

Pesticide residues in food – 1996

Toxicological evaluations

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FAO Panel of Experts on Pesticide Residues
in Food and the Environment
and the
WHO Core Assessment Group**

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* First full evaluation

Introduction

The toxicological monographs and monograph addenda contained in this volume were prepared by a WHO Core Assessment Group that met with the FAO Panel of Experts on Pesticide Residues in Food and the Environment in a Joint Meeting on Pesticide Residues (JMPR) in Rome, Italy, on 16–25 September 1996.

Nine of the compounds considered by the WHO Core Assessment Group at this Meeting had been evaluated at earlier meetings. For one of these, phorate, only information received since the previous evaluation is summarized, in a 'monograph addendum'. The appropriate earlier documents should be consulted in order to obtain a full toxicological profile of this chemical. Toxicological monographs were prepared on carbaryl, carbofuran, 2,4-D, dimethoate, ferbam, flumethrin, maleic hydrazide, mevinphos, tebufenozide, and ziram, summarizing new data and, where relevant, incorporating information from previous monographs and addenda. Reports and other documents resulting from previous Joint Meetings on Pesticide Residues are listed in Annex 1.

The report of the Joint Meeting will be published by the FAO in the *FAO Plant Production and Protection Paper* series. The report contains brief comments on the compounds considered, acceptable daily intakes established by the WHO Core Assessment Group, and maximum residue limits or guideline levels established by the FAO Panel of Experts. Monographs on residues prepared by the FAO Panel of Experts are published as a companion volume, as *Evaluations 1996, Part I, Residues*, in the FAO Plant Production and Protection Paper series.

The toxicological monographs and addendum contained in this volume are based on working papers that were prepared by temporary advisers before the 1996 Joint Meeting. A special acknowledgement is made to those advisers. The monographs were edited by Mrs E. Heseltine, St Léon-sur-Vézère, France.

The preparation and editing of this volume were made possible by the technical and financial contributions of the lead institutions of the International Programme on Chemical Safety (IPCS), which supports the activities of the JMPR. The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Central Unit of the IPCS concerning the legal status of any country, territory, city or area or of its authorities, nor concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the IPCS in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biology or toxicology of the compounds included in this volume should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Meeting on Pesticide Residues, International Programme on Chemical Safety, World Health Organization, Avenue Appia, 1211 Geneva 27, Switzerland.

2,4-DICHLOROPHENOXYACETIC ACID (2,4-D)

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Explanation

Data on the toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D) were reviewed by the JMPR in 1970, 1971, 1974, and 1975 (Annex I, references 14, 16, 22, and 24). The 1970 Meeting did not establish an ADI, owing to the absence of long-term studies. The 1971 Meeting established an ADI of 0-0.3 mg/kg bw on the basis of a NOAEL of 3.1 mg/kg bw per day in a two-year dietary study in rats. The 1974 Meeting reviewed data on the use and residues of 2,4-D and concluded that there was no need to modify the previously established ADI. The 1975 Meeting reviewed a study of tissue distribution in rats and a study of teratogenicity in mice and reaffirmed the established ADI. Since that meeting, studies have become available on acute toxicity; short-term toxicity in mice, rats, and dogs; long-term toxicity in mice, rats, and dogs; carcinogenicity in mice and rats; acute and chronic neurotoxicity; developmental toxicity in rats and rabbits; mutagenicity; and epidemiological studies of 2,4-D. These

studies and summaries from the previous monograph and monograph addenda (Annex 1, references 15, 17, 23, and 25), with studies of the acute, subchronic, and developmental toxicity and genotoxicity of the four amine salts, diethanolamine (DEA), dimethylamine (DMA), isopropylamine (IPA), and triisopropanolamine (TIPA) salts, and two esters, butoxyethylhexyl (BEH) ester and 2-ethylhexyl (EH) ester, are summarized below.

Evaluation for acceptable daily intake

1. Biochemical aspects

(a) Absorption, distribution, and excretion

The pharmacokinetics of ^{14}C -2,4-D (purity, 98%) was studied in groups of 26 male B6C3F1 mice after a single oral dose at 5, 45, or 90 mg/kg bw and a single intravenous administration of 90 mg/kg bw. In order to evaluate excretion balance, groups of five mice were given the same single doses of ^{14}C -2,4-D by gavage or an intravenous dose of 5 or 90 mg/kg bw. Plasma, liver, and kidneys were analysed for radiolabel at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 36, 48, and 72 h after treatment. Urine was collected before treatment and at 0–6, 6–12, 12–24, 24–36, 36–48, 48–72 h, and every 24 h thereafter for 168 h after treatment. Faeces were collected before treatment and 0–12, 12–24, and every 24 h up to 168 h after treatment. At 168 h after treatment, the animals were killed, and blood, liver, kidneys, and residual carcasses were sampled for radiolabel.

Disappearance of the label from plasma in animals at each dose was analysed by reiteratively weighted nonlinear regression analysis to obtain the apparent pharmacokinetic parameters by both the oral and intravenous routes. Because of the apparent lag in clearance at the higher doses and the high levels of 2,4-D-derived radiolabel in plasma during the first 4 h after administration of these doses, a two-compartment model with Michaelis-Menten limited clearance was chosen. The half-lives were calculated to be 28–45 h. At least 50% of the administered dose was cleared within 12 h, suggesting that the estimates are lower than the actual clearance constants.

After oral administration, the area under the curve (AUC) for time vs concentration was 95 and 1087 $\mu\text{g} \times \text{h}/\text{ml}$ at the 5 and 45 mg/kg bw doses, respectively; with 90 mg/kg bw given orally and intravenously, the AUCs were 2257 and 2548 $\mu\text{g} \times \text{h}/\text{ml}$, respectively. Therefore, the AUC increased with dose. The apparent volumes of distribution were also found to increase with dose: after oral administration, they were 143, 213, and 300 ml/kg at 5, 45, and 90 mg/kg bw, respectively; after intravenous administration of 90 mg/kg bw, the volume of distribution was 263 ml/kg.

The main route of elimination of radiolabel was the urine, accounting for 63, 84, 71, 53, and 65% of the dose excreted by animals receiving 5 mg/kg bw orally, 5 mg/kg bw intravenously, 45 mg/kg bw orally, 90 mg/kg bw orally, and 90 mg/kg bw intravenously, respectively. Faecal elimination represented 7.6% of the dose in animals receiving 5 mg/kg bw orally and 5.2% of the dose in those given 5 mg/kg bw intravenously. A greater portion of the 2,4-D-derived radiolabel appeared in the faeces in animals at the higher dose: 15% in those at 45 mg/kg bw orally, 16% with 90 mg/kg bw orally, and 12% with 90 mg/kg bw intravenously. Most of the radiolabel was eliminated in urine collected during the first 0–6 h after treatment with 5 mg/kg bw intravenously, within 0–12 h after treatment with 5 mg/kg bw orally, and within 6–24 h after treatment with 45 or 90 mg/kg bw.

At 168 h after treatment, very little radiolabel was detected. None was found in blood or plasma after the intravenous doses, and only one animal had a detectable, low level of radiolabel in plasma after oral administration. The liver and kidneys contained similar microgram equivalents per gram of tissue of 2,4-D-derived radiolabel at each dose. Less than 1.1% of the dose was retained in the animals seven

days after administration of ^{14}C -2,4-D, independently of the dose and route of administration. The urinary clearance of 2,4-D appeared to be a saturable process in male mice at doses ≥ 45 mg/kg bw (Eiseman, 1984).

The pharmacokinetics of ^{14}C -2,4-D (radiochemical purity, $> 99\%$) was examined after oral and intravenous administration to several groups of male Fischer 344 rats. The objective of the study was to investigate the dose-dependent fate of the compound and to identify the approximate dose at which the kinetics of elimination of ^{14}C -2,4-D begin to show evidence of saturation. In order to determine the time course of disappearance of the compound from plasma and the rate of excretion in urine, three groups of three rats with jugular cannulae received an oral dose of 10, 50, or 150 mg/kg bw, and two similar groups received intravenous doses of 5 or 90 mg/kg bw. The concentrations of radiolabel were determined 1, 2, 3, 6, 9, 12, 15, 18, 24, 36, 48, 60, and 72 h after treatment; urinary ^{14}C levels were measured at 6-h intervals for the first 24 h of collection and at 12-h intervals up to 72 h after treatment; faecal samples were collected at 24-h intervals. In order to determine the effect of dose on the ratio of the concentrations of ^{14}C in kidney and plasma, five groups of six rats were given single oral doses of 10, 25, 50, 100 or 150 mg/kg bw and were killed 6 h after treatment, when plasma, urine, and kidneys were analysed for ^{14}C activity.

Absorption of 2,4-D after oral administration was complete, as urinary excretion represented $> 85\%$ of the dose within the first 12 h after treatment, and 97% of the 10 mg/kg bw oral dose and 95% of the 150 mg/kg bw dose were recovered in urine. After intravenous administration, 99 and 86% of the 5- and 90-mg/kg bw doses were recovered within the first 12 h and 100 and 91% after 72 h, respectively. The absorption rate constant in plasma was 1.4 h^{-1} . Saturable clearance from the plasma was detected and was corroborated by the disproportionate increase in the AUC with increasing dose. This effect probably reflects saturable urinary excretion, in view of the increasing ratio of plasma:kidney ^{14}C concentrations with increasing dose.

The mean half-lives for the α phase by the intravenous and oral routes were 0.92 and 1.0 h, respectively. The mean half-lives for the β phase were 14 and 18 h for the intravenous and oral routes, respectively. Doses > 50 mg/kg bw were required to bring the plasma concentration above the K_m . At or above this dose, saturation of clearance became evident. The rapid elimination of ^{14}C -2,4-D in the urine and the small contribution of the β phase indicated low potential accumulation of 2,4-D in rats (Smith *et al.*, 1990).

The absorption, distribution, metabolism, and excretion of ^{14}C -2,4-D were further examined after oral and intravenous administration to Fischer 344 rats. Four groups of five male and five female rats received either a single oral administration of ^{14}C -2,4-D by gavage at 100 mg/kg bw, ^{14}C -2,4-D at 1 mg/kg bw as a single oral dose, ^{14}C -2,4-D at 1 mg/kg bw as a single intravenous dose, or 14 daily oral doses of non-radiolabelled 2,4-D at 1 mg/kg bw followed by a single oral dose of ^{14}C -2,4-D at 1 mg/kg bw on day 15. Two additional groups of four male rats were given a single oral dose and then 1 or 100 mg/kg bw through jugular cannulae in order to define the concentration-time course in plasma. Plasma concentrations were determined for 24 h after treatment.

In all groups, $> 94\%$ of the administered dose was recovered within 48 h after treatment. The primary route of excretion was the urine (85–94%), the faeces being a minor excretory pathway (2–11%). No sex-related difference was seen, and repeated oral treatment did not alter the excretory route. ^{14}C -2,4-D was rapidly and almost completely absorbed, as peak plasma levels were attained about 4 h after treatment and 85–94% of each dose was excreted in the urine. The non-proportional AUCs and the delayed urinary excretion of radiolabel strongly imply, however, dose-dependent non-linear kinetics. Although the elimination of radiolabel was saturated during the first few hours after the high dose of 100 mg/kg bw, the excretion of radiolabel was rapid, most of the administered dose having been excreted by 36 h after treatment in all groups. Rapid excretion of ^{14}C -2,4-D is also corroborated by the

approximate half-life of 5 h for urinary excretion after oral administration. The rapid clearance of 2,4-D from plasma and its rapid excretion in the urine indicate that it has little potential to accumulate in rats. Analysis of all major tissues and organs for residual ^{14}C activity indicated that only a small fraction of the dose was still present 48 h after treatment. Tissues and organs from animals at the low dose contained $< 0.7\%$ of the administered dose (Timchalk *et al.*, 1990). These results indicate that the fate of ^{14}C -2,4-D in the rat is independent of dose and sex, that the compound is rapidly and almost completely eliminated, essentially by the urinary route, and that it has little potential to accumulate. The main results on ^{14}C -2,4-D metabolism in rats are discussed below.

In a limited study of metabolism, male Fischer 344 rats were given either unlabelled IPA salt of 2,4-D at 2.7 mg/kg bw, ^{14}C -2,4-D at 10 mg/kg bw, or both unlabelled IPA salt at 2.7 mg/kg bw and ^{14}C -2,4-D at 10 mg/kg bw. The fate of the two compounds was unaffected by their co-administration. After the single dose of ^{14}C -2,4-D, alone or in combination with IPA salt, 2,4-D was rapidly absorbed and excreted, primarily in the urine. IPA salt, administered alone or in combination with ^{14}C -2,4-D, was readily absorbed and rapidly excreted as unchanged parent compound in the urine. For both groups, $> 90\%$ of the administered dose was excreted as IPA salt within the first 12 h after treatment (Dryzga *et al.*, 1993).

