated 10% of households with dogs or cats (EPA, 2002a).

(c) Public health

Tetrachlorvinphos has been used as a spray to control nuisance and public health pests in and around refuse sites, recreational areas, and for outdoor use as sprays for fleas, ticks, and mites, around kennels, yards, camping grounds, parks, foot paths, and roadways (EPA, 2002a).

(d) Regulation

No maximum residue limit (MRL) for tetrachlorvinphos was listed in the Codex Alimentarius (Codex Alimentarius, 2015).

Tetrachlorvinphos was revoked for use in the European Union as of 2003 under Directive 91/414/EEC (European Commission, 1991). It was used in some member states for slightly different periods, e.g. France, 1972–1998; and the Netherlands, 1973–1999 (CTGB, 2015; Ministère de l'Agriculture et de la Forêt, 2015).

Use of tetrachlorvinphos remains allowable for pets, livestock and poultry in the USA, but no tetrachlorvinphos products were currently registered for use on any plant commodity in the USA, as crop uses were voluntarily cancelled in 1987 (EPA, 2006). In 2006, the United States Environmental Protection Agency (EPA) modified the allowable use of tetrachlorvinphos to reduce risks (EPA, 2006). The EPA has established maximum tolerances for tetrachlorvinphos in eggs, milk, and other animal products (NIH, 2015).

Tetrachlorvinphos is reportedly registered for use in Canada, South Africa, and Australia, as well as in the USA (Paranjape et al., 2015).

No occupational exposure limits for tetrachlorvinphos were available to the Working Group.

1.3 Measurement and analysis

Tetrachlorvinphos can be measured in air, water, soil, dust, fruits and vegetables, and urine and faeces (Table 1.1). The metabolites found in urine include 2,4,5-trichlorophenylethanediol glucuronide and dimethylphosphate, a nonspecific metabolite of several organophosphate pesticides (Beynon et al., 1973; Bravo et al., 2004).

Tetrachlorvinphos is not persistent in the environment. It is broken down in air within 24 hours, and in soil over a few weeks (NIH, 2015).

Table 1.1 Methods of analysis for tetrachlorvinphos

Sample matrix	Assay procedure	Limit of detection	Reference
Air	GC/ECD	10 µg/m³	OSHA (2015)
Aqueous	GC/FPD or GC/NPD	NR	EPA (2007)
Water	GC/MS	11 ng/L	Beceiro-González et al. (2007)
Solids (soils, sediments, sludges)	GC/FPD or GC/NPD	NR	EPA (2007)
Dust	GC/MS-EI-MID	50 ng/g	Quirós-Alcalá et al. (2011)
Fruits and vegetables	GC/MS	70 µg/kg	Fillion et al. (2000)
Urine	Isotope dilution GC-MS/MS	0.6 µg/L (dimethyl phosphate)	Bravo et al. (2004)

ECD, electron-capture detection; EI, electron impact; FPD, flame-photometric detector; GC, gas chromatography; MID, multiple ion detection mode; MS, mass spectrometry; NPD, nitrogen-phosphorous detector; NR, not reported

1.4 Occurrence and exposure

1.4.1 Exposure

(a) Occupational exposure

Workers including farmers, ranchers and pesticide applicators may be exposed to tetrachlorvinphos during mixing, loading, application and entering treated areas (EPA, 2006). No data on occupational exposure levels were available to the Working Group.

(b) Community exposure

Adults and children in the general population can be exposed to tetrachlorvinphos when treating pets, or through dermal contact with pets treated with pet collars, powders, or aerosol sprays (EPA, 2006). Several studies have shown transferable residues from fur of pets treated with veterinary products containing tetrachlorvinphos (Davis et al., 2008; Rotkin-Ellman & Solomon, 2009). In one study, five dogs and five cats wearing flea collars containing tetrachlorvinphos were followed for 14 days. After 3 days with the flea collar, average residue levels were 57.98 µg/wipe in dogs and 43.40 µg/wipe in cats). After 14 days, the average residues were 5.67 µg/wipe in dogs and 8.19 µg/wipe in cats (Rotkin-Ellman & Solomon, 2009).

In a second study, the transfer of tetrachlorvinphos to humans from dogs treated with flea collars was estimated for a sample of 55 dogs. Researchers used cotton gloves to pet the dogs: the average amounts of tetrachlorvinphos transferred from the fur of the neck and the back to gloves were 22 400 ± 2900 and 80 ± 20 µg/glove, respectively, at 5 days after the collar application. The amounts transferred declined notably with time after application. T-shirts worn by children living with the treated dogs 7–11 days after treatment contained tetrachlorvinphos at 1.8 ± 0.8 µg/g shirt. 2,4,5-Trichloromandelic acid, a biomarker of exposure to tetrachlorvinphos, was detected in the urine of adults and children exposed to treated dogs (range, 1.4–582 ng/mL in adults and 2.1–1558 ng/mL in children) (Davis et al., 2008).

Other pathways are dietary exposure due to the use of tetrachlorvinphos on livestock and crops, and inhalation after outdoor application; exposure through drinking-water is expected to be minimal due to the localized nature of most applications (EPA, 2006). Very little information on environmental exposure to tetrachlorvinphos was available to the Working Group.

In Venezuela, tetrachlorvinphos was detected in 19% of red peppers and 25% of lettuces sampled (Quintero et al., 2008).

In 2006 in the USA, dust samples for 13 urban homes in Oakland, California, and 15 farmworker homes in Salinas, an agricultural community in California were collected. Detection frequencies

of tetrachlorvinphos were 4% in Oakland and 10% in Salinas. The concentration ranged from less than the limit of detection (LOD) to 15.8 ng/g in Oakland and from < LOD to 271 ng/g in Salinas (Quirós-Alcalá et al., 2011).

1.4.2 Exposure assessment and biological markers

(a) Exposure assessment

Exposure assessment methods in epidemiological studies on tetrachlorvinphos and cancer are discussed in Section 1.4.2 and Section 2.1.2 of the *Monograph* on Malathion, in the present volume.

(b) Biological markers

Urinary dimethyl phosphate reflects recent exposure to organophosphate insecticides. (EPA, 2006), but interpretation of such data is always difficult because the results cannot be attributed to any specific organophosphate.

Cholinesterase inhibition is often used as a marker of exposure to organophosphate insecticides; however, no changes in plasma or erythrocyte cholinesterase activity were observed during 4 weeks in a study in five subjects treated with a tetrachlorvinphos-based formulation (Rider & Puletti, 1969).

2. Cancer in Humans

Tetrachlorvinphos was previously evaluated by the Working Group as *Group 3*, *not classifiable as to its carcinogenicity to humans*, based on limited evidence in experimental animals (IARC, 1983, 1987). No data in humans were available at that time.

2.1 Summary of frequently cited epidemiological studies

A general discussion of the epidemiological studies on agents considered in Volume 112 of the *IARC Monographs* is presented in Section 2.2 of the *Monograph* on Malathion in the present volume. The scope of the available epidemiological studies is discussed in Section 2.1 of the *Monograph* on Malathion, and includes a consideration of chance, bias and confounding, and exposure assessment.

2.2 Cohort studies

There were no cohort studies available that provided a specific assessment of exposure to tetrachlorvinphos.

Settimi et al. (1999) reported results from a cohort of workers in a cigarette factory in Bologna, Italy, where tobacco was treated with tetrachlorvinphos, γ-hexane, and methyl bromide. The cohort of 1733 (972 women and 761 men) included workers who had been employed for at least 6 months in cigarette manufacturing and related jobs between 1 January 1962 and 1 January 1990. The cohort was traced for vital status until 1 July 1996 using municipal offices, finding 1250 living (715 women, 535 men), 467 deceased (247 women, 220 men), and 16 (10 women, 6 men) lost to follow-up (0.9%). Standardized mortality ratios (SMRs) were calculated using sex-specific mortality rates for the population of the Emilia Romagna region, adjusted for age and calendar period. Standardized mortality ratios for total mortality and total cancer ranged from 0.8 to 1.1. Mortality for cancer of the stomach was significantly lower among men (SMR, 0.5; 95% CI, 0.2–1.0; $P < 0.05$). Mortality for non-Hodgkin lymphoma (NHL) was significantly elevated among women (SMR, 2.7; 95% CI, 1–5.6; $P > 0.05$), especially among those employed for ≥ 15 years (SMR, 8.3; 95% CI, 2.3–21.4). No deaths from NHL occurred

among men. Mortality from cancer of the brain was elevated among men (SMR, 2.0; 95% CI, 0.5–5.0; 3 deaths) and women (SMR, 1.7; 95% CI, 0.5–4.3; 3 deaths). [This study was limited by the lack of specific information about individual exposure to tetrachlorvinphos, and by the small numbers of specific cancers. Lack of information on personal use of tobacco was not considered to be a significant limitation because the observed rate of cancer of the lung and respiratory disease was about that expected.]

There were other studies of workers in the cigarette/tobacco industry, but no use of tetrachlorvinphos was reported.

2.3 Case–control studies on lympho-haematopoietic cancers

See Table 2.1

Brown et al. (1990) evaluated the relationship between tetrachlorvinphos and leukaemia for a case–control study of white men in the USA (Iowa and Minnesota) (see the *Monograph* on Malathion, Section 2.2.2, for a detailed description of this study). The odds ratio for leukaemia among farmers reporting use of tetrachlorvinphos was 2.9 (95% CI, 0.8–10.6; 5 exposed cases and 5 exposed controls), compared with non-farmers, adjusted for age, state, tobacco use, family history of lymphopoietic cancer, high-risk occupations, and high-risk exposures [This study overlapped with Waddell et al., 2001 and De Roos et al., 2003. The strengths of this study were that it was population-based and enrolled incident cases, and there was detailed exposure assessment of exposure to pesticides from farmers who could provide such information. A limitation was that it was not possible to evaluate by level of exposure given the small number of exposed cases.]

Waddell et al. (2001) pooled data from three population-based case–control studies of NHL (748 cases, 2236 controls) among men in the midwestern USA (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) to evaluate several pesticides, including tetrachlorvinphos (see the *Monograph* on Malathion, Section 2.2.2, for a detailed description of this study). Comparing farmers using tetrachlorvinphos to non-farmers yielded an odds ratio of 1.8 (95% CI, 0.7–4.7; 9 exposed cases and 17 exposed controls) after adjusting for age, state, and respondent type. Adjustment for other potential confounders did not affect the odds ratios. The odds ratio for tetrachlorvinphos was not adjusted for reported use of other pesticides. [The strengths of this study were that it was population-based and enrolled incident cases, and there was detailed exposure assessment of pesticides from farmers who could provide such information. A limitation was that it was not possible to evaluate by level of exposure given the small number of exposed cases.]

De Roos et al. (2003) re-analysed data from the pooled studies of NHL in four midwestern states (Iowa, Kansas, Minnesota, and Nebraska) in the USA using logistic regression and hierarchical regression to distinguish between individual pesticides and scenarios of farmers' exposure to multiple pesticides (see the *Monograph* on Malathion, Section 2.2.2, for a detailed description of this study). This analysis included 650 cases of NHL and 1933 controls. Based on three exposed cases and 11 exposed controls, the odds ratio for tetrachlorvinphos (adjusted for age, state and other pesticides) was 0.4 (95% CI, 0.1–1.8) for logistic regression, and 0.8 (95% CI, 0.3–1.9) for hierarchical regression. [The results from De Roos et al. (2003) may have differed from analyses by Waddell et al. (2001): there were fewer cases (650 versus 748) and controls (19 033 versus 2236) in these analyses because of exclusion of individuals with missing information on other pesticides included in the hierarchical model, and because the hierarchical model adjusted for effects of other pesticides.]

Table 2.1 Case-control studies on cancer and exposure to tetrachlorvinphos

Reference, location enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled
Brown et al. (1990) Iowa and Minnesota, USA 1981–1984	Cases: 578 (response rate, 86%); white men, newly diagnosed, age ≥ 30 yr; cancer registry or hospital records Controls: 1245 (response rate, 77–79%); population-based; frequency matched on 5-yr age group, vital status, state of residence; random-digit dialling for those aged < 65 yr and Medicare for those aged ≥ 65 yr Exposure assessment method: questionnaire; in-person interview with subject or proxy; farming and pesticide use history for subjects who worked on farm, listing 23 animal insecticides, 34 crop insecticides, 38 herbicides, 16 fungicides. Exposure defined as ever personally handled, mixed or applied; ORs for diazinon refer to use on crops	Total leukaemia	Ever used TCVP	5	2.9 (0.8–10.6)	Age, vital status, state, tobacco use, family history of lympho-haematopoietic cancer, high-risk occupations, high-risk exposures
Waddell et al. (2001) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 748 (response rate, NR); white men, newly diagnosed, age ≥ 21 yr (Iowa & Minnesota: 462; Kansas: 150; Nebraska: 136) Controls: 2236 (response rate, NR); white men, population-based, frequency matched on: 5-yr age group, vital status, state of residence (Iowa & Minnesota: 927; Kansas: 823; Nebraska: 486) Exposure assessment method: questionnaire; use of pesticides obtained by questionnaire from subjects, or surrogates if subjects deceased. In-person interviews in Minnesota and Iowa, and telephone interviews in Kansas and Nebraska. Sought information on specific pesticides, when and frequency of use, type of application, and use of protective equipment	NHL	Ever used	9	1.8 (0.7–4.7)	Age, state of residence, respondent type (proxy/ direct)

Table 2.1 (continued)

Reference, location enrolment period	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/deaths	Risk estimate (95% CI)	Covariates controlled
De Roos et al. (2003) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 650 (response rate, 74.7%); cancer registries and hospital records; white men Controls: 1933 (response rate, 75.2%); random-digit dialling, Medicare, state mortality files; white men Exposure assessment method: see Waddell et al. (2001); questionnaire and interview (direct or next-of-kin); analyses focused on 47 pesticides to which ≥ 20 persons were exposed; any subject with a missing or "don't know" response for any of the 47 pesticides was excluded from all analyses	NHL	Ever (logistic regression) Ever (hierarchical regression)	3 3	0.4 (0.1–1.8) 0.8 (0.3–1.9)	Age, study site, all the other 46 pesticides to which 20 or more persons were exposed

CI, confidence interval; TCVP, tetrachlorvinphos; yr, year

3. Cancer in Experimental Animals

Studies of carcinogenicity previously assessed by and leading to the previous evaluation of *limited evidence* in experimental animals for the carcinogenicity of tetrachlorvinphos are also included in the present monograph (IARC, 1983, 1987).

3.1 Mouse

See Table 3.1

Oral administration

In a study by the National Cancer Institute (NCI), groups of 50 male and 50 female B6C3F$_1$ mice (age, 35 days) were given diets containing tetrachlorvinphos (purity, 98%) at a dose of 8000 or 16 000 ppm ad libitum 7 days per week for 80 weeks, and then held for an additional 12 weeks (NTP, 1978). Groups of 10 male and 10 female mice held untreated for 90–92 weeks served as matched untreated controls. Since the numbers of mice in the matched-control groups were small, pooled-control groups were also used for statistical comparisons. Matched controls from the study on tetrachlorvinphos were combined with matched controls from long-term studies performed on malathion, toxaphene, endrin, and lindane that were conducted at the same time in the same laboratory. The pooled untreated controls for tetrachlorvinphos consisted of a total of 50 male and 50 female mice. For this bioassay, mice receiving tetrachlorvinphos were maintained in a room housing mice treated with dieldrin or malathion, together with their respective matched controls. There was a dose-related decrease in mean body weights in treated male and female mice compared with the matched controls throughout the exposure period. Survival in all dose groups was similar to that of controls.

There was a significant positive trend in the incidence of hepatocellular carcinoma in treated males compared with either matched or pooled controls: matched controls, 0/9; pooled controls, 5/49 (10%); lower dose, 36/50 (72%); higher dose, 40/50 (80%); $P < 0.001$. Pairwise comparison of lower- and higher-dose groups of males with matched- or pooled-control groups showed significant increases in the incidences of hepatocellular carcinoma in the treated groups in every case. The incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) was also increased in treated males. In treated females, the incidence of hepatocellular carcinoma by itself was not significantly increased. However, the incidence of liver neoplastic nodules [hepatocellular adenoma] alone – pooled controls, 1/48 (2%); lower dose, 14/49 (29%), $P < 0.001$; higher dose, 9/47 (19%), $P = 0.007$) – and the incidence of liver neoplastic nodules [hepatocellular adenoma] or hepatocellular carcinoma (combined) – pooled controls, 3/48 (6%); lower dose, 19/49 (39%), $P < 0.001$; higher dose, 11/47 (23%), $P = 0.019$ – showed significant dose-related positive trends, and also significantly increased incidences in the groups at the lower and higher dose compared with pooled controls. The incidence of liver neoplastic nodules [hepatocellular adenoma] or hepatocellular carcinoma (combined) in females was also significantly increased in the group at the lower dose (19/49, $P = 0.020$) compared with matched controls (0/9). There were no significant increases in tumour incidence at any other site in treated mice (NTP, 1978). [The Working Group noted the small number of matched controls, and the exposure in a room where other chemicals were also being studied.]

Parker et al. (1985) treated groups of 80 male and 80 female B6C3F$_1$ mice (age, 7–8 weeks) with diets containing tetrachlorvinphos (purity, 98%) at a dose of 0, 17.5, 64, 320, 1600, 8000, or 16 000 ppm (in these groups, the test chemical was reported by the authors to be representative

Table 3.1 Studies of carcinogenicity with tetrachlorvinphos in mice

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
$B6C3F_1$ (M, F) ≤ 92 wk NTP (1978)	Diet containing TCVP (purity, 98%) at a concentration of 0 ppm (matched control), 0 ppm (pooled control), 8000 ppm, or 16 000 ppm, ad libitum, 7 days/wk for 80 wk, and then held untreated for an additional 10–12 wk 50 M and 50 F (age, 35 days)/treated group; 10 M and 10 F/matched untreated control group Due to the small number of mice in the matched-control group, pooled controls (50 M and 50 F) were also used for statistical comparisons; matched controls from this study were combined with matched controls from long-term studies with other chemicals that were conducted at the same time in the same laboratory	*Males* Liver: Neoplastic nodule [hepatocellular adenoma]: 0/9, 3/49 (6%), 11/50 (22%)*, 2/50 (4%) Hepatocellular carcinoma: 0/9‡, 5/49 (10%)‡, 36/50 (72%)**, 40/50 (80%)** [Hepatocellular adenoma] or carcinoma (combined): 0/9‡, 8/49 (16%)‡, 47/50 (94%)**, 42/50 (84%)** Kidney: Renal tubule carcinoma: 0/9, NR, 0/50, 1/50 *Females* Liver: Neoplastic nodule [hepatocellular adenoma]: 0/9, 1/48‡‡ (2%), 14/49 (29%)***, 9/47 (19%)† Hepatocellular carcinoma: 0/9, 2/48 (4%), 5/49 (10%), 2/47 (4%) [Hepatocellular adenoma] or carcinoma (combined): 0/9, 3/48 (6%)‡‡‡, 19/49 (39%)††, 11/47 (23%)††† Kidney: Renal tubule adenoma: 0/9, NR, 1/49, 0/46	Fisher exact test and Cochran-Armitage trend test *$P = 0.024$ (vs pooled controls) **$P < 0.001$ (vs matched and pooled controls) ***$P < 0.001$ (vs pooled controls) †$P = 0.007$ (vs pooled controls) ††$P < 0.001$ (vs pooled controls), $P = 0.020$ (vs matched controls) †††$P = 0.019$ (vs pooled controls) ‡ $P < 0.001$ for trend ‡‡ $P = 0.018$ for trend ‡‡‡ $P = 0.030$ for trend	Mice receiving TCVP were maintained in a room housing mice given dieldrin or malathion, together with their respective matched controls There was a small number of matched controls, and the study was conducted in same room as studies with other chemicals (malathion, toxaphene, endrin, and lindane) Dose-related decrease in mean body weights in treated males and females were observed throughout exposure period

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
$B6C3F_1$ (M, F) 103 wk Parker et al. (1985)	Diet containing TCVP (purity, 98%) at a concentration of 0, 17.5, 64, 320, 1600, 8000, 16 000 ppm (all groups receiving TCVP of current production), or 16 000 ppm (NCI test material) ad libitum, 7 days/wk for 103 wk 80 M and 80 F (age, 7–8 wk)/treated group; 160 M and 160 F mice/double control group; 10 treated and 20 control mice of each sex per group were killed and examined at 6, 12, and 18 mo Two samples of TCVP were used; mice at 16 000 ppm were fed test material from the previous NCI bioassay (NTP, 1978); mice in all other treatment groups received TCVP that was reported to be "more representative of current production" [of technical TCVP]	*Males* Liver: Hepatocellular adenoma: 2/99 (2%), 0/50, 0/49, 0/50, 1/50 (2%), 5/50 (10%), 3/50 (6%), 4/46 (9%) Hepatocellular carcinoma: 24/99 (24%), 14/50 (28%), 14/49 (29%), 9/50 (18%), 11/50 (22%), 13/50 (26%), 20/50 (40%), 31/46 (67%)* Hepatocellular adenoma or carcinoma (combined): 26/99 (26%), 14/50 (28%), 14/49 (29%), 9/50 (18%), 12/50 (24%), 18/50 (36%), 23/50 (46%), 35/46 (76%)*	*$P \leq 0.05$ (Cox test with Bonferroni correction)	Additional pathology review was conducted by a "consultant pathologist," and the results were as follows: *Males* Liver: Adenomatous nodules: 2/99 (2%), 3/50 (6%), 2/49 (4%), 1/50 (2%), 2/50 (4%), 3/50 (6%), 9/50 (18%), 9/46 (20%) Hepatocellular carcinoma: 14/99 (14%), 7/50 (14%), 6/49 (12%), 4/50 (8%), 4/50 (8%), 1/50 (2%), 2/50 (4%), 6/46 (13%) Adenoma or carcinoma (combined): 16/99 (16%), 10/50 (20%), 8/49 (16%), 5/50 (10%), 6/50 (12%), 4/50 (8%), 11/50 (22%), 15/46 (33%)