The absorption, distribution, and excretion of ^{14}C -TIPA salt of 2,4-D were also studied in male Fischer 344 rats, which received a targeted dose of 10.7 mg/kg bw TIPA salt or 10 mg/kg bw 2,4-D and 20–30 μCi of ^{14}C per animal. Blood was collected from each rat at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 18, 24, 48, and 72 h after treatment, and plasma was analysed for radiolabel. Urine was collected at 6, 12, 24, 48 and 72 h after treatment, and the radiolabel in urine and the cage rinse was combined for each collection interval and expressed as radiolabel excreted in the urine. Faeces were collected at 24-h intervals and analysed for radiolabel. Expired air was passed through a charcoal trap to capture expired organic ^{14}C and then through a monoethanolamine:1-methoxy-2-propanol trap to capture expired ^{14}C -carbon dioxide; the latter traps were changed at 12, 24, 36, 48, and 72 h after treatment and analysed for radiolabel. Samples of liver, kidneys, perirenal fat, skin, and remaining carcass were collected from animals killed 72 h after treatment and analysed for radiolabel. Metabolites were characterized in pooled urine samples collected 0–6 and 6–12 h after treatment.

^{14}C -TIPA salt was rapidly absorbed by the gastrointestinal tract and excreted in the urine unchanged. The concentration of radiolabel in the plasma peaked 0.25 h after treatment and then decreased in a tri-exponential manner. Owing to its rapid elimination, ^{14}C -TIPA salt did not accumulate in the tissues: $< 1\%$ of the administered radiolabel remained in the tissues and carcass in rats sacrificed 72 h after treatment. ^{14}C -TIPA salt did not undergo extensive metabolism, as essentially all of the radiolabel excreted in the urine represented unchanged TIPA salt. By the first 24 h after treatment, 80% had been excreted in the urine. Faecal excretion accounted for 4–7% of the dose, expired ^{14}C -carbon dioxide for 3–4%, and the final cage rinse for about 1% of the dose. ^{14}C -TIPA salt was thus well absorbed and rapidly excreted in the urine, primarily as unchanged compound; it does not accumulate in rat tissues. Excretion of the parent acid, 2,4-D, was not affected by addition of the TIPA salt (Dryzga *et al.*, 1992a).

The absorption, metabolism, and excretion of the BEH ester of 2,4-D were studied in a group of four male Fischer 344 rats given a single dose of 13.9 mg/kg bw ^{14}C -BEH ester in corn oil by gavage. Blood samples were collected 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 18, and 24 h after treatment; urine, cage rinse, and expired ^{14}C -carbon dioxide were collected from rats 6, 12, 24, and 48 h after treatment. The 0–6-, 6–12-, and 12–24-h pooled urine specimens were analysed for unchanged BEH ester, 2,4-D, ethylene glycol, and ^{14}C -2-butoxyethanol and its metabolites by gas chromatography–mass spectrometry (GC–MS) and high-performance liquid chromatography (HPLC) with radiochemical detection.

^{14}C -BEH ester was rapidly absorbed and hydrolysed to 2,4-D and ^{14}C -2-butoxyethanol. 2,4-D was eliminated unchanged in the urine. By 48 h after treatment, the mean recovery of radiolabel represented 78% of the administered dose. The urine was the major route of elimination (58% of the administered dose); expired ^{14}C -carbon dioxide contained 17% and the faeces 2.4% of the administered dose. Elimination was rapid, as indicated by a recovery of 49% in the urine 12 h after treatment; the elimination half-life of radiolabel in urine was 4.6 h. No unchanged parent compound was detected in blood or urine. The radiolabelled metabolites identified in urine included 2-butoxyethanol, 2-butoxyacetic acid, ethylene glycol, and their conjugates. The major metabolite was 2-butoxyacetic acid (Dryzga *et al.*, 1992b).

The absorption, distribution, excretion, and biotransformation of ^{14}C -EH ester of 2,4-D were studied in male Fischer 344 rats that received a single oral dose of 15 mg/kg bw ^{14}C -EH ester. Blood was collected from each rat 0.5, 1, 1.5, 2, 4, 6, 8, 12, 18, and 24 h after treatment, and plasma was analysed for radiolabel; urine was collected 6, 12, 24, and 48 h after treatment, and the radiolabel in urine and the cage rinse was combined for each collection interval and expressed as radiolabel excreted in the urine. Faeces were collected at 24-h intervals and analysed for radiolabel; expired ^{14}C -carbon dioxide collected at 6, 12, 24, and 48 h was trapped in a solution of monoethanolamine:1-methoxy-2-propanol, and the radiolabel was quantified. No tissue samples were analysed. Metabolites were characterized in pooled urine (0–6- and 6–12-h) and in faecal (0–24-h) samples by GC-MS, and unchanged EH ester in pooled urine and faecal extracts was determined by HPLC.

^{14}C -EH ester was rapidly absorbed, with a peak plasma concentration of 1.0 $\mu\text{g/g}$ 4 h after treatment, decreasing with a half-life of 9 h. Once absorbed, ^{14}C -EH ester was extensively metabolized and was eliminated in the urine, faeces, and as expired ^{14}C -carbon dioxide. It was rapidly hydrolysed to 2,4-D and ^{14}C -2-ethylhexanol, since no ^{14}C -EH ester was found in blood, urine, or faeces. The principal route of excretion was the urine (62–66%), less being eliminated in the faeces (14–21%) and expired carbon dioxide (9–12%). The metabolites found in both urine and faeces were 2-ethylhexanol, 2-ethylhexanoic acid, 2-ethyl-1,6-hexanedioic acid, and 2,4-D. Metabolites found in the urine but not in the faeces were 2-ethyl-5-ketohexanoic acid, 2-ethyl-5-hydroxyhexanoic acid, 2-heptanone, and 4-heptanone. These metabolites were previously reported as metabolites of ^{14}C -2-ethylhexanol. The EH ester of 2,4-D is thus converted rapidly to 2,4-D, which is then excreted in the urine. Therefore, the EH ester is toxicologically equivalent to 2,4-D itself (Dryzga *et al.*, 1992c).

Dermal absorption of 2,4-D, the DMA salt, and the EH ester was studied after topical application to male Sprague-Dawley rats, New Zealand white rabbits, and rhesus monkeys. About 1 μCi of ^{14}C -labelled compound dissolved in acetone was applied to the shaven mid-dorsal regions of rats and rabbits and to the mid-dorsal forearm or forehead of the monkeys. The area of application varied but provided a constant dose rate of 4 $\mu\text{g}/\text{m}^2$. Nonocclusive gauze patches were used to protect the treated areas, except the monkey forehead. Urine samples were collected after 4 and 8 h on the first day and then at 24-h intervals for 14 days after treatment. Total dermal absorption and excretion half-lives were calculated. The monkey forehead was more permeable than the monkey forearm; the rate of urinary excretion of radiolabel after dermal absorption was similar in all cases. Dermal absorption of 2,4-D was 36% in rabbits, 15% in monkey forearm, and 29% in monkey forehead; the half-lives were 2.1 days in rabbits, 1.9 days for monkey forearm, and 1.5 days for monkey forehead. Dermal absorption of the DMA salt was 14% in rats, 6% in monkey forearm, and 31% in monkey forehead; the half-lives were 1.4 days, 1.8 days, and 2.3 days in rats, monkey forearm, and forehead, respectively. Dermal absorption of the EH ester was 50% in rabbits, 40% in monkey forearm, and 56% in monkey forehead; the half-lives were 2.1 days in monkey forearm and 2.0 days in monkey forehead; no half-life was determined in rabbits (Moody *et al.*, 1990).

A study was conducted to determine the extent to which dogs absorb and excrete 2,4-D in urine after contact with treated lawns under natural conditions. Concentrations of $\geq 10.0 \mu\text{g/litre}$ were found in the urine of 33 of 44 neighborhood mixed-breed dogs (75%) potentially exposed to 2,4-D-treated lawns an average of 10.9 days after application, and concentrations of $\geq 50 \mu\text{g/litre}$ were found in 17 (39%). Of 15 dogs with no known exposure to a 2,4-D-treated lawn during the previous 42 days, 4 (27%) had 2,4-D in urine (1 at a concentration $\geq 50 \mu\text{g/litre}$). The odds ratio (OR) for an association between exposure to a 2,4-D-treated lawn and the presence of $\geq 50 \mu\text{g/litre}$ 2,4-D in urine was 8.8 (95% confidence interval [CI], 1.4–56). Dogs exposed to lawns treated within seven days before urine collection were more than 50 times as likely to have 2,4-D at concentrations $\geq 50 \mu\text{g/litre}$ than dogs exposed to a lawn treated more than one week previously (OR, 56; 95% CI, 10–312). The highest mean concentration of 2,4-D in urine (21.3 mg/litre) was found in dogs sampled within two days after application of the herbicide (Reynolds *et al.*, 1994).

The excretion, tissue residues, and metabolism of ^{14}C -2,4-D were investigated in a lactating goat given an oral dose of 483 ppm for three consecutive days in a capsule. On the basis of established tolerances, this dose is equivalent to 68% of the maximal exposure of dairy cattle to 2,4-D residues. Urine and faeces were collected daily and milk each morning and evening. At the end of the study, selected tissues were sampled for determination of radiolabel concentration. About 90% of the dose was recovered in the urine and faeces; milk, liver, kidneys, composite fat, and composite muscle accounted for $< 0.1\%$ of the total dose received. The residues in the milk were 0.22–0.34 ppm at the morning milking and 0.04–0.06 ppm in the evening. Kidneys accounted for the highest residue concentration, 1.4 ppm; liver contained 0.22 ppm, fat, 0.09 ppm, and muscle, 0.04 ppm (Guo & Stewart, 1993).

After a single oral dose of 5 mg/kg bw 2,4-D to a male human subject, the plasma level was 35 $\mu\text{g/ml}$ after 2 h, 25 $\mu\text{g/ml}$ after 24 h, and 3.5 $\mu\text{g/ml}$ after 48 h. The levels in whole blood decreased from 21 $\mu\text{g/ml}$ after 2 h to 2.1 $\mu\text{g/ml}$ after 48 h. A total of 73% of the dose was excreted in the urine within 48 h after treatment. On the basis of this study, it was estimated that 1 mg/kg bw of 2,4-D can be eliminated by humans within 24 h (Gehring & Gorden, personal communication, 1971).

The absorption and excretion of 2,4-D was investigated in six healthy volunteers (age, 22–30 years) after ingestion of 5 mg/kg bw in gelatin capsules. Blood samples were obtained 1, 2, 7, 12, 24, 32, 48, 56, and 168 h after ingestion; urine samples were collected during the first 24 h. 2,4-D was absorbed fairly quickly, a significant amount of the compound being detected 1 h after administration. The highest concentration of 2,4-D in plasma was reached in 7–24 h, after which the amount declined steadily. After absorption, 2,4-D was quickly excreted; about 75% of the dose was found unchanged in the urine 96 h after administration. No metabolites were detected in urine (Kohli *et al.*, 1974).

The pharmacokinetics of 2,4-D were studied in five male subjects (29–40 years; 70–90 kg) given 5 mg/kg bw 2,4-D (analytical grade) either as a slurry in milk or as the powder followed by water. Blood samples were collected at 1, 4, 8, 12, 24, 36, 48, 72, 96, 120, and 144 h after treatment, and urine samples were collected at 12-h intervals. The concentrations of 2,4-D were determined in the plasma of three subjects and in the urine of all five subjects at intervals after ingestion. The mean half-life for absorption of 2,4-D was 3.8 h (range, 1.7–4.2 h). The average half-life for clearance from plasma was 11.6 h, and that for urinary elimination was 17.1 h (range, 10.2–28.4 h). About 82% of the 2,4-D was excreted unchanged, and about 13% was excreted as conjugates. Plasma clearance followed first-order kinetics in two subjects, with plasma half-lives of 7.3 and 11 h, and biphasic kinetics in one subject, with half-lives of 4.3 and 16 h. Despite these apparent differences in clearance pharmacokinetics, the overall clearance rates were not markedly different. Urinary excretion followed first-order kinetics in all three subjects (Saurehoff *et al.*, 1977).