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
$B6C3F_1$ (M, F) 103 wk Parker et al. (1985) (cont.)		Kidney: Renal tubule adenoma: 1/99 (1%), 0/50, 0/49, 0/50, 1/50 (2%), 0/50, 2/50 (4%), 2/46 (4%) Renal tubule carcinoma: 0/99, 0/50, 0/49, 0/50, 1/50 (2%), 1/50 (2%), 9/50 (18%)*, 10/46 (22%)* Renal tubule adenoma or carcinoma (combined): 1/99 (1%), 0/50, 0/49, 0/50, 2/50 (4%), 1/50 (2%), 11/50 (22%)*, 12/46 (26%)* *Females* Liver: Hepatocellular adenoma: 0/99, 1/48 (2%), 0/49, 0/50, 1/49 (2%), 2/49 (4%), 3/50 (6%), 1/47 (2%) Hepatocellular carcinoma: 0/99, 0/48, 0/49, 0/50, 3/49 (6%), 5/49 (10%), 4/50 (8%), 5/47 (11%)* Hepatocellular adenoma or carcinoma (combined): 0/99, 1/48 (2%), 0/49, 0/50, 4/49 (8%), 7/49 (14%)*, 7/50 (14%)*, 6/47 (13%)* Kidney: Renal tubule adenoma or carcinoma (combined): 0/99, 0/48, 0/49, 0/50, 0/49, 0/49, 0/50, 2/47 (4%)		Kidney: Renal tubule adenoma: 0/99, 0/50, 0/49, 0/50, 0/50, 0/50, 10/50 (20%)*, 10/46 (22%)* Renal tubule carcinoma: 0/99, 0/50, 0/49, 0/50, 0/50, 0/50, 1/50 (2%), 2/46 (4%) Renal tubule adenoma or carcinoma (combined): 0/99, 0/50, 0/49, 0/50, 0/50, 0/50, 11/50 (22%)*, 12/46 (26%)* *Females* Liver: Adenomatous nodules: 0/99, 0/48, 0/49, 0/50, 0/49, 0/49, 1/50 (2%), 1/47 (2%) Hepatocellular carcinoma: 0/99, 0/48, 0/49, 0/50, 1/49 (2%), 0/49, 0/50, 1/47 (2%) Adenoma or carcinoma (combined): 0/99, 0/48, 0/49, 0/50, 1/49 (2%), 0/49, 1/50 (2%), 2/47 (4%) The total numbers of liver nodular lesions identified by the study and consultant pathologists were similar, but the subsequent histological diagnoses of these nodules depended on differences in classification used by each pathologist. Similarly, the study pathologist considered the majority of renal tumours to be carcinomas, while the consultant pathologist diagnosed most of these as adenoma

Table 3.1 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
$B6C3F_1$ (M, F) 103 wk EPA (1988)	Diet containing TCVP at a concentration of 0, 17.5, 64, 320, 1600, 8000, or 16 000 ppm, ad libitum, 7 days/wk for 103 wk 80 M and 80 F (age, 7–8 wk)/treated group; 160 M and 160 F/double control group; 10 treated and 20 control mice of each sex per group were killed at 6, 12, and 18 mo	*Males* Liver: Hepatocellular adenoma: 2/80 (2%), 1/37 (3%), 0/42, 0/35, 1/39 (3%), 5/47 (11%), 3/47 (6%) Hepatocellular carcinoma: 26/113 (23%), 17/58 (29%), 16/58 (28%), 10/51 (20%), 14/55 (25%), 13/60 (22%), 22/59 (37%) Hepatocellular adenoma or carcinoma (combined): 28/113 (25%)*, 18/58 (31%), 16/58 (28%), 10/51 (20%), 15/55 (27%), 18/60 (30%), 25/59 (42%)* Kidney: Renal tubule adenoma: 0/113**, 0/58, 0/68, 0/62, 1/65 (2%), 0/70, 4/69 (6%)* Renal tubule carcinoma: 0/71**, 0/37, 0/39, 0/31, 1/36 (3%), 1/47 (2%), 9/46 (20%)** Renal tubule adenoma or carcinoma (combined): 0/113**, 0/58, 0/68, 0/62, 2/65 (3%), 1/70 (1%), 13/69 (19%)** *Females* Liver: Hepatocellular adenoma: 0/113**, 1/57 (2%), 1/57 (2%), 0/59, 1/57 (2%), 2/56 (4%), 3/58 (5%)* Hepatocellular carcinoma: 1/119 (1%)**, 0/58, 0/69, 0/70, 4/69 (6%), 5/66 (8%)*, 5/68 (7%)* Hepatocellular adenoma or carcinoma (combined): 1/119 (1%)**, 1/58 (2%), 1/69, 0/70, 5/69 (7%)*, 7/66 (11%)**, 8/68 (12%)**	*$P \leq 0.05$ (pairwise comparison by Fisher exact test, or trend test by Cochran-Armitage test) **$P \leq 0.01$ (pairwise comparison by Fisher exact test, or trend test by Cochran-Armitage test)	Evaluation of the study published by Parker et al. (1985). The study reviewed by the EPA reported results on a single sample of TCVP ("current production" technical TCVP). Tumour incidences in the EPA (1988) evaluation were different to those reported by Parker et al. (1985), because the EPA used the number of tumour-bearing animals/number of animals at risk, excluding animals that died before appearance of first tumour, as the criteria for tumour incidence

EPA, United States Environmental Protection Agency; F, female; M, male; mo, month; NCI, National Cancer Institute; NR, not reported; TCVP, tetrachlorvinphos; wk, week

of "current production of technical tetrachlorvinphos"), and 16 000 ppm (the test chemical in this group was the same as in the NCI bioassay reported above; NTP, 1978). A group of 160 male and 160 female mice served as a double control. In each group, 10 treated and 20 control mice of each sex were killed and examined at 6, 12, and 18 months. Survival of males and females in the groups receiving the two highest doses was significantly greater than in the control group, while survival in all other dose groups was comparable to that of controls. Statistical evaluation of data on body weight revealed significantly lower mean values for all treatment groups compared with the control group at week 50. Exposure to tetrachlorvinphos caused a significant increase ($P \leq 0.05$) in the incidence of hepatocellular carcinoma – 31 out of 46 (67%) versus 24 out of 99 (24%) in controls – and hepatocellular adenoma or carcinoma (combined) – 35 out of 46 (76%) versus 26 out of 99 (26%) in controls) in males receiving the NCI study material at 16 000 ppm. In female mice exposed to the NCI study material at 16 000 ppm there was a significant increase ($P \leq 0.05$) in the incidence of hepatocellular carcinoma – 5 out of 47 (11%) versus 0 out of 99 in controls– and in the incidence of hepatocellular adenoma or carcinoma (combined)– 6 out of 47 (13%) versus 0 out of 99 in controls. Additionally, in the females exposed to "current production" technical tetrachlorvinphos at 8000 ppm – 7 out of 49 (14%) versus 0/99 in controls – or 16 000 ppm – 7 out of 50 (14%) versus 0 out of 99. Also, in both high-dose groups of male mice, there was a significant increase ($P \leq 0.05$) in the incidence of renal tubule carcinoma of the kidney –"current production" technical tetrachlorvinphos, 9 out of 50 (18%); and NCI study material, 10 out of 46 (22%) versus 0 out of 99 in controls – and the incidence of renal tubule adenoma or carcinoma (combined) of the kidney –"current production" technical tetrachlorvinphos, 11 out of 50 (22%); and NCI study material, 12 out of 46 (26%) versus 1 out of 99 (1%) in controls.

Parker et al. (1985) also reported an additional pathology review conducted by a "consultant" pathologist. The only statistically significant finding reported by the consultant pathologist was an increase in the incidences of renal tubule adenoma and renal tubule adenoma or carcinoma (combined) of the kidney in both high-dose groups of males fed tetrachlorvinphos at 16 000 ppm; renal tubule adenoma or carcinoma (combined): "current production" technical tetrachlorvinphos, 11 out of 50 (22%); and NCI study material, 12 out of 46 (26%) versus 0/99 in controls. The study pathologist considered the majority of renal tumours to be carcinomas, whereas the consultant pathologist diagnosed most of these tumours as adenomas.

The EPA (1988) evaluated the study described above (Parker et al., 1985). This evaluation reported results on only one sample of tetrachlorvinphos ("current production" technical tetrachlorvinphos). The tumour incidences reported in the EPA (1988) evaluation differed with those reported by Parker et al. (1985), because the EPA used the number of tumour-bearing animals per number of animals at risk, excluding animals that died before appearance of first tumour, as the criteria for tumour incidence. Using this criterion, the EPA found that exposure to tetrachlorvinphos caused a significant increase ($P \leq 0.05$) in the incidence of hepatocellular adenoma or carcinoma (combined): 25 out of 59 (42%) in male mice at 16 000 ppm versus 28 out of 113 (25%) in controls. In female mice, there was a significant increase ($P \leq 0.05$) in the incidence of hepatocellular carcinoma in groups at 8000 ppm (5 out of 66 (8%) versus 1 out of 119 (1%) in controls) and 16 000 ppm (5 out of 68 (8%) versus 1 out of 119 (1%) in controls), and a significant increase ($P \leq 0.01$) in the incidence of hepatocellular adenoma or carcinoma (combined) in the groups at 8000 ppm (7 out of 66 (11%) versus 1 out of 119 (1%) in controls) and 16 000 ppm (8 out of 68 (12%) versus 1 out of 119 (1%) in controls). Also, in male mice at the highest dose there was

a significant increase ($P \leq 0.01$) in the incidences of renal tubule carcinoma of the kidney (9 out of 46 (20%) versus 0 out of 71 in controls) and renal tubule adenoma or carcinoma (combined) (13 out of 69 (19%) versus 0 out of 113 in controls). All the above significant increases in tumour incidence by pairwise comparison were associated with a significant positive trend in the incidence of the related tumour.

[The Working Group noted that while the tumour incidences reported by EPA (1988) and Parker et al. (1985) differed because of the different criteria used, the two reported significance mainly at the same tumour sites.]

3.2 Rat

See Table 3.2

Oral administration

In a study by the NCI, groups of 50 male and 50 female Osborne-Mendel rats (age, 35 days) were given feed containing tetrachlorvinphos (purity, 98%) at a time-weighted average dose of 4250 ppm (8000 ppm for 5 weeks, then lowered to 4000 ppm for 75 weeks), or 8500 ppm (16 000 ppm for 5 weeks, then lowered to 8000 ppm for 75 weeks) ad libitum 7 days per week for 80 weeks, and then held untreated for an additional 31 weeks (NTP, 1978). Initial doses were lowered by 50% at 5 weeks on study because toxicity was observed that indicated excessive mortality might occur before the end of the study. Groups of 10 male and 10 female rats held untreated for 111 weeks served as matched untreated controls. Since the numbers of rats in the matched-control groups were small, pooled-control groups were also used for statistical comparisons. Matched controls from the study on tetrachlorvinphos were combined with matched controls from long-term studies on malathion, toxaphene, endrin, and lindane that were conducted at the same time in the same laboratory. The pooled controls for statistical tests consisted of 55 males and 55 females. There was a dose-related decrease in mean body weights in treated male and female rats compared with the matched controls throughout the exposure period. Survival of males at the higher dose was only 48% at the end of the study. Survival of males and females in all other dosed groups was similar to or higher than that of controls (NTP, 1978).

In female rats at the higher dose, there was a significant increase in the incidence of thyroid C-cell adenoma (7 out of 46 versus 1 out of 46 in pooled controls; $P = 0.027$,) and in the incidence of cortical adenoma of the adrenal gland (5 out of 50 versus 0 out of 50 in pooled controls; $P = 0.022$). There was also a significant positive trend ($P < 0.02$) in the incidence of both types of neoplasm. Haemangioma of the spleen was also reported at a significantly higher incidence in males at the lower dose compared with the corresponding pooled controls (4 out of 48 (8%) versus 0 out of 47; $P = 0.049$) [the Working Group considered that these tumours may not have been associated with treatment, since there were only four tumours in the group at the lower dose, none at the higher dose, and the statistical test result for a positive dose-related trend was not significant]. There were no significant increases in tumour incidence at any other site in treated rats (NTP, 1978). [The Working Group noted the small number of matched controls, the early toxicity causing halving of doses at week 5 of the study, and that the rats were dosed for only 80 weeks and held for 31 weeks before termination.]

The EPA (1988) provided information on a long-term study in which groups of Porton Wistar rats [age not reported] were given diets containing tetrachlorvinphos [purity not reported] at a dose of 0 (60 males and 60 females; controls), 5 (40 males and 40 females), 25 (40 males and 40 females), 125 (40 males and 40 females), or 2000 (20 males and 20 females) ppm, ad libitum, 7 days per week for 2 years. In groups of treated male and female rats, final

Table 3.2 Studies of carcinogenicity with tetrachlorvinphos in rats

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
Osborne-Mendel (M, F) 111 wk NTP (1978)	Diet containing TCVP (purity, 98%) at a concentration of 0 ppm (matched control), 0 ppm (pooled control), 4250 ppm TWA (8000 ppm for 5 wk, then lowered to 4000 ppm for 75 wk), or 8500 ppm TWA (16 000 ppm for 5 wk, then lowered to 8000 ppm for 75 wk), ad libitum, 7 days/wk for 80 wk, and then held untreated for an additional 31 wk 50 M and 50 F (age, 35 days)/treated groups; 10 M and 10 F/matched untreated control group Since the numbers of rats in the matched-control groups was small, pooled-control groups also were used for statistical comparisons; matched controls from this study were combined with those from long-term studies with malathion, toxaphene, endrin, and lindane that were conducted at the same time in the same laboratory; the pooled controls for statistical tests consisted of 55 M and 55 F	*Males* Spleen: Haemangioma: 0/10, 0/52, 4/48 (8%)*, 0/47 *Females* Thyroid: C-cell adenoma: 1/9 (11%), 1/46 (2%)†, 2/50 (4%), 7/46 (15%)** Adrenal: Cortical adenoma: 0/9, 0/50†, 2/49 (4%), 5/50 (10%)**	*$P = 0.049$ (Fisher exact test, vs pooled controls) **$P < 0.03$ (Fisher exact test, vs pooled controls) † $P < 0.02$ for trend (Cochran-Armitage test)	Initial doses were lowered by 50% at wk 5 because observed toxicity indicated that excessive mortality might occur before the end of the study Study had a small number of matched controls and early toxicity led to halving of doses at experimental wk 5 of study. The animals were dosed for only 80 wk and then held for 31 wk Dose-related decrease in mean body weights in treated males and females were observed throughout exposure period; survival of males at the highest dose was only 48% at the end of the study
Porton Wistar (M, F) 2 yr EPA (1988)	Diet containing TCVP at a concentration of 0, 5, 25, 125, or 2000 ppm, ad libitum, 7 days/wk for 2 yr 60 M and 60 F/control group; 20 M and 20 F/highest-dose group; 40 M and 40 F/all other dose groups	No TCVP-related tumours reported	NS	Purity of study material and age of animals at study start, NR Final body weights significantly lower than those of controls for both sexes Small number of animals at the highest dose

Table 3.2 (continued)

Strain (sex) Duration Reference	Dosing regimen, Animals/group at start	Incidence (%) of tumours	Significance	Comments
Sprague-Dawley (M, F) 2 yr EPA (1995b)	Diet containing TCVP at a concentration of 0, 100, 1000, or 2000 ppm, ad libitum, 7 days/wk for 2 yr 50 M and 50 F/group	*Males* Thyroid: C-cell adenoma: 8/47 (17%), 10/47 (21%), 7/48 (15%), 13/45 (29%) C-cell carcinoma: 0/47, 1/47 (2%), 1/48 (2%), 0/45 Adrenal: Pheochromocytoma, benign: 4/49 (8%)*, 2/49 (4%), 6/49 (12%), 9/50 (18%) Pheochromocytoma, malignant: 0/49, 0/49, 1/49 (2%), 0/50 Pheochromocytoma, benign or malignant (combined): 4/49 (8%)*, 2/49 (4%), 6/49 (12%), 9/50 (18%) *Females* Thyroid: C-cell adenoma: 6/64 (9%), 4/50 (8%), 5/49 (10%), 4/65 (6%) C-cell carcinoma: 0/64, 0/50, 1/49 (2%), 1/65 (2%) Adrenal: Pheochromocytoma, benign: 0/48, 1/50 (2%), 2/50 (4%), 0/49 Pheochromocytoma, malignant: 1/48 (2%), 0/50, 0/50, 0/49	*$P = 0.018$, trend test	Purity of study material and age of animals at study start, NR

F, female; M, male; mo, month; NR, not reported; NS, not significant; TCVP, tetrachlorvinphos; TWA, time-weighted average; vs, versus; wk, week; yr, year

body weights were significantly lower than those of the controls. There were no compound-related lesions reported. [The Working Group noted the small number of rats at the highest dose.]

The EPA (1995b) also provided information on a long-term study in which groups of 50 male and 50 female Sprague-Dawley rats [age not reported] were given diets containing tetrachlorvinphos [purity not reported] at a dose of 0, 100, 1000, or 2000 ppm ad libitum 7 days per week for 2 years. [No information on survival or body weight was provided.] In male rats, there was a significant positive trend ($P = 0.018$) in the incidence of adrenal pheochromocytoma (benign or malignant, combined). The incidences were: controls, 4 out of 49; lowest dose, 2 out of 49; intermediate dose, 6 out of 49; highest dose, 9 out of 50. In male rats at the highest dose, there was also a non-significant increase in the incidence of thyroid C-cell adenoma (13 out of 45 (29%) versus 8 out of 47 (17%) in controls).

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

4.1.1 Absorption

(a) Humans

Very little literature on absorption of tetrachlorvinphos was available to the Working Group. No studies on oral absorption in humans were identified by the Working Group. Because of the lipophilicity of tetrachlorvinphos, it is expected that oral or dermal absorption occurs via passive diffusion. One study showed that tetrachlorvinphos used in dog flea collars could be transferred to the pet owner's clothing (Davis et al., 2008). Urinary concentrations of 2,4,5-trichloromandelic acid (a tetrachlorvinphos metabolite) excreted by the dog owners were significantly higher on days immediately after placement of the flea collar on dogs than before treatment. [This result, although it summarizes excretion data, suggested that dermal absorption of tetrachlorvinphos might occur in humans.]

(b) Experimental systems

A study in rats (Porton strain) given a single oral dose of [^{14}C]-tetrachlorvinphos (16.5–22 mg/kg bw; radiolabelled at both vinyl carbon atoms) indicated efficient absorption from the gastrointestinal tract; 78% of the administered dose was eliminated in the urine within 4 days (Akintonwa & Hutson, 1967). In dogs given [^{14}C]-tetrachlorvinphos orally (0.24–0.47 mg/kg bw), 92% of the radiolabel was excreted in the urine and faeces within 4 days (Akintonwa & Hutson, 1967), again indicating effective absorption of tetrachlorvinphos.

In dairy cows given diet containing [^{14}C]-tetrachlorvinphos at a concentration of 50 ppm for five consecutive days resulted in absorption and subsequent rapid metabolism of tetrachlorvinphos to several polar metabolites, as assessed by thin-layer chromatography (Akhtar & Foster, 1980b). Nearly all the metabolites, and only trace amounts of parent compound, were excreted in the urine (Akhtar & Foster, 1980b).

Following dermal application to male CD rats of radiolabelled tetrachlorvinphos (0.1 mg/cm^2 for 10 hours), 9.57% of the administered dose was recovered in the skin, urine, faeces, and carcass, while 84% was recovered unabsorbed (EPA, 2000).

4.1.2 Distribution

(a) Humans

No data on the distribution of tetrachlorvinphos in human tissue were available to the Working Group.

(b) Experimental systems

Administration of tetrachlorvinphos to dairy cows resulted in its delivery to the liver and kidney (Akhtar & Foster, 1980b). In addition, studies of toxicokinetics in rodents indicated that tetrachlorvinphos was available systemically after oral dosing. After 4 days, 0.5% of the radiolabel was present in the skin and hair of rats (Akintonwa & Hutson, 1967).

4.1.3 Metabolism and modulation of metabolic enzymes

(a) Metabolism

(i) Overview

Tetrachlorvinphos is somewhat unique among organophosphate pesticides in that it does not require a bioactivation step in vivo to elicit its toxicological effects (see Fig. 4.1). Tetrachlorvinphos, a phosphoric acid triester, is essentially a "preformed" oxon that can directly inhibit serine hydrolases, such as acetylcholinesterase and other B-esterases. Cytochrome P450 (CYP)-catalysed demethylation and esterase-catalysed hydrolysis are both important routes of tetrachlorvinphos detoxification (Fig. 4.1). It was reported that horse plasma butyrylcholinesterase activity was significantly inhibited by tetrachlorvinphos, indicating that esterases can interact with tetrachlorvinphos (Karanth et al., 2008). For example, a single oral dose of tetrachlorvinphos (500 mg/kg) significantly inhibited liver carboxylesterase activity in rats (Moroi & Kuga, 1982). [The Working Group could not identify any evidence that paraoxonase 1 (PON-1) hydrolysed tetrachlorvinphos, but again, based on structural precedent, and the fact that 2,4,5-trichlorphenacyl chloride and downstream metabolites are formed in vitro and in vivo (Akhtar & Foster, 1980a, b), it seems reasonable and likely that PON-1 will hydrolyse tetrachlorvinphos.]

(ii) Humans

No data on metabolism in humans were available to the Working Group.