The percutaneous absorption and urinary excretion of ^{14}C -2,4-D (purity unspecified) after dermal or intravenous administration were studied in male subjects (age and weight unspecified). ^{14}C -2,4-D (1 μCi ; 4 $\mu\text{g}/\text{cm}^2$) was dissolved in acetone and applied to the volunteers' forearms. The area of application was not covered, but the subjects were asked not to wash the area for 24 h. Within 120 h after dermal administration, $5.8 \pm 2.4\%$ of the dose was excreted in the urine. After intravenous administration of a tracer dose of 1 μCi , 100% was excreted in the urine, with a half-life for excretion of 13 h (Feldman & Maibach, 1974).

The percutaneous absorption of 2,4-D (purity, 99.5%) and the DMA salt was examined in male subjects aged 27–48 years after dermal application in acetone at a rate of 10 mg per 9 cm^2 on the back of the hand. The area of skin was not protected, to reduce the possibility of a covering dislodging the test material; subjects were instructed to avoid contact of the application site with clothing or other materials. In order to determine the amount of 2,4-D that could be removed from the skin, the marked area was rinsed with distilled water and scrubbed with a toothbrush 6 h after the application, and the resulting wash water was analysed. Urine samples collected over 144 h after application were analysed for 2,4-D. An average of 4.5% was recovered in the urine after application of 2,4-D, and 1.8% after application of the DMA salt. More DMA salt (7.6%) than acid (5.4%) was found in the hand wash, indicating that the amount of chemical absorbed is related inversely to the amount washed off. Urinary excretion of neither material was complete: 96 h after application, averages of 85% of the total dose of the acid and 77% of the salt were recovered in the urine; the approximate average half-lives for excretion were 40 h for the acid and 59 h for the DMA salt (Harris & Solomon, 1992).

The dermal absorption of the DMA salt and the EH ester was studied after application to four male volunteers. A dose of 0.7 mCi of ^{14}C -labelled compound, dissolved in water or acetone, was applied to a 45- cm^2 area of the forehead of each volunteer. The site was washed with soap and water 24 h after application. Urine samples were collected 4, 8, and 12 h daily for seven days. Dermal absorption represented 58% of the total dose of DMA salt in water and 6% EH ester in acetone (Moody *et al.*, 1990).

(b) *Biotransformation*

The metabolism of 2,4-D in Fischer 344 rats was investigated by analysing urine samples collected after oral and intravenous administration of ^{14}C -2,4-D at 1 or 100 mg/kg bw. The nature and amounts of the 2,4-D metabolites excreted were determined in urine samples by direct HPLC and/or GC-MS after an extraction step. ^{14}C -2,4-D was eliminated primarily unchanged, representing > 97% of urinary radiolabel, by both males and females. Two minor metabolites were detected in urine from most groups, which accounted for 0.5–3.2% of the radiolabel excreted in the first 12-h urine. Because of the limited amount available, no attempt was made to identify these minor metabolites; but their HPLC elution indicated that they might be 2,4-D conjugates (Timchalk *et al.*, 1990).

2,4-D metabolites were investigated in samples and tissues collected from a lactating goat after oral administration of ^{14}C -2,4-D. Urine was analysed directly by HPLC. The ^{14}C residues in various matrices and liver were extracted with organic and aqueous solvents, then analysed by thin-layer chromatography, HPLC, and MS. 2,4-D was the major ^{14}C component in urine, milk, and various extracts. Some polar conjugates of the parent compound, which were readily hydrolysed to 2,4-D under acidic conditions, were found in milk. A non-polar ^{14}C component detected in milk was identified as 2,4-dichloroanisole. Low levels of 2,4-dichlorophenol were tentatively identified in milk and fat (Guo & Stewart, 1993).

In four of five human subjects given a single oral dose of 2,4-D at 5 mg/kg bw, an acid-hydrolysable conjugate was detected in the urine, representing 4.8–27% of the administered dose (Sauerhoff *et al.*, 1977).

No metabolites were found in the urine of male subjects given a single oral dose of 5 mg/kg bw 2,4-D in gelatin capsules. About 75% of the administered dose was detected unchanged in the urine after 96 h (Kohli *et al.*, 1974).

(c) *Effects on enzymes and other biochemical parameters*

The acute effects of 2,4-D on the activities of lactate dehydrogenase, alkaline phosphatase, aspartate transaminase, alanine transaminase, amylase, creatinine, glucose, total protein, and albumin were investigated in male Wistar rats given a single oral dose of 0.6 g/kg bw, which is close to the lower limit of the LD₅₀ (0.6–1.3 g/kg). The serum levels of lactate dehydrogenase, alkaline phosphatase, and creatinine increased by one- to fourfold 5, 8, and 24 h after treatment, whereas the activities of aspartate and alanine transaminases were higher only at 8 and 24 h. Amylase activity was increased only 8 h after administration of 2,4-D and then returned to normal. In contrast, 2,4-D reduced the serum levels of glucose and total protein 5, 8, and 24 h after treatment and serum albumin levels by 5 h. Thus, an acute dose of 2,4-D disrupts the serum levels of several entities considered to be indicators of tissue injury. The authors speculated that these alterations reflected mainly hepatic and muscular tissue damage, but they suggested that significant pancreatic and kidney toxicity may also have occurred (Paulino & Palermo-Neto, 1991).

The effect of 2,4-D on the biogenesis of liver mitochondrial and peroxisomal proteins was tested in male Fischer 344 rats fed a diet containing 100 ppm (5 mg/kg bw) for 26 weeks. The parameters investigated were somatic index, histochemistry, enzymatic activities in purified peroxisomes and mitochondria, protein (by electrophoresis and immunolabelling), and mRNA hybridization with specific DNA probes. 2,4-D was a peroxisomal proliferator, while mitochondria were weakly affected. The compound modified mitochondrial protein patterns (Cherkaoui Malki *et al.*, 1991).

Metabolic alterations were investigated in hepatocytes from male Wistar rats (200–250 g) treated with 2,4-D at 1–10 mmol/litre. Cell viability was determined by measurement of cytosolic lactate dehydrogenase leakage into the medium. Intracellular glutathione and oxidized glutathione levels were determined and adenine (ATP, ADP and AMP) and pyridine nucleotides (NADH, NAD⁺) were extracted and analysed. 2,4-D was cytotoxic to the hepatocytes, as indicated by increased leakage of lactate dehydrogenase, and it caused dose- and time-dependent cell death accompanied by depletion of intracellular glutathione, mirroring increases in oxidized glutathione. ATP and NADH levels were also rapidly depleted by 2,4-D metabolism in the millimolar range. 2,4-D completely depleted cellular ATP, resulting in cell death. The compound appeared to be hepatotoxic and to initiate the process of cell death by decreasing cellular glutathione. After this primary disturbance, alteration of adenine and pyridine nucleotide content is a critical event in the induction of irreversible cell injury (Palmeira *et al.*, 1994).

The inhibitory activity of several compounds, including 2,4-D, on the Ca²⁺-transport-ATPase of human erythrocyte membranes was determined to ascertain the usefulness of this screening test for characterizing cellular toxicity. Some compounds, particularly those with lipophilic properties, are incorporated into membranes, where they disintegrate the structure. Since the Ca²⁺-transport-ATPase of human erythrocytes is a membrane-bound enzyme, incorporation of such compounds into membranes impairs membrane function and results in the reduction of enzyme activity. Although several other compounds were successful in inhibiting the enzyme, 2,4-D did not do so, probably because it is negatively charged under physiological conditions (pH 7.4 or 7.0 in this assay) and was therefore unable to penetrate biological membranes (Janik & Wolf, 1992).

2. Toxicological studies

(a) Acute toxicity

The acute toxicity of 2,4-D, the DEA, DMA, IPA, and TIPA salts, and the BEH and EH esters are summarized in Table 1. After their oral administration, the clinical signs of toxicity observed consistently were ataxia, myotonia, and decreased limb tone. No dermal or systemic toxicity was seen in rabbits treated dermally, and no deaths were seen after inhalation. Clinical signs of toxicity seen during exposure were decreased activity and closed eyes; all rats had dried white material on their fur (presumably test material). Signs seen at the end of and during the week after exposure were salivation, lacrimation, mucoid nasal discharge, laboured breathing, dried red or brown material around the eyes and nose, matted fur, and staining of the fur in the anogenital region. None of these signs was seen within three to seven days of treatment. There was no significant finding *post mortem* (Myer, 1981a,b,c,d,e,f; Carreon *et al.*, 1983; Squibb *et al.*, 1983; Streeter & Young, 1983; Auletta & Daly, 1986; Jeffrey *et al.*, 1987a,b; Streeter *et al.*, 1987; Schults *et al.*, 1990a,b; Cieszlak, 1992).

(b) Short-term toxicity

Mice

Groups of 20 male and 20 female B6C3F1 mice were fed diets designed to provide technical-grade 2,4-D (purity, 96.1%) at doses of 0, 5, 15, 45, or 90 mg/kg bw per day for 13 weeks. No treatment-related effects were seen on survival, clinical signs, or body weight, or by ophthalmology, haematology, or

Table 1. Acute toxicity of 2,4-D, amine salts, and esters in male and female animals

Compound	Species	Route	LD ₅₀ (mg/kg bw) or LC ₅₀ (mg/litre)	Reference
2,4-D	Rat	Oral	699	Myer (1981a)
2,4-D	Rat	Oral	443	Squibb <i>et al.</i> (1983)
DEA salt	Rat	Oral	910	Schults <i>et al.</i> (1990a)
DMA salt	Rat	Oral	949	Myer (1989b)
IPA salt	Rat	Oral	2322 (m) 1646 (f)	Carreon <i>et al.</i> (1983)
TIPA salt	Rat	Oral	1220 (m) 1074 (f)	Berdasco <i>et al.</i> (1989a)
BEH ester	Rat	Oral	866	Jeffrey <i>et al.</i> (1987a)
EH ester	Rat	Oral	896	Myer (1981c)
2,4-D	Rabbit	Dermal	> 2000	Myer (1981d)
DEA salt	Rabbit	Dermal	> 2000	Schults <i>et al.</i> (1990b)
DMA salt	Rabbit	Dermal	> 20000	Myer (1981e)
IPA salt	Rabbit	Dermal	> 2000	Carreon <i>et al.</i> (1983)
TIPA salt	Rabbit	Dermal	> 2000	Berdasco <i>et al.</i> (1989b)
BEH ester	Rabbit	Dermal	> 2000	Jeffrey <i>et al.</i> (1987b)
EH ester	Rabbit	Dermal	> 2000	Myer (1981f)
2,4-D	Rat	Inhalation	> 1.8	Auletta & Daly (1986)
DEA salt	Rat	Inhalation	> 3.5	Jackson & Hardy (1990)
DMA salt	Rat	Inhalation	> 3.5	Streeter <i>et al.</i> (1990)
IPA salt	Rat	Inhalation	> 3.2	Streeter <i>et al.</i> (1983)
TIPA salt	Rat	Inhalation	> 0.84	Nitschke & Lomax (1990)
BEH ester	Rat	Inhalation	4.6	Streeter <i>et al.</i> (1987)
EH ester	Rat	Inhalation	> 5.4	Cieszlak (1992)

DEA, diethanolamine; DMA, dimethylamine; IPA, isopropanolamine; TIPA, triisopropylamine; BEH, butoxyethylhexyl; EH, 2-ethylhexyl

gross pathology. Increases in the weights of the pituitary and adrenals in animals of each sex and the kidneys of females were observed, the latter effect being correlated with histopathological lesions in that organ. Treatment-related histopathological alterations were seen in the kidneys of 3, 9, 18, and 20 males and 1, 4, 6, 12, and 14 females at 0, 5, 15, 45, and 90 mg/kg bw per day, respectively. The effects were characterized by increased homogeneity and altered tinctorial properties of the cytoplasm and decreased intracellular and intraluminal vacuolization in the renal cortex of the males, and increased homogeneity and altered tinctorial properties of the cytoplasm with or without cytoplasmic swelling in the renal cortex of the females. Since renal lesions occurred in a dose-related manner, including the lowest dose tested (5 mg/kg bw per day), there was no NOAEL (Serota, 1983a).