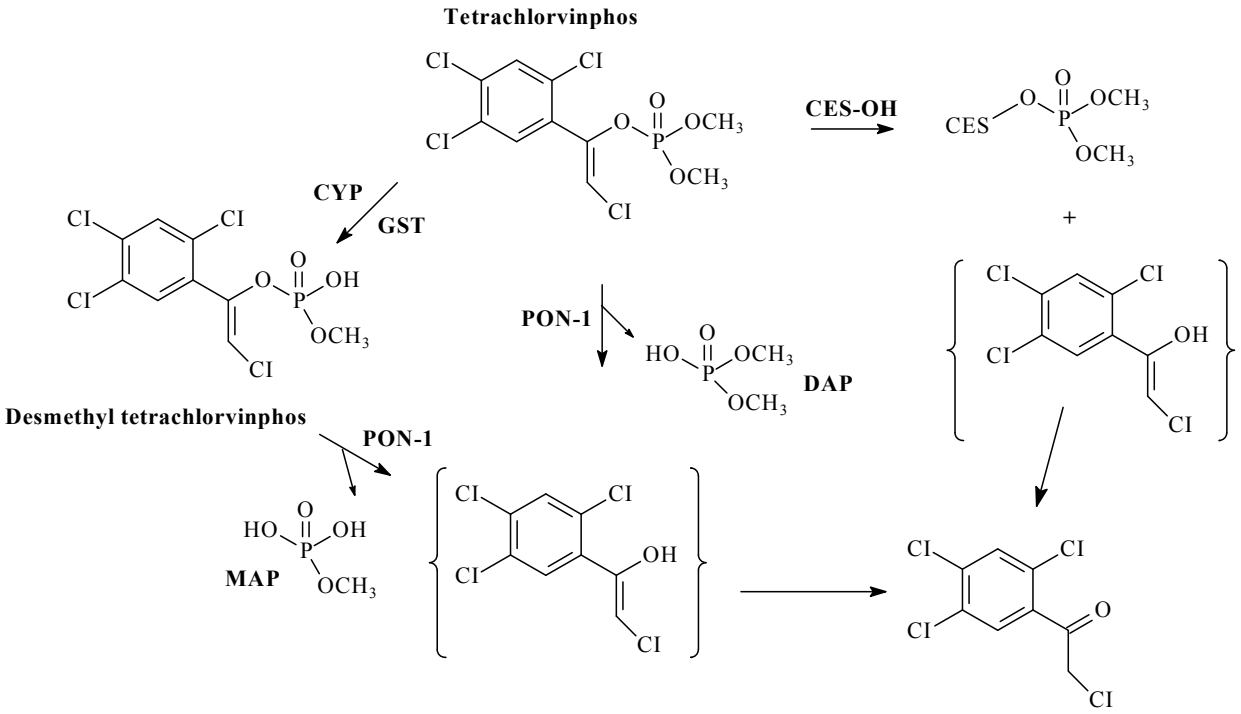
(iii) Experimental systems

Dogs were shown to metabolize tetrachlorvinphos more rapidly than rats; this was attributed to the higher activities of CYP [isoform not specified] and glutathione transferases that metabolize tetrachlorvinphos in dog liver compared with rat liver (Crawford et al., 1976). Addition of glutathione to the soluble fraction of liver from mouse, rat, rabbit, and pig caused the demethylation of tetrachlorvinphos, thus forming desmethyl tetrachlorvinphos (Fig. 4.1; Hutson et al., 1972). Glutathione acts as the acceptor of the transferred methyl group yielding *S*-methyl glutathione. In addition, demethylation of tetrachlorvinphos can also be catalysed by the hepatic microsomal fraction in an NADPH-dependent reaction, thus implicating CYP (Fig. 4.1; Crawford et al., 1976).

In lactating cows, oral administration of food containing [^{14}C]-tetrachlorvinphos at a concentration of 50 ppm for five consecutive days resulted in rapid metabolism to several polar metabolites, as assessed by thin-layer chromatography (Akhtar & Foster, 1980b). Extensive demethylation of tetrachlorvinphos was also noted in this study. Similar biotransformation pathways of tetrachlorvinphos were observed using the soluble fraction of goose and turkey liver homogenates (Akhtar & Foster, 1980a).

Hydrolytic degradation of tetrachlorvinphos and desmethyl tetrachlorvinphos are also likely important routes of degradation (Akhtar & Foster, 1980b). The resulting metabolites are mono- and di-alkyl phosphates and 2,4,5-trichlorophenacyl chloride (Fig. 4.1). 2,4,5-Trichlorophenacyl chloride can be further metabolized to 2,4,5-trichloroacetophenone via the spontaneous formation of *S*-(2,4-dichlorophenacyl) glutathione, which is converted to the ketone by an enzyme-catalysed glutathione-dependent reaction. In dairy cows, it

Fig. 4.1 Biotransformation of tetrachlorvinphos



Cytochrome P450 (CYP)-catalysed reactions produce desmethyl tetrachlorvinphos. On the basis of structural similarity with other oxons, it is likely that PON-1 catalyses the hydrolysis of tetrachlorvinphos and that carboxylesterases will react directly with tetrachlorvinphos. GST, glutathione transferase. CES-OH represents carboxylesterase; the OH functionality represents the catalytic (nucleophilic) serine residue in the active site that reacts with the electrophilic phosphoric acid triester. Metabolites in brackets have not been isolated, but are likely intermediates in the formation of 2,4,5-trichlorophenacyl chloride. MAP, monoalkyl phosphate; DAP, dialkyl phosphate.
Compiled by the Working Group

was suggested that 2,4,5-trichloroacetophenone could be converted to 1-(2,4,5-trichlorophenyl) ethanol by a keto reductase activity (Akhtar & Foster, 1980b). Alternatively, 2,4,5-trichlorphenacyl chloride can be converted to 2,4,5-trichloromandelic acid, as shown in (Akhtar & Foster, 1980b). These metabolites are readily excreted in the urine as glucuronide conjugates.

(b) Modulation of metabolic enzymes

Tetrachlorvinphos did not inhibit CYP19 aromatase activity in human placental microsomes in vitro (Vinggaard et al., 2000). In hepatocytes harvested from human liver biopsies, and in rat primary hepatocytes, tetrachlorvinphos induced CYP1A1, as measured by 7-ethoxyresorufin-*O*-deethylase (EROD) activity (Delescluse et al., 1998). No similar activity was seen in a human carcinoma cell line (HepG2) or an immortalized human keratinocyte cell line (HaCaT).

In rats, two hepatic monooxygenase enzyme activities were induced in a dose-related manner after oral administration of tetrachlorvinphos (60 and 250 mg/kg) for 10 days (Moroi et al., 1976). The increases were observed in aminopyrine demethylase *o*-ethyl *O*-*p*-nitrophenylphenylphosphonothioate detoxification.

4.1.4 Excretion

(a) Humans

No data on excretion of tetrachlorvinphos in humans were available to the Working Group.

(b) Experimental systems

In rats (Porton strain) given a single oral dose of [^{14}C]-tetrachlorvinphos (16.5–22 mg/kg bw; radiolabelled at both vinyl carbon atoms), on average 78% of the administered dose was excreted in the urine, 16.5% in the faeces, and 0.5% in expired gases over 4 days (Akintonwa & Hutson, 1967). A significant fraction of the faecal radiolabel was identified as [^{14}C]-tetrachlorvinphos, indicating incomplete absorption. Similarly, in dairy cows given diets containing [^{14}C]-tetrachlorvinphos (5 or 50 ppm), ~76–82% of the administered dose was also eliminated in the urine, as metabolites (Gutenmann et al., 1971; Akhtar & Foster, 1980b). Only trace amounts of parent compound were detectable (Akhtar & Foster, 1980b). After hydrolysis of glucuronide and sulfate conjugates in the urine, the metabolites identified were (percentage of administered dose indicated): desmethyl tetrachlorvinphos (13.2%), 1-(2,4,5-trichlorophenyl) ethanol (34.8%), (2,4,5-trichlorophenyl)ethane-1,2-diol (28.1%), and 2,4,5-trichloromandelic acid (6.1%) (Gutenmann et al., 1971; Akhtar & Foster, 1980b).

4.2 Mechanisms of carcinogenesis

This section summarizes evidence for the key characteristics of carcinogens (IARC, 2014) for which there were adequate data for evaluation, concerning whether tetrachlorvinphos is genotoxic, modulates receptor-mediated events, and alters cell proliferation, cell death or nutrient supply.

4.2.1 Genetic and related effects

Table 4.1, Table 4.2, Table 4.3, and Table 4.4 summarize the results of studies carried out in human cells in vitro, in experimental animals in vivo, in non-human mammalian cells in vitro, and in non-mammalian systems in vitro, respectively.

(a) Humans

See Table 4.1

No data in exposed humans were available to the Working Group.

A significant increase in the frequency of chromosomal aberrations was observed in human cultured lymphocytes exposed to tetrachlorvinphos in the absence of metabolic activation (Kurinnyĭ & Pilinskaia, 1977).

(b) Experimental systems

See Table 4.2, Table 4.3, Table 4.4

In one study, tetrachlorvinphos significantly increased the frequency of micronucleus formation in the bone marrow of Swiss mice after repeated doses administered intraperitoneally (100 mg/kg bw) or orally (3000 ppm, in the diet) (Amer & Fahmy, 1983). In the same study, no increase in the frequency of micronucleus formation was seen after dermal exposure (1350 mg/kg bw, twice per week, for 2 weeks).

In primary cultures of mouse spleen cells, tetrachlorvinphos significantly increased the frequency of chromosomal aberrations and sister-chromatid exchanges in the absence of metabolic activation (Amer & Aly, 1992). In Chinese hamster ovary cells, tetrachlorvinphos induced chromosomal aberrations in the absence but not in the presence of metabolic activation with S9 (EPA, 2002b).

In bacterial studies, tetrachlorvinphos did not induce primary DNA damage in *Escherichia coli PQ37* (Ruiz & Marzin, 1997), or mutations in *Salmonella typhimurium* (Dean, 1972; Bartsch et al., 1980; Brooks et al., 1982; Moriya et al.,

Table 4.1 Genetic and related effects of tetrachlorvinphos in humans cells in vitro

Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Cultured lymphocytes	Chromosomal damage	Chromosomal aberrations	+	NT	2 µg/mL	$P < 0.05$	Kurinnyĭ & Pilinskaia (1977)

[a] +, positive
HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested

Table 4.2 Genetic and related effects of tetrachlorvinphos in non-human mammals in vivo

Species, strain	Tissue	End-point	Test	Results[a]	Doses (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, Swiss	Bone marrow	Chromosomal damage	Micronucleus formation	+ [a]	50 and 100 (LED) mg/kg bw	Intraperitoneal, 1–4×		Amer & Fahmy (1983)
Mouse, Swiss	Bone marrow	Chromosomal damage	Micronucleus formation	+	3000 (LED) and 6000 ppm, in diet	Oral, ≤ 10 wk		Amer & Fahmy (1983)
Mouse, Swiss	Bone marrow	Chromosomal damage	Micronucleus formation	(–)	1350 mg/kg bw	Dermal, 2× per wk, for 2 wk	Only one dose tested	Amer & Fahmy (1983)

[a] +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality
bw, body weight; HID, highest ineffective dose; LED, lowest effective dose (units as reported); NT, not tested; vs, versus; wk, week

Table 4.3 Genetic and related effects of tetrachlorvinphos in non-human mammalian cells in vitro

Species, strain	Tissue, cell line	End-point	Test	Results		Concentration (LEC or HIC)	Reference
				Without metabolic activation	With metabolic activation		
Mouse, Swiss	Spleen cell primary cultures	Chromosomal damage	Chromosomal aberrations	+ [a]	NT	0.50 µg/mL	Amer & Aly (1992)
Mouse, Swiss	Spleen cell primary cultures	Chromosomal damage	Sister-chromatid exchange	+	NT	0.50 µg/mL	Amer & Aly (1992)
Chinese hamster	Ovary	Chromosomal damage	Chromosomal aberrations	NT	+	75.1 µg/mL	EPA (2002b)
Chinese hamster	Ovary	Chromosomal damage	Chromosomal aberrations	–	NT	59.9 µg/mL	EPA (2002b)

[a] +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality
HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested

Table 4.4 Genetic and related effects of tetrachlorvinphos in non-mammalian systems in vitro

Phylogenetic class	Test system (species, strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Reference
				Without metabolic activation	With metabolic activation		
Prokaryote (bacteria)	*Escherichia coli* PQ37	DNA damage	SOS chromotest	–	–	NR	Ruiz & Marzin (1997)
	Salmonella typhimurium TA98, TA100, TA102, TA1535, and TA1537	Mutation	Reverse mutation	–	–	500 µg/plate	Ruiz & Marzin (1997)
	Salmonella typhimurium TA98, TA100, TA1535, and TA1538	Mutation	Reverse mutation	–	–	2000 µg/plate	Brooks et al. (1982)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, and TA1538	Mutation	Reverse mutation	–	–	5000 µg/plate	Moriya et al. (1983)
	Salmonella typhimurium TA98, TA100	Mutation	Reverse mutation	–	–	3 µmol/plate	Bartsch et al. (1980)
	Escherichia coli WP2 *hcr*	Mutation	Reverse mutation	–	–	5000 µg/plate	Moriya et al. (1983)
	Escherichia coli WP2 and WP2 *uvrA*	Mutation	Reverse mutation	–	–	2000 µg/plate	Brooks et al. (1982)
	Escherichia coli WP2	Mutation	Reverse mutation	–	NT	Tested dose, NR; semiquantitative paper disc method	Dean (1972)
Lower eukaryote (yeast)	*Saccharomyces cerevisiae* D4	Mutation	Gene conversion	–	NT	400 µg/mL	Brooks et al. (1982)
Plant systems	*Vicia faba*	Chromosomal damage	Chromosomal aberrations	+	NT	Saturated and 0.5-saturated solutions of TCVP tested;LEC, 0.5 saturated solution	Amer & Mikhael (1983)

[a] +, positive; –, negative; (+) or (–), positive or negative in a study of limited quality

HIC, highest ineffective concentration; LEC, lowest effective concentration, NR, not reported; NT, not tested; TCVP, tetrachlorvinphos

1983; Ruiz & Marzin, 1997) or *E. coli* (Brooks et al., 1982; Moriya et al., 1983). Moreover, tetrachlorvinphos failed to cause gene conversion in yeast *Saccharomyces cerevisiae* D4 (Brooks et al., 1982). On the other hand, tetrachlorvinphos did increase the frequency of chromosomal aberration in root-tip meristems of *Vicia faba* (Amer & Mikhael, 1983).

4.2.2 Receptor-mediated mechanisms

(a) Neurotoxicity-pathway receptors

Tetrachlorvinphos is a reactive oxon. It can covalently modify the catalytic serine residue of several B-esterases and inhibit their catalytic activity, including the canonical target acetylcholinesterase (Akintonwa & Hutson, 1967; Moroi et al., 1976), resulting in the acute neurotoxicity elicited in insects and mammalian species (Ogawa et al., 1990; see Section 4.5). Acetylcholinesterase is responsible for terminating the signalling action of the neurotransmitter acetylcholine in the central and peripheral nervous systems. The inhibition of acetylcholinesterase results in acetylcholine overload and the overstimulation of nicotinic and muscarinic acetylcholine receptors. The relevance of these effects of tetrachlorvinphos to mechanisms of carcinogenesis is unknown.

(b) Humans

No data from exposed humans were available to the Working Group.

In an in-vitro assay for competitive binding, tetrachlorvinphos did not displace 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (40 nM) from the human aryl hydrocarbon receptor (AhR) when administered at a ~2000-fold molar excess (Delescluse et al., 1998).

In human androgen and estrogen receptor reporter-gene assays in the Chinese hamster ovary cell line (CHO-K1), tetrachlorvinphos was not an antagonist or agonist of human androgen receptor, nor an antagonist or agonist of estrogen receptors α and β (Kojima et al., 2004, 2010). In the same studies, tetrachlorvinphos was found to be an agonist for human pregnane X receptor in transfected CHO-K1 cells.

(c) Experimental systems

(i) In vivo

The effect of tetrachlorvinphos on thyroid function was studied in two experiments in animals. In the first study, thyroid uptake of iodine was significantly reduced 2, 6, and 24 hours after exposure to tetrachlorvinphos (a single intraperitoneal dose at 500 mg/kg) in male albino rats (Bojadziev & Manolov, 1975). No effect was seen on triiodothyronine (T3) or thyroxine (T4). A second study examined T3 or T4 in 10 horses of various breeds and ages (Berger et al., 2008). Six horses received a dietary supplement containing tetrachlorvinphos for 30 days and four horses served as controls. Tetrachlorvinphos significantly decreased serum cholinesterase activity (to < 50%) during and for 13 days after exposure, and induced behavioural changes. Thyroid hormone levels were highly variable and no significant changes were observed; the authors noted that, "detection of possible effects of thyroid hormones associated with tetrachlorvinphos exposure may require a larger number of horses and/or a longer treatment period."

Technical-grade tetrachlorvinphos substantially reduced oocyte maturation in freshwater catfish native to southern India (Haider & Upadhyaya, 1986). A subsequent study of in-vitro exposures reported significant inhibition of luteinizing hormone-induced germinal vesicle breakdown in isolated fish oocytes at all three concentrations used (1, 10, and 100 ppb) (Haider & Upadhyaya, 1986).

Other effects of tetrachlorvinphos on the thyroid, testis, ovary, and adrenal glands of rodents are discussed in Sections 4.2.3 and 4.5.

(ii) In vitro

Tetrachlorvinphos was not an agonist for the AhR in mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing copies of dioxin-responsive element (Takeuchi et al., 2008; Kojima et al., 2010). Tetrachlorvinphos was also not an agonist for mouse peroxisome proliferator-activated receptors α or γ in reporter-gene assays in CV-1 monkey kidney cells (Takeuchi et al., 2006; Kojima et al., 2010).

4.2.3 Cell proliferation and death

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

Lesions indicative of altered cell proliferation and death were observed in studies of carcinogenicity in mice and rats (NTP, 1978; Parker et al., 1985). Other adverse effects reported in studies of carcinogenicity in rodents are discussed in Section 4.5.

In a study of carcinogenicity in B6C3F$_1$ mice, necropsy at study week 26 revealed hyperplasia of the renal inner cortical tubular epithelium in males and females fed diets containing tetrachlorvinphos at 8000 or 16 000 ppm (NTP, 1978; see Section 3.1). At 78 weeks, intraluminal necrotic debris was present at high doses in both sexes, and in males the parietal epithelium of the Bowman's capsule was devoid of cuboid cells.

In the mouse liver at 26 weeks, lesions (e.g. hepatocyte enlargement) but not hyperplasia, were reported in groups at 8000 and 16 000 ppm. At a necropsy at week 53, "scattered necrotic hepatocytes" and bile duct hyperplasia were reported for these groups (NTP, 1978). Liver-weight increases were reported in 28-day and 13-week studies in rats (Ogawa et al., 1990; EPA, 2002c).

In female mice, corpora lutea were not observed in the ovaries at any necropsy of mice in the group at the highest dose (Parker et al., 1985). In male mice, hyperchromatic degenerate cells were observed in the seminiferous tubules after week 26 in the groups at 8000 and 16 000 ppm. The size and secretory activity of seminal vesicles was decreased in males at the highest dose. In males and females, adrenal hypertrophy was observed in the group at 16 000 ppm.

In a separate study of ovarian follicles explanted from C57Bl/6J female mice after treatment of the colony with tetrachlorvinphos for skin parasites, Nayudu et al. (1994) reported premature termination of follicular growth and release of oocytes with immature nuclei and without cumulus cells. The duration and pattern of in-vitro growth was markedly altered in follicles isolated from exposed mice. In follicles isolated from the offspring (age, 21 days) of exposed parents (C57BI/6J females and CBA/J males), in-vitro growth was improved, but did not follow the linear growth pattern seen in follicles of unexposed mice.

In a study of carcinogenicity, parafollicular cell (C-cell) and follicular cell hyperplasia was observed in male and female rats (NTP, 1978). The C-cell hyperplasia was described as mostly unilateral, and microscopically as having a fairly uniform and diffuse increase of C-cells scattered between the thyroid follicles. The follicular cell hyperplasia was sometimes bilateral, appearing as nodular alterations on the surface of the thyroid. Microscopically they were described as variable, "multifocal and cystic or having inward papillary projections of variable thickness," lined by regular appearing follicular cells.

4.2.4 Other mechanisms

In six horses exposed to tetrachlorvinphos for 30 days (see also Section 4.2.2), there was no effect on the expression of cytokines (interferon-γ, INF-γ) and interleukin (IL-12p40), or cyclooxygenase-2 in concanavalin A-stimulated peripheral blood mononuclear cells (Berger et al.,

2008). However, when assessed in non-stimulated cells of treated animals, INF-γ was decreased (≈-20-fold transcription compared with reference value) non-significantly towards the end of treatment and even more so (≈70-fold) after treatment ($P = 0.064$) (Berger et al., 2008).

4.3 Data relevant to comparisons across agents and end-points

4.3.1 General description of the database

The analysis of the in-vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 112 (i.e. malathion, parathion, diazinon, and tetrachlorvinphos) was informed by data from high-throughput screening assays generated by the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast™) research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013). At its meeting in 2014, the Advisory Group to the *IARC Monographs* programme encouraged inclusion of analysis of high-throughput and high-content data (including from curated government databases) (Straif et al., 2014).

Diazinon, malathion, and parathion, as well as the oxon metabolites, malaoxon and diazoxon, are among the approximately 1000 chemicals tested across the full ToxCast/Tox21 assay battery as of 3 March 2015. This assay battery includes 342 assays, for which data on 821 assay end-points are publicly available on the web site of the ToxCast research programme (EPA, 2015a). *Z*-Tetrachlorvinphos (CAS No. 22248–79–9; a structural isomer of tetrachlorvinphos) and the oxon metabolite of parathion, paraoxon, are among an additional 800 chemicals tested as part of an endocrine profiling effort using a subset of these assays. Glyphosate was not tested in the ToxCast/Tox21 assays.

Detailed information about the chemicals, assays and associated data analysis procedures is also publicly available (EPA, 2015b). It should be noted that the metabolic capacity of the cell-based assays is variable, and generally limited. [The Working Group noted that the limited activity of the oxon metabolites in in-vitro systems may be attributed to the high reactivity and short half-life of these compounds, hindering interpretation of the results of in-vitro assays.]

4.3.2 Aligning in-vitro assays to 10 "key characteristics" of known human carcinogens

To explore the bioactivity profiles of the agents being evaluated in *IARC Monographs* Volume 112 with respect to their potential impact on mechanisms of carcinogenesis, the Working Group first mapped the 821 available assay end-points in the ToxCast/Tox21 database to the key characteristics of known human carcinogens (IARC, 2014). Independent assignments were made by the Working Group members and *IARC Monographs* staff for each assay type to the one or more "key characteristics." The assignment was based on the biological target being probed by each assay. The consensus assignments comprise 263 assay end-points that mapped to 7 of the 10 "key characteristics" as shown below.