Groups of 10 male and 10 female B6C3F1 mice were fed diets containing technical-grade 2,4-D (purity, 96.1%) at 0, 1, 15, 100, or 300 mg/kg bw per day for 13 weeks. Treatment at 1, 15, or 100 mg/kg bw per day had no adverse effect on survival, body weight, body-weight gain, or food consumption, or on ophthalmological, haematological, clinical chemical, or gross pathological parameters. Treatment-related changes at 100 mg/kg bw per day included significant ($p \leq 0.05$) decreases in glucose and thyroxine levels in females and males, increased mean absolute and relative kidney weights in females and a liver lesion in one female. Treatment-related changes at 300 mg/kg bw per day included transient decreases in food consumption (only up to week 7); decreases in glucose and thyroxine levels in females and males, respectively; a significant decrease in the kidney:brain weight ratio in males; histopathological lesions in the kidneys of males characterized as karyomegaly, loss of brush border, and decreased size of tubular lining cells; and histopathological lesions in the livers of animals of each sex, characterized as nuclear hyperchromatism, and decreased glycogen in periportal hepatocytes. The NOAEL was 15 mg/kg bw per day on the basis of renal toxicity (Schulze, 1991a).

Rats

Groups of 20 male and 20 female Fischer 344 rats were fed diets designed to provide technical-grade 2,4-D (purity, 97.5%) at doses of 0, 1, 5, 15, or 45 mg/kg bw per day for 13 weeks. No treatment-related effects were seen on survival, clinical signs, body weights, food consumption, ophthalmoscopic findings, or haematological parameters. Clinical chemistry indicated decreases in alanine and aspartate transaminase and alkaline phosphatase activities and blood urea nitrogen in animals of each sex at 15 and 45 mg/kg bw per day and increased thyroxine values in males at 5 and 15 mg/kg bw per day. No treatment-related gross pathological changes were seen. Both the absolute and relative kidney weights were significantly ($p \leq 0.05$) increased in animals of each sex at 45 mg/kg bw per day. The absolute and relative thyroid weights were significantly increased in males at all doses and in females at 5, 15, and 45 mg/kg bw per day; however, there were no corroborative thyroid lesions. Histopathological examination revealed renal lesions in animals of each sex at 5, 15, and 45 mg/kg bw per day, characterized by increased homogeneity, altered tinctorial properties, and fine vacuolization of the cytoplasm in the renal cortex. The lesions were generally diffuse, most frequent, and more severe at the high dose, with a multifocal, less severe pattern at the lower doses. Renal lesions were seen in only one female at 1 mg/kg bw per day; none of the males at this dose exhibited this lesion. The NOAEL was 1 mg/kg bw per day on the basis of renal toxicity (Serota, 1983b).

Groups of 20 male and 20 female Fischer 344 rats received technical-grade 2,4-D (purity, 96.1%) in the diet to provide doses of 0, 1, 15, 100, or 300 mg/kg bw per day, for 13 weeks. Treatment at 1 or 15 mg/kg bw per day caused no adverse effects, but toxicity was seen in animals of each sex at 100 and 300 mg/kg bw per day. At termination, significant ($p < 0.05$) reductions in body-weight gain were observed in males at 100 and 300 mg/kg bw per day and in females at 300 mg/kg bw per day. Rats of

each sex at 100 and 300 mg/kg bw per day had alterations in some haematological and clinical chemical parameters and changes in organ weights. Histopathological lesions in the liver, adrenals, and kidneys were seen at 100 mg/kg bw per day and in the eye, liver, testis, adrenals, kidneys, thymus, bone marrow, spleen, thyroid, and lungs at 300 mg/kg bw per day. In some instances, the histopathological changes correlated well with the alterations seen on haematology, clinical chemistry, and organ weight measurement. Increases in liver weight and in alanine and aspartate transaminase activity were associated with centrilobular hepatocellular hypertrophy in animals of each sex at 100 and 300 mg/kg bw per day. The decreased thyroxine levels were correlated with follicular-cell hypertrophy of the thyroid gland in females at 300 mg/kg bw per day. An increase in adrenal weight may have been correlated with hypertrophy of cells of the zona glomerulosa of the adrenal glands in animals of each sex at 100 and 300 mg/kg bw per day. The decreased mean thymic weight may have been correlated with the atrophy seen in males and females at 300 mg/kg bw per day. Atrophy of mesenteric adipose tissue in the peritoneal cavity may have been correlated with the mean decreases in body weight seen in animals of each sex at 300 mg/kg bw per day. Additional treatment-related histological lesions seen were bilateral cataracts in females at 300 mg/kg bw per day, brush border loss in proximal tubular cells of the kidneys in animals of each sex at 100 and 300 mg/kg bw per day, and alveolar macrophage accumulation and hypocellularity of the bone marrow in animals of each sex at 300 mg/kg bw per day. The NOAEL was 15 mg/kg bw per day, and the doses selected for the two-year study of toxicity and carcinogenicity were 0, 5, 75, and 150 mg/kg bw per day (Schultze, 1991b).

The toxicity of purified 2,4-D was evaluated in groups of 15 Fischer 344 rats of each sex, which received diets designed to provide doses of 0, 15, 60, 100, or 150 mg/kg bw per day for 13 weeks. The toxicity of the purified 2,4-D was similar to that of technical-grade 2,4-D. At 15 mg/kg bw per day, a minimal increase in epithelial vacuolization of the renal convoluted tubules was seen in females but not in males. Treatment at 60 mg/kg bw per day induced a slight reduction in body-weight gain in females, a decrease in thyroxine levels in females, increases in absolute kidney and relative liver weights in females, and renal lesions in males and females. Treatment at 100 or 150 mg/kg bw per day decreased body-weight gains, altered alanine and aspartate transaminase and alkaline phosphatase activities and thyroxine levels, increased absolute and/or relative kidney weights, increased relative liver weights, and induced histopathological lesions in the kidneys (renal tubular changes) and liver (hepatocellular cytoplasmic swelling and homogeneity of the liver cells) of animals of each sex. The NOAEL was 15 mg/kg bw per day (Gorzinski *et al.*, 1981).

Groups of 10 male and 10 female Fischer 344 rats were fed diets containing the DEA salt of 2,4-D (purity, 73.8%) at doses of 0, 1.5, 27, 150, and 440 mg/kg bw per day (equivalent to 0, 1, 18, 100, or 300 mg/kg bw of the acid per day) for 13 weeks. The DEA salt had no adverse effects at 1.5 or 27 mg/kg bw per day. Treatment-related effects observed at higher doses included mortality; decreases in mean body-weight gain and mean food consumption; alterations in some haematological and clinical chemical parameters; changes in organ weights; gross pathological changes; and histopathological changes in the bone marrow, eyes, kidneys, liver, lungs, lymphoid tissues, stomach, thyroid, thymus, testes, epididymides, seminal vesicles, prostate, ovaries, and uterus. Histopathological alterations at the two highest doses included diffuse regeneration of the renal tubular epithelium of the kidneys; hypertrophy and necrosis of individual centrilobular hepatocytes; bilateral retinal degeneration; foamy macrophage accumulation in the lungs; follicular-cell hypertrophy in the thyroid glands accompanied by decreased colloid; necrosis and regeneration of the epithelium with submucosal oedema in the stomach; decreased cellularity in the sternal bone marrow; lymphoid atrophy of the spleen and cervical lymph node; atrophy of the thymus; degeneration of seminiferous epithelium in the testes; decreased spermatozoa in the epididymides; decreased secretory content in the prostate and seminal vesicles; and

atrophy of the ovaries and uterus. The NOAEL was 27 mg/kg bw per day, equivalent to 18 mg/kg bw per day of acid (Serrone *et al.*, 1991).

Groups of 10 male and 10 female Fischer 344 rats received diets containing the DMA salt of 2,4-D (purity, 66.2%) at doses of 0, 1.2, 18, 120, or 360 mg/kg bw per day (equivalent to 0, 1, 15, 100, or 300 mg/kg bw per day of the acid) for 13 weeks. No adverse effects were seen at 1.2 or 18 mg/kg bw per day. There were decreases in mean body weight, body-weight gain, and food consumption and alterations in some haematological and clinical chemical parameters at 120 mg/kg bw per day. The highest dose was associated with decreases in body-weight gain, reduced food consumption, alterations in haematological and clinical chemical parameters, changes in various organ weights, and histopathological changes consisting of bilateral retinal degeneration and cataract formation, centrilobular hepatocellular hypertrophy, and hypoplasia of the spleen in females, atrophy of the testes in males, and hypertrophy of thyroid follicular cells, brush border loss in proximal tubular cells in the kidney, and hypoplasia of the bone marrow in males and females. The NOAEL was 18 mg/kg bw per day, equivalent to 15 mg/kg bw per day of the acid (Schultze, 1991c).

Groups of 10 male and 10 female Fischer 344 rats were fed diets containing the IPA salt of 2,4-D (purity, 35.6%) at 0, 1, 19, 130, or 380 mg/kg bw per day (equivalent to 0, 1, 15, 100, or 300 mg/kg bw per day of the acid) for 13 weeks. The IPA salt at 1 or 19 mg/kg bw per day had no adverse effects. The treatment-related effects seen at 130 mg/kg bw per day were decreased mean body weight, body-weight gain, and food consumption in animals of each sex; minor alterations in haematological, clinical chemical, and urinary parameters in animals of each sex; increased relative kidney weights in males and females; and histopathological changes in the liver and kidneys of males and the kidneys, adrenals, and thyroid glands of females. Primary treatment-related effects observed at 380 mg/kg bw per day included decreases in mean body weight, body-weight gain, and food consumption in animals of each sex; alterations in haematological parameters in females and in clinical chemical and urinary parameters in animals of each sex; changes in organ weights; gross pathological changes; and histopathological lesions in the eyes, kidneys, liver, and thyroids of animals of each sex. Effects secondary to decreased weight gain, the debilitated condition of the rats, and/or toxicity in other organs occurred in the adrenals, bone marrow, mesenteric fat, lungs, spleen, thymus, and testes. The NOAEL was 19 mg/kg bw per day, equivalent to 15 mg/kg bw per day of the acid (Yano *et al.*, 1991a).

Groups of 10 male and 10 female Fischer 344 rats received diets designed to provide the TIPA salt of 2,4-D (purity, 72.2%) at doses of 0, 2, 28, 190, or 560 mg/kg bw per day (equivalent to 0, 1, 15, 100, or 300 mg/kg bw per day of the acid) for 13 weeks. The TIPA salt had no adverse effects at 2 or 28 mg/kg bw per day. The treatment-related effects seen at 190 mg/kg bw per day were decreased body-weight gain in females; minor alterations in haematological, clinical chemical, and urinary parameters in animals of each sex; and histopathological changes in the liver and kidneys of males and in the kidneys and adrenal glands of females. Treatment-related effects seen in animals of each sex at 560 mg/kg bw per day were decreased mean body weight, body-weight gain, and food consumption, alterations in haematological, clinical chemical, and urinary parameters, changes in organ weights, gross pathological changes, and histopathological lesions in the eyes, kidneys, liver, and thyroids. The NOAEL was 28 mg/kg bw per day, equivalent to 15 mg/kg bw per day of the acid (Yano *et al.*, 1991b).

Groups of 10 male and 10 female Fischer 344 rats received the BEH ester of 2,4-D (purity, 94.6%) in their diet to provide doses of 0, 1.5, 22, 140, or 440 mg/kg bw per day (equivalent to 0, 1, 15, 100, or 300 mg/kg bw per day of the acid) for 13 weeks. No adverse effects were seen at 1.5 or 22 mg/kg bw per day. Treatment at 140 mg/kg bw per day decreased mean body weight, body-weight gain, and food consumption; caused alterations in some haematological and clinical chemical parameters;

changed thyroid hormone concentrations; and induced histopathological lesions in the thyroids. The high dose was associated with decreased body-weight gain, reduced food consumption, alterations in haematological and clinical chemical parameters, changes in various organ weights, changes in thyroid hormone concentrations, and histopathological lesions in the eye, liver, kidneys, and thyroids. The NOAEL was 22 mg/kg bw per day, equivalent to 15 mg/kg bw per day of 2,4-D (Szabo & Rachunek, 1991).

Groups of 10 male and 10 female Fischer 344 rats were fed diets designed to provide the EH ester of 2,4-D (purity, 98%) at 0, 1.5, 23, 150, or 450 mg/kg bw per day (equivalent to 0, 1, 15, 100, or 300 mg/kg bw per day of the acid) for 13 weeks. The EH ester had no adverse effects at 1.5 or 23 mg/kg bw per day. Treatment at 150 mg/kg bw per day decreased mean body weight, body-weight gain, and food consumption and induced alterations in some haematological and clinical chemical parameters. The high dose was associated with decreased body-weight gain and food consumption, alterations in haematological and clinical chemical parameters, changes in various organ weights, and histopathological lesions which included bilateral retinal degeneration and cataract formation in females; lymphoid hypoplasia of the thymus in females and of the spleen in animals of each sex; centrilobular hepatocellular hypertrophy; hypoplasia of the bone marrow; hypertrophy of thyroid follicular cells; atrophy of the testes; vacuolization of the tubular cells of the kidney; and brush border loss in proximal tubular cells in the kidneys of females. The NOAEL was 23 mg/kg bw per day, equivalent to 15 mg/kg bw per day of the acid (Schultze, 1991d).

Dogs

Groups of five male and five female beagle dogs were given gelatin capsules containing 2,4-D (purity, 96.1%) at doses of 0, 0.3, 1, 3, or 10 mg/kg bw per day for 13 weeks. No treatment-related effects were observed at 0.3 or 1 mg/kg bw per day. At 3 mg/kg bw per day 2,4-D caused significant ($p \leq 0.05$) increases in blood urea nitrogen and creatinine levels and renal lesions characterized as cellular alterations in the proximal convoluted tubules in three male dogs. Treatment-related changes at 10 mg/kg bw per day included morbidity in two males and one female; clinical signs of toxicity (thin and languid appearance, anorexia, emesis, and swollen testes); decreased mean body weights (-8% in males and -14% in females) and body-weight gains (-50% in males and -83% in females); alterations in haematological (decreased haemoglobin, haematocrit, and platelet counts) and clinical chemical (increased blood urea nitrogen and creatinine levels) parameters in animals of each sex; decreased absolute testicular weights; increased relative kidney weights in females; and renal lesions in animals of each sex. The renal lesions were characterized as cellular alterations in the proximal convoluted tubules in three of three males and one of four females. The NOAEL was 1 mg/kg bw per day (Schultze, 1990a).

Groups of four male and four female beagle dogs received 2,4-D (purity, 96.7%) in their diet to provide doses of 0, 0.5, 1, 3.8, or 7.5 mg/kg bw per day for 13 weeks. No treatment-related effects were observed at 0.5 or 1 mg/kg bw per day. No mortality, clinical signs of toxicity, ophthalmological changes, alterations in haematological or urinary parameters, gross pathological changes, or changes in organ weights were seen at 3.8 or 7.5 mg/kg bw per day. Body-weight gains were decreased in males (-50%) and females (-47%) at 3.8 mg/kg bw per day and in males (-39%) and females (-42%) at 7.5 mg/kg bw per day. Food consumption was decreased (by about 15%) in animals of each sex at 3.8 and 7.5 mg/kg bw per day. Clinical chemical analyses showed significant ($p \leq 0.05$) increases in blood urea nitrogen, creatinine, and alanine transaminase levels at four- and 13-week intervals in animals at 3.8 and 7.5 mg/kg bw per day. The toxicological significance of these increases is unknown as there were no alterations in organ weights or corroborative histopathological renal lesions. Except for a

moderate, chronic, active perivascular inflammation of the liver in one male and one female at 7.5 mg/kg bw per day, no treatment-related histopathological lesions were seen. There was no correlation between the severity of liver lesions and the increased **alanine** transaminase activity. The NOAEL was 1 mg/kg bw per day on the basis of the effects on body weight (Dalgard, 1993a; Charles et al., 1996).

Groups of four male and four female beagle dogs were fed diets containing the DMA salt of 2,4-D (purity, 55.45%) at doses equivalent to 0, 1, 3.8, or 7.5 mg/kg bw of the acid per day for 13 weeks. The DMA salt had effects similar to those of the acid. No treatment-related effects were observed on survival, clinical signs of toxicity, ophthalmological, haematological, or urinary parameters, gross pathological appearance, organ weights, or histopathological appearance in animals of either sex. At 3.8 mg/kg bw per day, there was a nonsignificant decrease in body-weight gain (-40%) in females and statistically significant ($p \leq 0.05$) increases in blood urea nitrogen, **alanine** transaminase, and creatinine levels in animals of each sex at four- and 13-week intervals. The toxicological significance of these increases is unclear as no corroborative changes in organ weights or histopathology were seen at this dose. The treatment-related changes seen at 7.5 mg/kg bw per day included decreased body-weight gains in males (-31%) and females (-67%; $p \leq 0.05$); reduced food consumption (by about 15%); significant ($p \leq 0.05$) increases in blood urea nitrogen, creatinine, and **alanine** transaminase levels at four- and 13-week intervals in animals of each sex; and decreases in absolute (-42%) and relative (-38%) testicular weights. The toxicological significance of these increases is unknown as no alterations in organ weights or corroborative histopathological lesions were seen in the kidneys or testes. A minimal increase in the average severity of perivascular, chronic, active inflammation in the liver was seen in two males and one female; however, there was no correlation between the severity of liver lesions and the increase in **alanine** transaminase activity. The NOAEL was 1 mg/kg bw per day on the basis of the decreases in body-weight gain (Dalgard, 1993b; Charles et al., 1996).

Groups of four male and four female beagle dogs received diets containing the EH ester of 2,4-D (purity, 62.7%) at doses equivalent to 0, 1, 3.8, or 7.5 mg/kg bw of the acid per day for 13 weeks. The effects were similar to those seen with the acid and the DMA salt. No treatment-related effects were observed at 1 mg/kg bw per day, and no mortality, clinical signs of toxicity, ophthalmological changes, alterations in haematological or urinary parameters, gross pathological changes, altered organ weights, or histopathological alterations were seen at 3.8 or 7.5 mg/kg bw per day. Body-weight gains were decreased in males (-48%) and females (-62%) at 3.8 mg/kg bw per day and in males (-85%) and females (-50%) at 7.5 mg/kg bw per day. Food consumption was decreased (by about 15%) in animals of each sex at 3.8 and 7.5 mg/kg bw per day. Significant ($p \leq 0.05$) increases in blood urea nitrogen, creatinine, and **alanine** transaminase levels were seen at four- and 13-week intervals in animals of each sex at 3.8 and 7.5 mg/kg bw per day. The toxicological significance of these increases is unknown as no alterations in organ weights or corroborative lesions were seen in the kidneys. Liver lesions, characterized as perivascular, chronic, active inflammation, were seen in two male dogs; no liver lesions were seen in females. There was no correlation between the severity of the liver lesions and the increased **alanine** transaminase activity. The NOAEL was 1 mg/kg bw per day on the basis of the effects on body weight (Dalgard, 1993c; Charles et al., 1996).

(c) *Long-term toxicity and carcinogenicity*

Mice

Groups of 50 male and 50 female B6C3F1 mice were fed diets designed to provide 2,4-D (purity, 97.5%) at 0, 1, 15, or 45 mg/kg bw per day for two years. Ten mice of each sex at each dose were killed at 12 months. Survival, body weight, food consumption, and clinical signs were noted; haematological

parameters were evaluated at 12, 18, and 24 months and organ weights and histopathological changes at 12 and 24 months. There were no treatment-related effects on survival, clinical signs, body weights, haematological or urinary parameters, or gross pathology. An increased relative kidney weight was seen in females at 15 mg/kg bw per day, increased absolute kidney weight in males at 45 mg/kg bw per day, and increased relative kidney weight in animals of each sex at 45 mg/kg bw per day. Histopathology revealed treatment-related kidney lesions only in male mice at 15 and 45 mg/kg bw per day. Renal lesions, characterized as cytoplasmic homogeneity of the renal tubule epithelium, were seen in 11/60 control males (18%), 15/60 at 1 mg/kg bw per day (25%), 48/60 at 15 mg/kg bw per day (80%, $p < 0.0001$), and 58/59 at 45 mg/kg bw per day (98%, $p < 0.0001$). This change was associated with a reduction in the cytoplasmic vacuoles that are normally present in the renal tubular epithelium. No evidence of carcinogenicity was seen; the tumour types and incidence were similar in the treated and control groups. The NOAEL was 1 mg/kg bw per day on the basis of the increase in kidney weights and renal lesions (Serota, 1987).

2,4-D (purity, 96.4%) was administered in the diet of groups of 50 male B6C3F1 mice at doses of 0, 5, 62, or 120 mg/kg bw per day and to groups of 50 female B6C3F1 mice at doses of 0, 5, 150, or 300 mg/kg bw per day for two years. Ten mice of each sex at each dose were killed at 12 months. Survival, body weight, food consumption, and clinical signs were noted; haematological parameters were evaluated at 12, 18, and 24 months and organ weights and histopathological changes at 12 and 24 months. No treatment-related effects were seen on survival, body-weight gain, clinical signs, haematological parameters, or gross pathological appearance in males at any dose. Except for a transient decrease in body-weight gain (–14% at three months but comparable to that of controls at termination) in females at 300 mg/kg bw per day, treatment did not affect survival, induce clinical signs, alter haematological parameters, or cause gross pathological changes in females at any dose. Treatment-related changes in organ weights were limited to the kidney. Dose-related increases in absolute (5 and 7%) and relative (6 and 10%) weights were seen in males at 62 and 120 mg/kg bw per day, respectively, only at 24 months. In females at 150 and 300 mg/kg bw per day, absolute kidney weights were increased by 14 and 17% and relative weights by 22 and 30% at the two doses, respectively, at 12 months; at 24 months, the absolute weights were increased by 14 and 22% and the relative weights by 12 and 20%, respectively.

Histopathological examination revealed dose-related renal lesions in males at 62 and 120 mg/kg bw per day, comprised of a constellation of changes that involved five different diagnoses. Degeneration with regeneration of the descending limb of the proximal tubule was seen in 25/50 (50%) and 48/50 (96%) animals at 62 and 120 mg/kg bw per day, respectively, but not in the controls or in animals at 5 mg/kg bw per day. Decreased vacuolization of the renal proximal tubule was seen in 39/50 (78%) and 48/50 (96%) animals at 62 and 120 mg/kg bw per day, respectively. Both of these lesions were also seen in a dose-related manner at the interim sacrifice. Mineralization of the tubules occurred in 29/50 (58%) and 36/50 (72%) animals and multifocal cortical cysts in 22/50 (44%) and 20/50 (40%) rats at 62 and 120 mg/kg bw per day, respectively. In females at 150 and 300 mg/kg bw per day, the renal lesions were characterized by hypercellularity in 32/50 (64%) and 25/50 (50%) animals and degeneration with regeneration of the tubules in 38/50 (76%) and 34/50 (68%), respectively. A variety of benign and malignant tumours at different sites was seen in both the control and treated mice but were similar in number and type to those commonly seen in this strain and age of mice. The NOAEL was 5 mg/kg bw per day in animals of each sex on the basis of the renal lesions (Sott *et al.*, 1995).

Rats

Groups of 25 male and 25 female Osborne-Mendel rats were given diets containing 2,4-D to provide doses of 0, 5, 125, 625, or 1250 ppm (equal to 0, 0.25, 6.2, 31, and 62 mg/kg bw per day) for two years.

Treatment had no adverse effect on survival, clinical signs, body weight, organ weights, or haematological parameters. Slight hepatitis was seen in one rat each at 5 and 25 ppm and three rats each at 625 and 1250 ppm, with none in the controls. The authors reported that 15, 14, 18, 20, 23, and 22 rats were still alive after two years, but the numbers were not given by sex. The total numbers of males with malignant tumours were 1, 2, 4, 2, 5, and 6, and the numbers of females were 5, 6, 3, 5, 3, and 8, respectively, at 0, 5, 25, 125, 625, and 1250 ppm. The NOAEL was 625 ppm, equal to 31 mg/kg bw per day (Hansen *et al.*, 1971). Two working groups convened by the IARC considered this study to be inadequate for assessing carcinogenicity (IARC, 1977, 1982).