1. *Is electrophilic or can undergo metabolic activation (31 end-points)*: that were mapped to this characteristic measure cytochrome p450 (CYP) inhibition (29 end-points) and aromatase inhibition (2 end-points). All 29 assays for CYP inhibition are cell-free. These assay end-points are not direct measures of electrophilicity or metabolic activation.
2. *Is genotoxic (9 end-points):* the only assay end-points that mapped to this characteristic measure TP53 activity. [The Working Group noted that while these assays are not direct measures of genotoxicity, they are an indicator of DNA damage.]

3. *Alters DNA repair or causes genomic instability (0 end-points):* no assay end-points were mapped to this characteristic.
4. *Induces epigenetic alterations (11 end-points):* assay end-points mapped to this characteristic measure targets associated with DNA binding (4 end-points) and histone modification (7 end-points) (e.g. histone deacetylase, HDAC).
5. *Induces oxidative stress (18 end-points):* a diverse collection of assay end-points measure oxidative stress via cell imaging, and markers of oxidative stress (e.g. nuclear factor erythroid 2-related factor, NRF2). The 18 assay end-points that were mapped to this characteristic are in subcategories relating to metalloproteinase activity (5), oxidative stress (7), and oxidative-stress markers (6).
6. *Induces chronic inflammation (45 end-points):* the assay end-points that were mapped to this characteristic include inflammatory markers and are in subcategories of cell adhesion (14), cytokines (e.g. interleukin 8, IL8) (29), and nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) activity (2).
7. *Is immunosuppressive (0 end-points):* no assay end-points were mapped to this characteristic.
8. *Modulates receptor-mediated effects (81 end-points):* a large and diverse collection of cell-free and cell-based nuclear and other receptor assays was mapped to this characteristic. The 81 assay end-points that were mapped to this characteristic are in subcategories of AhR (2), androgen receptor (11), estrogen receptor (18), farnesoid X receptor (FXR) (7), others (18), peroxisome proliferator-activated receptor (PPAR) (12), pregnane X receptor-vitamin D receptor (PXR-VDR) (7), and retinoic acid receptor (RAR) (6).
9. *Causes immortalization (0 end-points):* no assay end-points were mapped to this characteristic.
10. *Alters cell proliferation, cell death, or nutrient supply (68 end-points):* a collection of assay end-points was mapped to this characteristic in subcategories of cell cycle (16), cytotoxicity (41), mitochondrial toxicity (7), and cell proliferation (4).

Assay end-points were matched to a "key characteristic" to provide additional insights into the bioactivity profile of each chemical under evaluation with respect to their potential to interact with, or have an effect on, targets that may be associated with carcinogenesis. In addition, for each chemical, the results of the in-vitro assays that represent each "key characteristic" can be compared with the results for a larger compendium of substances with similar in-vitro data, so that particular chemical can be aligned with other chemicals with similar toxicological effects.

The Working Group then determined whether a chemical was "active" or "inactive" for each of the selected assay end-points. The decisions of the Working Group were based on raw data on the concentration–response relationship in the ToxCast database, using methods published previously (Sipes et al., 2013) and available online (EPA, 2015b). In the analysis by the Working Group, each "active" was given a value of 1, and each "inactive" was given a value of 0.

Next, to integrate the data across individual assay end-points into the cumulative score for each "key characteristic," the toxicological prioritization index (ToxPi) approach (Reif et al., 2010) and associated software (Reif et al., 2013) were used. In the analyses of the Working Group, the ToxPi score provides a measure of the potential for a chemical to be associated with a "key characteristic" relative to 178 other chemicals that have been previously evaluated by the *IARC Monographs* and that had been screened by ToxCast. Assay end-point data were available in ToxCast for these 178 chemicals, and not for other chemicals previously evaluated by the *IARC*

Monographs. ToxPi is a dimensionless index score that integrates multiple different assay results and displays them visually. The overall score for a chemical takes into account the score for all other chemicals in the analysis. Different data are translated into ToxPi scores to derive slice-wise scores for all compounds as detailed below, and in the publications describing the approach and the associated software package (Reif et al., 2013). Within the individual slice, the values are normalized from 0 to 1 based on the range of responses across all chemicals that were included in the analysis by the Working Group.

The list of ToxCast/Tox21 assay end-points included in the analysis by the Working Group, description of the target and/or model system for each end-point (e.g. cell type, species, detection technology, etc.), their mapping to 7 of the 10 "key characteristics" of known human carcinogens, and the decision as to whether each chemical was "active" or "inactive" are available as supplemental material in the present volume (see Annex 1). The output files generated for each "key characteristic" are also provided in the supplemental material, and can be opened using ToxPi software that is freely available for download without a licence (Reif et al., 2013).

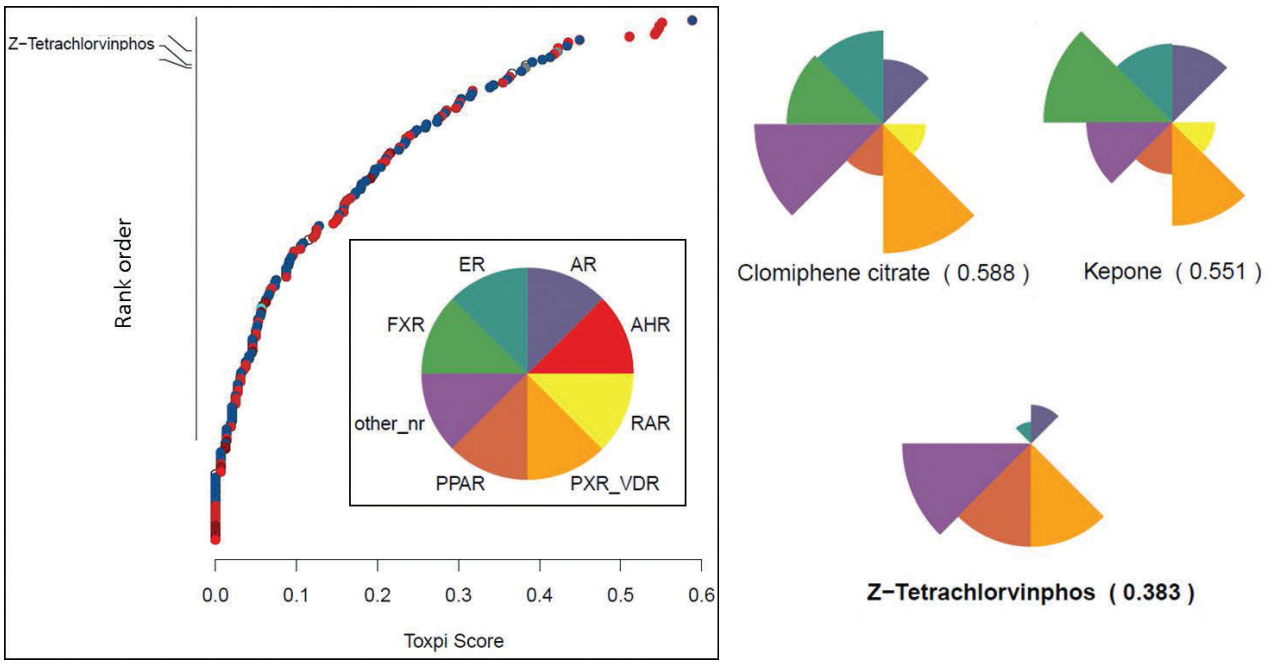
4.3.3 Specific effects across 7 of the 10 "key characteristics" based on in-vitro screening data

The relative effects of tetrachlorvinphos were compared with those of 178 chemicals selected from the more than 800 chemicals previously evaluated by the *IARC Monographs* and also screened by the ToxCast/Tox21 programmes, and with those of the other three compounds evaluated in the present volume of the *IARC Monographs* (Volume 112) and with three of their metabolites (see Fig. 4.2). Of these 178 chemicals previously evaluated by the *IARC Monographs* and screened in the ToxCast/Tox21 programmes, 8 are classified in Group 1 (*carcinogenic to humans*), 16 are in Group 2A (*probably carcinogenic to humans*), 58 are in Group 2B (*possibly carcinogenic to humans)*, 95 are in Group 3 (*not classifiable as to its carcinogenicity to humans*), and 1 is in Group 4 (*probably not carcinogenic to humans)*. The results are presented as a rank order of all compounds in the analysis arranged in the order of their relative effect. The relative position of *Z*-tetrachlorvinphos in the ranked list is also shown on the *y* axis. The inset in the scatter plot shows the components of the ToxPi chart as subcategories that comprise assay end-points in each characteristic, as well as their respective colour-coding. On the right-hand side, the two highest-ranked chemicals in each analysis are shown to represent the maximum ToxPi scores (with the scores in parentheses). Because *Z*-tetrachlorvinphos was not tested against many of the assay end-points for most characteristics discussed below, the ToxPi chart of *Z*-tetrachlorvinphos is shown only for the "modulates receptor-mediated effects" key characteristic.

Characteristic (1). *Is electrophilic or can undergo metabolic activation: Z*-tetrachlorvinphos was tested only for the two assay end-points relating to aromatase inhibition, demonstrating activity for a one cell-based end-point, but not for an end-point in a cell-free inhibition assay. *Z*-tetrachlorvinphos was not tested for any of the other 29 end-points in cell-free CYP-inhibition assays.

Characteristic (2) *Is genotoxic*: *Z*-tetrachlorvinphos was tested for 6 of the 9 assay end-points related to TP53 activity, showing activity for 2 end-points. In comparison, the most active chemical in the data set, chlorobenzilate, showed activity for 7 out of the 9 assay end-points for which it was tested. One of the active assay end-points, from a multiplexed transcription factor assay platform, has been demonstrated to be

Fig. 4.2 ToxPi ranking for *Z*-tetrachlorvinphos using ToxCast assay end-points mapped to receptor-mediated effects



On the left-hand side, the relative rank of Z-tetrachlorvinphos is shown (*y*-axis) with respect to its toxicological prioritization index (ToxPi) score (*x*-axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in Toxicity ForeCaster (ToxCast™) assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, clomiphene citrate and kepone) and the target chemical (*Z*-tetrachlorvinphos) are shown with their respective ToxPi score in parentheses.
Compiled by the Working Group

confounded by oxidative stress (Martin et al., 2010).

Characteristic (4) *Induces epigenetic alterations: Z*-tetrachlorvinphos was active for all 4 of the DNA-binding assay end-points, but was not tested for any of the 7 cell-free enzymatic assay end-points assigned to the transformation assay grouping. [The Working Group noted that the positive response in the multiplexed transcription factor profiling assay platform was most likely due to the activation of oxidative stress.]

Characteristic (5) *Induces oxidative stress*: *Z*-tetrachlorvinphos was tested for all 6 assay end-points related to oxidative stress markers and exhibited intermediate activity, being active for 3 out of 6 assay end-points. Comparison with the most active chemicals, carbaryl and tannic acid, is limited due to the incomplete testing of *Z*-tetrachlorvinphos. *Z*-tetrachlorvinphos did activate NRF2, metal-response element and antioxidant-response element transcription.