Groups of 50 male and 50 female Fischer 344 rats were fed diets containing technical-grade 2,4-D (purity, 97.5%) at doses of 0, 1, 5, 15, or 45 mg/kg bw per day for two years. Haematological, clinical chemical, and urinary parameters were evaluated before treatment and after 2, 52, and 78 weeks of treatment. Necropsies were conducted on 10 rats of each sex at each dose after 52 weeks and on all surviving animals after two years. No treatment-related effects were seen on survival, clinical signs, or gross pathological appearance. Body weight gain was significantly ($p \leq 0.05$) decreased in females at 45 mg/kg bw per day at 12 months (-7%) and at 24 months (-9%); no adverse effects were seen in females at the lower doses or in males at any dose. Food consumption was decreased (-2.4%) in females at 45 mg/kg bw per day. No treatment-related effects were seen on haematological parameters. Clinical chemistry revealed a significant ($p \leq 0.05$) increase in alanine transaminase activity in males (50%) and females (43%) and a decrease in thyroxine level in females (-18%) at 45 mg/kg bw per day at termination. No treatment-related effects were seen on urinary parameters. Males at 45 mg/kg bw per day had significant ($p \leq 0.05$) increases in the absolute (9%) and relative (13%) weights of the kidneys after 52 weeks, while females at this dose had significant increases in absolute (10%) and relative (16%) kidney weights after 104 weeks. Thyroid weights were significantly increased at termination in males (absolute, 22%; relative, 26%) and females (absolute, 24%; relative, 26%) at 15 mg/kg bw per day and in males (absolute, 26%; relative, 29%) at 45 mg/kg bw per day; females at 45 mg/kg bw per day also showed increases in absolute (2%) and relative (16%) thyroid weights, but the increases were not statistically significant. Histopathological examination revealed renal lesions in males and females at 5, 15, and 45 mg/kg bw per day, including an increased frequency of a brown tubular epithelial-cell pigment, pelvic microcalculi, and transitional epithelial-cell hyperplasia secondary to microcalculi. Brown tubular-cell pigment was seen in 2/50 (4%), 1/50 (2%), 9/50 (18%), 18/50 (36%), and 19/50 (38%) males and 8/50 (16%), 10/50 (20%), 23/50 (46%), 20/50 (40%), and 15/50 (30%) females at 0, 1, 5, 15, and 45 mg/kg bw per day, respectively. The increases reached statistical significance ($p \leq 0.05$) at doses ≥ 5 mg/kg bw per day. Increased incidences of pelvic microcalculi were seen in 2/50 (4%), 2/50 (4%), 4/50 (8%), 8/50 (16%), and 11/50 (22%) males and in 19/50 (38%), 11/50 (22%), 15/50 (30%), 23/50 (46%), and 35/50 (70%) females at 0, 1, 5, 15, and 45 mg/kg bw per day, respectively. A slight increase in transitional epithelial cell hyperplasia was seen in females at 45 mg/kg bw per day (11/50; 22%) in comparison with controls (0%).

No treatment-related neoplastic lesions were seen at any dose. There was an increase in the incidence of brain astrocytomas in male rats, with 1/50 (2%), 0/50, 0/50, 2/48 (4%), and 6/50 (12%) seen in the controls and in rats at 1, 5, 15, and 45 mg/kg bw per day, respectively. Although there was a positive trend ($p = 0.002$), a pairwise test did not show statistical significance ($p = 0.055$) when the incidence at the high dose (6/60) was compared with that of the controls (1/50). The brain astrocytomas are not attributable to treatment because they did not occur earlier in treated rats than in controls (no decreased latency); there were no preneoplastic lesions such as gliosis in treated rats, and all the tumours were solitary; the tumours in the treated rats were no larger or more anaplastic than generally seen in control rats (the largest and most lethal tumour was seen in a control rat); and the tumours were seen only in animals of one sex. In another study in Fischer 344 rats (Jeffris *et al.*, 1995; discussed below),

no brain tumours or any evidence of carcinogenicity was seen in the same strain of rats treated at more than three times the dose (175 mg/kg bw per day) that was tested in this study. The NOAEL was 1 mg/kg bw per day on the basis of the histopathological lesions seen **in the kidneys of animals of each sex (Serota, 1986).**

Groups of 50 male and 50 female Fischer 344 rats were fed diets designed to provide 2,4-D (purity, 96.4%) at doses of 0, 5, 75, or 150 mg/kg bw per day for up to two years. Ten animals of each sex at each dose were killed at 12 months. Survival, body weight, food consumption, and clinical signs were noted; haematological parameters were evaluated at 12, 18, and 24 months and organ weights and histopathological changes at 12 and 24 months. Treatment had no adverse effect on survival, and there were no treatment-related clinical signs of toxicity. At termination, the body weights were lower than those of the respective controls for females at 75 mg/kg bw per day (-14%) and males (-8%) and females (-26%) at 150 mg/kg bw per day. The body-weight gains were also lower than those of the respective controls for females at 75 mg/kg bw per day (-24%) and males (-17%) and females (-48%) at 150 mg/kg bw per day. A concomitant decrease in mean food consumption occurred in females at 75 mg/kg bw per day (-4%) and in males (-5%) and females (-12%) at 150 mg/kg bw per day. Statistically significant ($p \leq 0.05$) increases in the plasma levels of alanine and aspartate transaminases, alkaline phosphatase, and/or cholesterol were seen in females at 75 mg/kg bw per day and in males and females at 150 mg/kg bw per day at various times. These increases may be due to treatment as hepatic lesions were observed at the interim sacrifice in females at 75 mg/kg bw per day and at terminal sacrifice in males and females at 150 mg/kg bw per day. It should be noted, however, that the hepatic lesions were limited to altered tinctorial properties involving all hepatocytes within the hepatic nodules and were not associated with hepatocellular degeneration or necrosis. Thyroxine levels were decreased at 6, 12, and 24 months in males and females at 75 and 150 mg/kg bw per day. Increases in absolute and relative thyroid weights were seen, however, only in females at 75 mg/kg bw per day and in animals of each sex at 150 mg/kg bw per day at the interim and terminal sacrifices. Histopathological lesions of the thyroid glands were seen only in females at 150 mg/kg bw per day at the interim sacrifice. Gross pathological examination revealed opacity of the lens and a general decrease in fat in females and pale foci in the lungs of animals of each sex at 150 mg/kg bw per day. The only treatment-related effects on organ weights were the increases in thyroid weight.

After 12 months of treatment, the non-neoplastic lesions seen were decreased haematopoiesis in the bone marrow of females at 150 mg/kg bw per day; altered tinctorial properties in the livers of females at 75 mg/kg bw per day and in animals of each sex at 150 mg/kg bw per day; bilateral retinal degeneration in females at 150 mg/kg bw per day; multifocal alveolar histiocytosis of the lungs in females at 75 mg/kg bw per day and animals of each sex at 150 mg/kg bw per day; degeneration of the descending portion of the proximal convoluted tubules of the kidneys in animals of each sex at 75 and 150 mg/kg bw per day; atrophy of the adipose tissue in females at 75 and 150 mg/kg bw per day; atrophy of the testes at 150 mg/kg bw per day; and decreased secretory material in the thyroid follicles of females at 150 mg/kg bw per day.

After 24 months of treatment, the non-neoplastic lesions were limited to the eyes, liver, lung, and mesenteric fat. The eye lesions were characterized as slight to severe bilateral retinal degeneration and lenticular cataracts in animals of each sex at 150 mg/kg bw per day. Liver lesions manifested as enlarged hepatocytes, often accompanied by altered tinctorial properties that involved all hepatocytes within the hepatic lobule of animals of each sex at 150 mg/kg bw per day. Lesions of the respiratory system included subacute to chronic inflammation of the lungs in females at 75 mg/kg bw per day and animals of each sex at 150 mg/kg bw per day. Atrophy of the adipose tissue was increased in animals of each sex at 150 mg/kg bw per day. It should be noted that the lesions seen in the spleen, kidneys, testes, and thyroid glands of rats killed at 12 months were not seen in those killed at 24 months. A variety of benign

and malignant tumours at different sites was seen in both the control and treated mice, but they were similar in number and type to those commonly seen in this strain and age of rats. The NOAEL was 75 mg/kg bw per day in males and 5 mg/kg bw per day in females on the basis of the decreases in body weights, body-weight gain, and food consumption, increases in liver enzymes, decrease in thyroxine concentration, increases in absolute and relative thyroid weights, and histopathological lesions (Jeffries *et al.*, 1995).

Dogs

Groups of three beagle dogs of each sex were fed diets containing 2,4-D at concentrations providing doses of 0, 10, 50, 100, or 500 ppm (equal to 0, 0.25, 1.2, 2.5, or 12 mg/kg bw per day) for two years. No gross or microscopic lesions were seen in any major organ (Hansen *et al.*, 1971).

Groups of five beagle dogs of each sex were fed diets containing 2,4-D (purity, 96.5%) at doses of 0, 1, 5, or 7.5 mg/kg bw per day for 52 weeks. No treatment-related effects were seen on survival, clinical signs, ophthalmological, haematological, or urinary parameters, organ weights, or gross pathological appearance at any dose. The body-weight gains of dogs at 1 mg/kg bw per day were comparable to those of the controls; the weight gains of animals of each sex at 5 and 7.5 mg/kg bw per day were decreased, the effect being most pronounced in females at the high dose. Increased blood urea nitrogen, creatinine, total cholesterol, and alanine transaminase levels were seen in dogs at 5 and 7.5 mg/kg bw per day, and these alterations were corroborated by histopathological changes in the livers and kidneys of these dogs. The increases in blood urea nitrogen and creatinine are compatible with either dehydration or mild compromise of the renal tubular epithelium, while the elevations in alanine transaminase activity are indicative of hepatocellular injury. The increases in total cholesterol are nonspecific but are typically seen with alterations in lipid metabolism by the liver. Histopathological examination revealed a minimal increase in the frequency and average severity of sinusoidal lining cells of the livers in females at 5 and 7.5 mg/kg bw per day; minimal increases in the frequency and average severity of perivascular, chronic, active inflammation of the liver; and an increase in pigment in the tubular epithelium of the kidneys of animals of each sex at 5 and 7.5 mg/kg bw per day. The NOAEL was 1 mg/kg bw per day on the basis of alterations in serum chemical parameters and histopathological lesions in the liver and kidneys (Dalgard, 1993d).

(d) Reproductive toxicity

Rats

In a two-generation study, groups of 30 male and 30 female Fischer 344 rats were fed diets containing 2,4-D (purity, 97.5%) at doses of 0, 5, 20, or 80 mg/kg bw per day for 105 days before mating (F_0 generation). The rats were dosed in an analogous manner during each mating, each gestation, and each lactation. The total and continuous dosing of the F_0 rats lasted 40 weeks, which included two weeks of rest between the end of lactation of the F_{1a} litters and the beginning of mating for the F_{1b} litters and 30 days after weaning of the latter litters. The F_1 generation, selected from the F_{1b} pups, was exposed to 2,4-D *in utero* and continuously via the milk or the feed for 125 days postnatally as well as prior to and throughout mating, gestation, and lactation of the F_{2a} litters. Dosing continued through a two-week rest period and during mating, gestation, and lactation of the F_{2b} litters and for at least 30 days after weaning of the F_{2b} litters. The dose of 80 mg/kg bw per day caused excessive toxicity in the F_1 generation and was deleted, leaving groups dosed at 5 and 20 mg/kg bw per day.

No adverse effects on fertility were seen in males or females at any dose or in any generation. The length of gestation was prolonged by one day in F_0 females at 80 mg/kg bw per day producing the F_{1b}

pups. This effect may have been the result of delayed implantation, hormonal imbalance, or problematic parturition. The mean body weights of F_0 males and females at 80 mg/kg bw per day were significantly ($p \leq 0.05$) lower than those of the controls. The F_0 dams fed 80 mg/kg bw per day and producing the F_{1a} litter had significantly ($p \leq 0.05$) lower body weights on days 7, 13 and 20 of gestation, while those producing the F_{1b} litter had significantly ($p \leq 0.05$) lower body weights only on day 20 of gestation. While the body weights of F_1 dams producing the F_{2a} litters were comparable to those of the controls at all doses during gestation, the F_1 dams fed 20 mg/kg bw per day and producing the F_{2b} litters had significantly ($p \leq 0.05$) lower body weights on days 7, 13, and 20 of gestation. The weights of pups of each sex of the F_{1a} generation at 80 mg/kg bw per day were significantly ($p \leq 0.05$) decreased during days 1-28 of lactation, as were those of the F_{1b} generation at 20 mg/kg bw per day on day 28 of lactation and those at 80 mg/kg bw per day on days 1-28. The weights of the F_{2a} and F_{2b} pups were comparable to those of the respective controls. The viability of F_{1a} and F_{1b} pups was affected only by treatment at 80 mg/kg bw per day. Live litter sizes were reduced in the F_{1a} (9%; 10.1% in controls) and F_{1b} (5.1%; 0.5% in controls; $p \leq 0.01$) generations. There was a significant ($p \leq 0.01$) decrease in the sex ratio of the F_{1a} pups (109 males and 71 females) when compared with controls (99 males and 114 females). Pup mortality was significantly ($p \leq 0.01$) increased in the F_{1b} generation (110 dead pups) in comparison with controls (5 dead pups), but the viability of the F_{2a} and F_{2b} pups was not affected. Examination of the F_{1b} pups at 80 mg/kg bw per day that died before lactation on day 28 revealed bent ribs in 30 fetuses in six litters, with none in the control fetuses; 14th rudimentary ribs in 12 fetuses in six litters, with none in the control fetuses; and slight or moderately malaligned sternbrae in 23 fetuses in nine litters, and in one fetus per litter in the control pups. None of these increases, however, showed statistical significance. Histopathological examination revealed increased focal nuclear density in the medullary renal tubules in animals of the F_0 generation at 20 mg/kg bw per day (7/30; 23%) and at 80 mg/kg bw per day (73%) and in the F_1 adults at 20 mg/kg bw per day (4/29; 14%) when compared with controls (0%). These lesions are indicative of degenerative or atrophic change of the epithelial cells. No changes were seen at 5 mg/kg bw per day or in any of the F_n weanlings. The NOAEL for systemic parental toxicity in the F_0 and F_1 generations and for reproductive and developmental toxicity was 5 mg/kg bw per day (Rodwell, 1985).

(e) Developmental toxicity

Mice

The teratogenic potential of 2,4-D and its esters was investigated in AKR, C57Bl/6, C3H, and A/Ha mice by subcutaneous injection of doses of 24-106 mg/kg bw per day on days 6-14 or 15 of gestation. Groups of positive and negative controls were used periodically throughout the study, but they were not matched with respect to either route or time of administration. 2,4-D increased the proportion of abnormal litters only in the AKR strain, in some tests but not others, depending on when the tests were conducted. No significant increase in the incidence of anomalies was noted with 2,4-D in C57, C3H, or hybrid C57 x AKR mice; with the isooctyl ester of 2,4-D in C3H, A/Ha, or AKR mice; with the butyl ester of 2,4-D in C57 or AKR mice; with the isopropyl ester of 2,4-D in C57 or AKR mice; with the methyl ester of 2,4-D in AKR mice in a hybrid fetus resulting from mating a C57Bl/6 female with an AKR male; or with the EH ester of 2,4-D in C57 or AKR mice (Bage et al., 1973).

Rats

Groups of 15-19 pregnant Sprague-Dawley rats were given 2,4-D (purity, 98.7%) in corn oil by gavage at doses of 12.5, 25, 50, 75, or 88 mg/kg bw per day on days 6-15 of gestation. Two control groups were used: one for the animals at 88 mg/kg bw per day and another for those at the lower doses.

Fetuses were delivered by caesarean section on day 20 of gestation and were examined grossly, measured, weighed, and examined for soft tissue and skeletal anomalies. There were no deaths, and the body-weight gains of treated dams were comparable to those of the controls. Statistically significant ($p \leq 0.05$) decreases in fetal body weights were observed at doses $> 50 \text{ mg/kg}$ bw per day. Fetal anomalies, such as subcutaneous oedema, lumbar and wavy ribs, and delayed ossification of bones including the skull were observed with increasing doses; however, these anomalies were also seen in both control groups. Significant ($p \leq 0.05$) increases were observed in both the fetal (8/119; 7%) and litter incidences (5/19; 26%) in comparison with controls (fetal, 2/205, 1%; litter, 2/25, 6%). The NOAELs were 88 mg/kg bw per day for maternal toxicity and 25 mg/kg bw per day for developmental toxicity (Schwetz *et al.*, 1971).

Groups of 35 pregnant Fischer 344 rats were given technical-grade 2,4-D (purity, 97.5%) in corn oil by gavage at doses of 8, 25, or 75 mg/kg bw per day during days 6-15 of gestation. The control group received the vehicle alone by the same schedule. Dams were sacrificed on day 20 of gestation; post-mortem examination included gross macroscopic examination of all internal organs with emphasis on the uterus, uterine contents, position of fetuses in the uterus, and the number of corpora lutea. Fetuses were weighed, sexed, and examined for gross external abnormalities; they were prepared by Wilson's slicing technique for visceral examination, after which they were stained with alizarin red S for skeletal examination. Treatment did not alter survival or induce clinical signs. Maternal toxicity was limited to decreased body-weight gain in dams at 75 mg/kg bw per day during treatment, which reached -43% during days 6-10 and -21% during days 6-15. No treatment-related effects were observed on the numbers of viable fetuses, early or late resorptions, pre-implantation losses, or corpora lutea or on the fetal sex distribution, fetal weights, or fetal crown-rump length. No gross external or visceral anomalies (malformations or variations) were seen at any dose. The incidence of skeletal variations was increased in fetuses at 75 mg/kg bw per day and included 7th cervical ribs in 4/127 (3%) fetuses and 3/26 (12%) litters, 14th rudimentary ribs in 4/127 (3%) fetuses and 3/26 (12%) litters, and missing sternalbrae in 15/26 (58%) fetuses and 10/26 (38%) litters; none were seen in the controls. Although these increases were not statistically significant, they are attributable to treatment since the same skeletal variations were also found at a high incidence in the F_{1b} pups of dams fed 80 mg/kg bw per day 2,4-D in a study in the same strain of rats (Rodwell, 1985) and in the fetuses of Sprague-Dawley dams fed 87.5 mg/kg bw per day (Schwetz *et al.*, 1971). Thus, the weight of the evidence from the two-generation study of reproductive toxicity and the studies of developmental toxicity in two strains of rats indicates that the lowest observed effect level for developmental toxicity was 75 mg/kg bw per day. The NOAEL was 25 mg/kg bw per day for maternal and developmental toxicity (Rodwell, 1983).

The salts and esters of 2,4-D were tested for developmental toxicity in rats in a series of experiments with similar protocols: The compounds were given by gavage on days 6-15 of gestation. The control groups received the vehicle by the same schedule. The dams were killed on day 20 of gestation, and post-mortem examination included gross macroscopic examination of all internal organs with emphasis on the uterus, uterine contents, position of fetuses in the uterus, and the number of corpora lutea. Fetuses were weighed, sexed, examined for gross external abnormalities, and prepared by Wilson's slicing technique for visceral examinations, after which they were stained with alizarin red S for skeletal examination.

The DEA salt of 2,4-D (purity, 73.1%) was administered at doses of 15, 75, or 150 mg/kg bw per day (equivalent to 11, 55, or 110 mg/kg bw per day of the acid) in distilled water to groups of 25 pregnant Sprague-Dawley CrI:CD rats. Maternal toxicity at 75 mg/kg bw per day was limited to a significant ($p \leq 0.05$) decrease in mean body-weight gain during days 6-9 of gestation. At 150 mg/kg bw per day, maternal toxicity consisted of significant ($p \leq 0.05$) decreases in mean body-weight gain during days

6–9 of gestation and reductions in mean food consumption during days 6–9 and 6–15 of gestation. No effects attributable to treatment were observed on the mean number of viable fetuses, early or late resorptions, pre- or post-implantation losses, or corpora lutea or on the fetal sex distribution or fetal crown–rump length. Singular fetotoxicity observed at 150 mg/kg bw per day was a significant ($p \leq 0.01$) reduction (–8%) in fetal body weight. Skeletal examination revealed fetal variations in animals at 75 and 150 mg/kg bw per day. At 75 mg/kg bw per day, there was a significant ($p \leq 0.05$) increase in the incidence of reduced ossification of the skull in 14/21 litters (67%) in comparison with the control (5/23 litters; 22%) and an increase in the incidence of bent ribs (6/21 litters, 29%; control, 0%). At 150 mg/kg bw per day, there was a nonsignificant increase in the incidence of reduced ossification of the skull (12/23 litters, 52%; control, 5/23 litters, 22%). Significant ($p \leq 0.05$) increases were seen in the incidences of 14th rudimentary ribs (15/21 litters, 65%; control, 4/23 litters, 17%) and of 7th cervical ribs (7/23 litters, 30%; control, 1/23 litters, 4%). These fetal anomalies were due to treatment because the incidences were statistically significantly higher than those in concurrent controls and exceeded the historical control range of the testing laboratory. The NOAEL was 15 mg/kg bw per day, equivalent to 11 mg/kg bw per day of the acid, for both maternal and developmental toxicity (Siglin *et al.*, 1990).

Groups of 25 pregnant Sprague-Dawley Crl:CD rats received the DMA salt of 2,4-D (purity, 66.2%) at doses equivalent to 12, 50, or 100 mg/kg bw per day of the acid in deionized water. Maternal toxicity was seen at 50 mg/kg bw per day as a decrease in body-weight gain (–8%) during treatment. Dams at 100 mg/kg bw per day had decreased motor activity and ataxia and a significant ($p \leq 0.01$) decrease in body-weight gain (–14%) during treatment. No effects attributable to treatment were observed on the mean numbers of viable fetuses, early or late resorptions, pre- or post-implantation losses, or corpora lutea or on the fetal sex distribution or fetal crown–rump length. Fetotoxicity was limited to significant ($p \leq 0.01$) decreases in male (–7%) and female (–8%) fetal body weights at 100 mg/kg bw. No external or visceral malformations or variations were observed at any dose. The treatment-related skeletal variation observed was a significant ($p \leq 0.01$) increase in the incidence of wavy and/or incompletely ossified ribs at 100 mg/kg bw (5/225 fetuses, 2.2%; 4/25 litters, 16%) in comparison with the vehicle controls (0%) and historical controls (range, 0–4%). The NOAEL was 12 mg/kg bw per day acid for maternal toxicity and 50 mg/kg bw per day acid for developmental toxicity (Lochry, 1990).

The IPA salt of 2,4-D (purity, 50.2%) was administered at doses of 22, 65, or 190 mg/kg bw per day (equivalent to 9, 25, and 74 mg/kg bw per day of the acid) in deionized water to groups of 30 pregnant Sprague-Dawley rats. Maternal toxicity at 190 mg/kg bw per day was seen as a significant ($p \leq 0.05$) decrease in body-weight gain (–57%) and reductions in food consumption (–9%) on days 6–11 of gestation. No treatment-related effects were observed on the mean numbers of viable fetuses, early or late resorptions, pre- or postimplantation losses, or corpora lutea or on the fetal sex distribution, fetal body weight, or fetal crown–rump length. No gross external, visceral, or skeletal malformations or variations were observed at any dose. The NOAEL was 65 mg/kg bw per day (9 mg/kg bw per day acid) for maternal toxicity; the NOAEL for developmental toxicity was 190 mg/kg bw per day (equivalent to 74 mg/kg bw per day of the acid), the highest dose tested (Schroeder, 1990a).

The TIPA salt of 2,4-D (purity, 72.2%) was given to groups of 30 pregnant Sprague-Dawley rats at doses of 32, 100, or 320 mg/kg bw per day (equivalent to 12, 37, or 120 mg/kg bw per day of the acid) in deionized water. Maternal toxicity included deaths, clinical signs, and decreases in body-weight gain and food consumption at 320 mg/kg bw per day: 4/30 dams (13.3%) died, 29 exhibited stiffness of the limbs, 10 had excessive salivation; body-weight gain was significantly ($p \leq 0.05$) decreased (–42%) throughout treatment, and food consumption was significantly ($p \leq 0.05$) reduced (–6.8%) during days 0–20 of gestation. Embryotoxicity at 320 mg/kg bw per day included increases in preimplantation loss

(9.6%; controls, 5.4%; 78% increase); increases in the number of resorptions per dam (2.6; controls, 0.8); and increases in postimplantation loss (16.6%; controls, 5.3%; 217% increase). Fetotoxicity at 320 mg/kg bw per day included decreases in the number of males per litter (–38%) and in fetal body weights of males (–11%) and females (–17%). Teratogenicity at 320 mg/kg bw per day was evidenced by external and visceral malformations and skeletal malformations and variations; the external and visceral variations were comparable to those of the controls. The incidence of external malformations was significantly ($p \leq 0.05$) increased for both fetuses (5/272; 1.8%) and litters (5/23; 22%) in comparison with controls (0%) and included filamentous tail (two fetuses in two litters) and small bulging eyes (one fetus). The incidence of visceral malformations was significantly ($p \leq 0.05$) increased for both fetuses (7/144; 4.9%) and litters (6/23; 26%) in comparison with controls (0%) and included microphthalmia (two fetuses in two litters), anophthalmia (two fetuses in two litters), and cardiovascular defects (one fetus). The incidence of skeletal malformations was significantly ($p \leq 0.05$) increased for both fetuses (16/131; 12%) and litters (9/23; 39%) in comparison with controls (0%) and included defects of vertebrae, vertebral transverse processes, sternbrae, and ribs. The incidence of skeletal variations was increased significantly ($p \leq 0.05$; 127/131; 97%) in comparison with controls (179/203; 88%) and included wavy ribs in five fetuses in four litters each at 100 and 320 mg/kg bw per day, and fused ribs in five fetuses in four litters at 320 mg/kg bw per day. The NOAEL for maternal and developmental toxicity was 100 mg/kg bw per day, equivalent to 37 mg/kg bw per day of the acid (Schroeder, 1990b).