Characteristic (6) *Induces chronic inflammation*: *Z*-tetrachlorvinphos was active for one out of two NF-kB assay end-points for which only 7 of the 185 chemicals in the analysis were active. *Z*-tetrachlorvinphos was not tested in the panel of 43 assay end-points that comprise the cytokine and cell-adhesion molecule assay groupings.

Characteristic (8) *Modulates receptor-mediated effects: Z*-tetrachlorvinphos was tested for 81 of the assay end-points mapped to this

characteristic. When compared with other chemicals evaluated by the *IARC Monographs*, *Z*-tetrachlorvinphos demonstrated appreciable capacity to interact with nuclear and other receptors similar to the two highest-ranking chemicals (clomiphene citrate and kepone). *Z*-tetrachlorvinphos showed consistent PXR activation and activity in assay end-points representative of antagonists of PPAR and other nuclear receptors. *Z*-tetrachlorvinphos activated 2 of the 18 estrogen-receptor assay end-points, both cell-based transcriptional assays. Of the 11 androgen receptor assay end-points, *Z*-tetrachlorvinphos was active in both assays run in an antagonist mode and in 1 out of 2 protein complementation assay end-points that test for agonist and antagonist activity (Fig. 4.2).

Characteristic (10) *Alters cell proliferation, cell death, or nutrient supply: Z*-tetrachlorvinphos was tested for 27 out of the 68 assay end-points. *Z*-tetrachlorvinphos showed moderate impact on the assay end-points in this group when compared with the two highest-ranking chemicals, clomiphene citrate and ziram. *Z*-tetrachlorvinphos was active in the only assay for mitochondrial toxicity in which it was tested.

Overall, *Z*-tetrachlorvinphos was active for 36 of the 137 assay end-points for which it was tested. The results of ToxPi analysis of the ToxCast/Tox21 data for *Z*-tetrachlorvinphos supported findings in other model systems, as described in Section 4.2. These include aromatase inhibition, multiple nuclear receptor activities, oxidative stress, and some cytotoxic effects.

4.4 Susceptibility

No relevant studies of susceptibility to tetrachlorvinphos in humans or rodents were available to the Working Group.

4.5 Other adverse effects

4.5.1 Human

Few data on toxicity in humans were available to the Working Group.

4.5.2 Experimental systems

Regulatory submissions and published studies in rodents and dogs provide evidence for adverse effects including in the cholinergic system, liver, kidney, adrenals, and thyroid.

Effects on cholinesterase activity have been observed in different species, including rodents (Ogawa et al., 1990; EPA, 2002d), dogs (EPA, 1994) and horses (Berger et al., 2008). In a 28-day study in Slc:Wistar rats, serum and erythrocyte cholinesterase was inhibited in a dose-dependent manner from a dose of 10 mg/kg bw per day administered by intragastric gavage (Ogawa et al., 1990). Similarly, dose-dependent inhibition of plasma cholinesterase activity was observed after single oral doses of tetrachlorvinphos in rats, beginning at 8 mg/kg in males (19% inhibition) and at 20 mg/kg in females (35.5% inhibition), while inhibition of brain cholinesterase activity occurred at higher doses (EPA, 2002d). In a study of six exposed horses (Berger et al., 2008), tetrachlorvinphos significantly decreased serum cholinesterase activity (to < 50%) during and for 13 days after exposure, and induced behavioural changes.

Liver granuloma was observed in male and female $B6C3F_1$ mice at both dietary doses in a study of carcinogenicity (1200 and 2400 mg/kg per day) (NTP, 1978). Liver granuloma was also observed in a study of carcinogenicity in Osborne-Mendel rats, in females (at 212.5 and 425 mg/kg per day) and in males at the highest dose (NTP, 1978). Histological changes in the liver were seen in a 2-year study in male and female Sprague-Dawley rats given tetrachlorvinphos at a dose of 43 mg/kg per day (EPA, 1995c). In Slc:Wistar rats, liver weights increased and there was

accompanying vacuolization and necrosis at the highest dose (10, 100, and 1000 mg/kg per day) in a 28-day study (Ogawa et al., 1990). Centrolobular hepatocellular hypertrophy was seen in female Sprague Dawley rats, and in males at the intermediate dose, in a 13-week dietary study with tetrachlorvinphos (0, 100, 2000, or 5000 ppm; 0, 6.7, 142 or 375 mg/kg per day in males; 0, 10, 197 or 467 mg/kg per day in females) (EPA, 2002c). Liver weights increased, while body weights and body weight gains were reduced in males and females at the two highest doses.

Increased incidence and severity of bilateral basophilic tubules of the kidneys were also reported in male Sprague Dawley rats fed diets containing tetrachlorvinphos at 2000 or 5000 ppm in the 13-week study. In females, adrenal gland weights increased, as did fat deposition in the adrenal cortex. In the 28-day study in rats given tetrachlorvinphos by oral gavage, adrenal gland and kidney weight increases were observed at the highest dose, with accompanying pathology (Ogawa et al., 1990).

Effects on the thyroid gland reported in the study of carcinogenicity in Osborne-Mendel rats included C-cell and follicular cell hypertrophy in males and females at both doses (NTP, 1978). In the 28-day study in rats, thyroid gland weights were increased at 1000 mg/kg per day (Ogawa et al., 1990). In the 13-week study in rats, thyroid follicular cell hypertrophy was seen in males and females fed diets containing tetrachlorvinphos at 2000 or 5000 ppm (EPA, 2002c).

Decreased body weights were observed in rats and mice at both doses in studies of carcinogenicity, and there was increased mortality in male rats at the highest dose (NTP, 1978). In a study of developmental neurotoxicity in rats treated with tetrachlorvinphos (10, 50, or 200 mg/kg per day), pup weight decreased at the highest dose (EPA, 2005). Decreased thickness of the striatum, corpus callosum, and hippocampus were observed in males and females at the highest dose, and decreased thickness of the cerebellum was observed in males at the highest dose.

5. Summary of data reported

5.1 Exposure data

Tetrachlorvinphos is an organophosphate insecticide with anticholinesterase activity, which was first used commercially in 1966. It is effective against a wide range of flies, moths, fleas, ticks, and other insects; it can be sprayed on surfaces or applied to animals dermally, orally, or on treated collars and ear tags. Tetrachlorvinphos is banned for all uses in the European Union, and is not permitted on crops in the USA. It is still used in flea collars for dogs and cats in the USA, and this is one of the main sources of exposure for the general population. No data were available on occupational exposure to tetrachlorvinphos.

5.2 Human carcinogenicity data

Very few studies were available on the carcinogenicity of tetrachlorvinphos in humans. Excesses in the incidence of cancer of the brain among men and women, and of non-Hodgkin lymphoma (NHL) among women were reported in a cohort study of workers in a cigarette factory where tobacco was treated with tetrachlorvinphos; however, numbers were small, and individual exposure to the pesticide was not characterized. Analyses of data pooled from three case–control studies found an excess of NHL, but the excess was attenuated in further analyses that adjusted for exposure from other pesticides. An excess incidence of leukaemia (not otherwise specified) reported in a case–control study was based on only five exposed cases. Although excesses of cancer of the brain, NHL, and leukaemia (not otherwise specified) were observed, there were few studies for each cancer

site, small numbers, and lack of information on exposure specifically to tetrachlorvinphos, and therefore these data were considered inadequate to make an evaluation regarding carcinogenicity.

5.3 Animal carcinogenicity data

Tetrachlorvinphos was tested for carcinogenicity in male and female mice in two feeding studies, and in male and female rats in three feeding studies.

In the first study in mice, tetrachlorvinphos significantly increased the incidence of hepatocellular adenoma, hepatocellular carcinoma, and hepatocellular adenoma or carcinoma (combined) in treated males; there was also a significant positive trend in the incidence of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined). In treated females, there was a significant increase in the incidence of hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined).

In the second study in mice, tetrachlorvinphos significantly increased the incidence of hepatocellular carcinoma, and of hepatocellular adenoma or carcinoma (combined) in treated males and females. There was also a significant increase in the incidence of renal tubule carcinoma, and of renal tubule adenoma or carcinoma (combined), in treated males.

In one study in rats, tetrachlorvinphos caused a significant increase in the incidence of thyroid C-cell adenoma and adrenal cortical adenoma in females at the highest dose (with a significant positive trend for both types of tumours), and of haemangioma of the spleen in males at the lowest dose. In another study in rats, there was a significant positive trend in the incidence of adrenal pheochromocytoma (benign or malignant, combined) in treated males. There were no significant increases in the incidence of any tumours in the third study in rats.

5.4 Mechanistic and other relevant data

Tetrachlorvinphos is efficiently absorbed in rats, dogs, and cattle after oral administration; however, other routes have not been explored. In humans, one study suggested that tetrachlorvinphos can be absorbed through dermal exposure. Wide systemic distribution into parenchymal tissues and in blood was demonstrated in studies of tetrachlorvinphos in cattle and rats. Tetrachlorvinphos itself is a reactive oxon moiety that is able to react with proteins, with greatest affinity for esterases. No data on metabolism in humans were available. Data were available for rats and dogs and showed relatively complete metabolism of tetrachlorvinphos through cytochrome P450, demethylation, and hydrolytic degradation. Urine is the primary route of elimination for tetrachlorvinphos, as established from studies in rats and cattle. Primary excreted metabolites are desmethyl tetrachlorvinphos, 1-(2,4,5-trichlorophenyl)ethanol, 1-(2,4,5-trichlorophenyl)ethane-1,2-diol,and2,4,5-trichloromandelic acid.

With respect to the key characteristics of human carcinogens, adequate data were available to evaluate whether tetrachlorvinphos is genotoxic, modulates receptor-mediated events, and alters cell proliferation, cell death or nutrient supply.

The evidence is *moderate* that tetrachlorvinphos is genotoxic. The overall database is sparse but consistent. The evidence includes chromosomal damage in one in-vivo study in mice treated by intraperitoneal and oral, but not dermal, routes of exposure, two in-vitro studies in rodents, and one in-vitro study in human lymphocytes. Studies of gene mutation in bacteria gave clearly negative results in the presence or absence of metabolic activation.

The evidence is *weak* that tetrachlorvinphos modulates receptor-mediated effects. A well-established mechanism for the neurotoxic effects

of tetrachlorvinphos is inhibitory binding to acetylcholinesterase. The relevance of these effects to carcinogenesis is not clear. In animals in vivo, no effect was seen on thyroid hormones, although tetrachlorvinphos reduced iodine uptake in rats. In vitro, tetrachlorvinphos was not an agonist of the aryl hydrocarbon receptor, or mouse peroxisome proliferator activated receptors α and gamma. In human cells in vitro, tetrachlorvinphos interacted with multiple nuclear and other receptors, with mixed effects.

The evidence is *moderate* that tetrachlorvinphos alters cell proliferation in the mouse kidney and biliary tract, and rat thyroid gland, as demonstrated by hyperplasia.

No data were available to the Working Group concerning susceptibility to cancer after exposure to tetrachlorvinphos.

Overall, the mechanistic data are uninformative for carcinogenicity related to tetrachlorvinphos.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of tetrachlorvinphos.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of tetrachlorvinphos.

6.3 Overall evaluation

Tetrachlorvinphos is *possibly carcinogenic to humans (Group 2B).*

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