Groups of 30 pregnant Sprague-Dawley CD rats were given the BEH ester of 2,4-D (purity, 95.6%) in corn oil at doses of 25, 75, or 180 mg/kg bw per day (equivalent to 17, 50, 120 mg/kg bw per day of the acid). Maternal toxicity at 180 mg/kg bw per day was shown by decreased body-weight gain during days 6–9 (–33.3%) and 9–12 (–19%) of gestation. No treatment-related effects were seen on the mean numbers of viable fetuses, early or late resorptions, pre- or postimplantation losses, or corpora lutea or on the fetal sex distribution, fetal body weight, or fetal crown–rump length. No gross external or visceral malformations or variations or skeletal malformations were observed at any dose. The total incidences of fetuses with skeletal variations were significantly ($p \leq 0.05$) increased at the high dose (162; 89.5%) when compared with the controls (138; 79.3%). Fetuses at 180 mg/kg bw per day had non-statistically significant increases in incompletely ossified supraoccipital (29 fetal variations in 181 fetuses, 16%; controls, 14/174, 8%), squamosal (26/181, 12%; controls, 7/174, 4%), maxilla (6/181, 3%; controls, 0%), and 4th sternbrae (20/184, 11%; controls, 12/174, 7%). The litter incidences were: supraoccipital (12/27, 44%; controls, 6/25, 24%), squamosal (14/27, 52%; controls, 28%), maxilla (4/27, 15%; controls, 0%), and 4th sternbrae (11/27, 41%; controls, 6/25, 24%). The NOAEL for maternal and developmental toxicity was 75 mg/kg bw per day, equivalent to 50 mg/kg bw per day of the acid (Schroeder, 1990c).

The EH ester of 2,4-D (purity, 95%) was administered to groups of 20 pregnant Sprague-Dawley rats at doses equivalent to 10, 30, or 90 mg/kg bw per day of the acid in 1% aqueous carboxymethylcellulose. Maternal toxicity seen at 90 mg/kg bw per day consisted of clinical signs (ataxia, decreased motor activity, and bradypnoea), significant ($p \leq 0.05$) decreases in body-weight gain during days 6–9 of gestation (–28%), and decreased food consumption throughout treatment. No treatment-related effects were observed on the mean numbers of viable fetuses, early or late resorptions, pre- or postimplantation losses, or corpora lutea or on the fetal sex distribution, fetal body weight, or fetal crown–rump distance. No treatment-related external or visceral malformation or variations or skeletal malformations were seen at any dose. There was a significant ($p \leq 0.05$) increase in the incidence of incomplete or unossified sternbrae at 90 mg/kg bw per day (18/207, 9%; controls, 8/182, 4%); the litter incidences were comparable to those of controls. The NOAEL for maternal and developmental toxicity was 30 mg/kg bw per day of the acid (Martin, 1992a).

Rabbits

2,4-D and its salts and esters were tested for developmental toxicity in groups of 20 New Zealand white rabbits in a series of experiments with similar protocols. The compounds were given orally or by gavage on days 6–18 of gestation. The control groups received the vehicle by the same schedule. The does were killed on day 29 of gestation. The thoracic, abdominal, and pelvic cavities were examined for gross lesions; if any were seen, the tissues were preserved in 10% formalin. The uterus was removed, examined externally, weighed, and then opened for internal examination. Uteri that appeared to be from nonpregnant rabbits were stained with 10% ammonium sulfide to determine pregnancy status. Corpora lutea were counted, and the numbers and placements of implantations, early and late resorptions, and live and dead fetuses were recorded. Fetuses were weighed, sexed, examined for gross external abnormalities, and prepared by Wilson's slicing technique for visceral examination, after which they were stained with alizarin red S for skeletal examination. No treatment-related effects were observed on the mean numbers of viable fetuses, early or late resorptions, pre- or postimplantation losses, or corpora lutea or on the fetal sex distribution, fetal body weight or fetal crown–rump length.

Rabbits were given 2,4-D (purity, 96.1%) in 0.5% carboxymethylcellulose orally at 10, 30 or 90 mg/kg bw per day. Maternal toxicity at 90 mg/kg bw per day was shown by clinical signs such as ataxia, decreased motor activity, loss of righting reflex, and cold extremities in the two does that aborted; a decrease in body-weight gain during (–27%) and after the treatment period (–16%); and a nonsignificant reduction in corrected body-weight gain during the entire period (–23%). No gross external, visceral, or skeletal malformations or variations were seen at any dose. The NOAEL for maternal toxicity was 30 mg/kg bw per day; the NOAEL for developmental toxicity was 90 mg/kg bw per day, the highest dose tested (Hoberman, 1990).

Rabbits received the DEA salt of 2,4-D (purity, 73.09%) in distilled water by oral administration at doses equivalent to 15, 30, or 60 mg/kg bw per day of the acid. Maternal toxicity at 30 mg/kg bw per day was characterized by significant ($p \leq 0.01$) decreases in body-weight gain (–41% and –26%) and food consumption (–15% and –13%) during days 6–19 and 0–29 of gestation, respectively. In animals at 60 mg/kg bw per day, maternal toxicity was seen, manifested as deaths (one doe on day 19), abortion (one doe on day 23), and decreases in body-weight gain (–51% and –24%) and food consumption (–28% and –17%) during days 6–19 and 0–29 of gestation, respectively. No gross external, visceral, or skeletal malformations were seen at any dose. Skeletal variations seen at 60 mg/kg bw per day included a significant ($p \leq 0.05$) increase in the number of litters with 7th cervical ribs (4/17, 24%; controls, 0%). This increase was outside the historical control range (0–6.7%) of the testing laboratory. This anomaly was also observed in 30% of pregnant rats given DEA at 150 mg/kg bw per day. The NOAEL was 15 mg/kg bw per day of the acid for maternal toxicity and 30 mg/kg bw per day of the acid for developmental toxicity (Rodwell, 1991).

Rabbits were given the DMA salt of 2,4-D (purity, 66.2%) in deionized water orally at doses equivalent to 10, 30, or 90 mg/kg bw per day of the acid. Maternal toxicity at 90 mg/kg bw per day included the deaths of four does (two on day 10 and two on day 18), clinical signs of toxicity such as decreased motor activity, myotonia, ataxia, and impaired or lost righting reflexes, and a significant ($p \leq 0.05$) decrease in food consumption on days 6–9 of gestation. Except for the body weight loss in the does that died, no treatment-related effects were observed on body-weight gain. No gross external, visceral, or skeletal malformations or variations were seen at any dose. The skeletal variation (wavy and/or incompletely ossified ribs) observed in pregnant rats given 100 mg/kg bw per day was not seen in rabbits. The NOAEL for maternal toxicity was 30 mg/kg bw per day of the acid; the NOAEL for developmental toxicity was 90 mg/kg bw per day of the acid, the highest dose tested (Martin, 1991).

Does received the IPA salt of 2,4-D (purity, 50.2%) in deionized water orally at 13, 38 or 95 mg/kg bw per day (equivalent to 10, 30, or 75 mg/kg bw per day of the acid). Maternal toxicity at 38 and 95 mg/kg bw per day included the deaths of two does at 38 mg/kg bw per day and three at 95 mg/kg bw per day, morbidity in four does at 95 mg/kg bw per day, clinical signs of toxicity (decreased faeces and myotonia at 38 and 95 mg/kg bw per day and lateral recumbency at 95 mg/kg bw per day), significant ($p \leq 0.05$) decreases in body-weight gain (-44% at 38 mg/kg bw per day and -56% at 95 mg/kg bw per day) on days 7-20 of gestation, and a significant ($p \leq 0.05$) increase (13%) in relative kidney weights. No gross external, visceral, or skeletal malformations or variations were seen at any dose. The NOAEL for maternal toxicity was 13 mg/kg bw per day (equivalent to 10 mg/kg bw per day of the acid); the NOAEL for developmental toxicity was 95 mg/kg bw per day (equivalent to 75 mg/kg bw per day of the acid), the highest dose tested (Breslin *et al.*, 1991).

Does were given the TIPA salt of 2,4-D (purity, 73.1%) in deionized water by oral administration at 19, 56, or 140 mg/kg bw per day (equivalent to 10, 30, or 75 mg/kg bw per day of the acid). Maternal toxicity at 56 and 140 mg/kg bw per day included the death of one doe at 56 mg/kg bw per day, morbidity in three does at 140 mg/kg bw per day, clinical signs of toxicity (decreased faeces, myotonia, and lateral recumbency) at 56 and 140 mg/kg bw per day, and significant ($p \leq 0.05$) decreases in body-weight gain (-46% at 56 mg/kg bw per day and -59% at 140 mg/kg bw per day) on days 7-20 of gestation. No gross external, visceral, or skeletal malformations or variations were seen at any dose. The NOAEL for maternal toxicity was 19 mg/kg bw per day, equivalent to 10 mg/kg bw per day of the acid; the NOAEL for developmental toxicity was 140 mg/kg bw (equivalent to 75 mg/kg bw per day of the acid), the highest dose tested (Liberacki *et al.*, 1991).

Groups of 20 artificially impregnated New Zealand rabbits were given the BEH ester of 2,4-D (purity, 95.6%) in corn oil by gavage at 15, 45, or 110 mg/kg bw per day (equivalent to 10, 30, or 75 mg/kg bw per day of the acid). Maternal toxicity at 45 and 110 mg/kg bw per day included the deaths of one doe at 45 and four at 110 mg/kg bw per day, morbidity in one doe at 45 and four at 110 mg/kg bw per day, clinical signs (decreased activity, myotonia, lateral recumbency, and prostration), and decreases in the body weights of does that died or were killed at these doses. No fetal gross external, visceral, or skeletal malformations or variations were seen at any dose. The NOAEL for maternal toxicity was 15 mg/kg bw per day, equivalent to 10 mg/kg bw per day of the acid; the NOAEL for developmental toxicity was 110 mg/kg bw per day (equivalent to 75 mg/kg bw per day of the acid), the highest dose tested (Zablotny *et al.*, 1991).

Pregnant does were given the EH ester of 2,4-D (purity, 95.6%) in 1% methylcellulose by oral administration at doses equivalent to 10, 30, or 75 mg/kg bw per day of the acid. Maternal toxicity at 75 mg/kg bw per day consisted of the deaths of two does, morbidity in two does, and abortion by one doe; clinical signs of toxicity (decreased activity, ataxia, impaired righting reflexes, loss of righting reflex and bradypnoea); and decreases in body weight (-19%) on days 6-19 of gestation. No gross external, visceral, or skeletal malformations or variations were seen in fetuses at any dose. The NOAEL for maternal toxicity was 30 mg/kg bw per day of the acid; the NOAEL for developmental toxicity was 75 mg/kg bw per day of the acid, the highest dose tested (Martin, 1992b).

(f) Genotoxicity

The mutagenic potential of 2,4-D, the DEA, DMA, IPA, and TIPA salts and the BEH and EH esters has been evaluated in numerous assays. The results are presented in Table 2.

Table 2. Results of tests for the genotoxicity of 2,4-D, its salts and its esters

End-point	Test system	Concentration	Purity (%)	Results	Reference
2,4-D					
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	100-1 0 000 µg/ plate with S9; 66.74670 µg/ plate without S9	96.1	Negative	Lawlor & Valentine 1990a)
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	0-1 000 µg/plate	NR	Negative	Rashid ef a/. (1984)
Reverse mutation	<i>S. typhimurium</i>	0-3333 µg/plate	NR	Negative	Soler-Neidzieler et al. (1988)
Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537, TA1538	0-1.000 µg/plate	NR	Negative	Kappas (1988)
Reverse mutation	<i>S. typhimurium</i> TA1538, TA1535	0-2000 µg/plate	NR	Negative	Rashid & Muma (1986)
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1538	≤ 5000 µg /plate	NR	Negative	Simmon et a/. (1977)
DNA damage	<i>E. coli</i> K12, WP2	0-2000 µg/plate	NR	Negative	Rashid & Mumma (1986)
DNA damage	<i>E. coli</i> PQ 37	0-200 µg/plate	NR	Negative	Sundermann et al. (1989)
Sex-linked recessive lethal mutation	<i>Drosophila melano-gaster</i> larvae	0.010 000 ppm	NR	Positive	Kale et al. (1995)
Sex-linked recessive lethal mutation	<i>Drosophila melano-gaster</i> adults	1000-10 000 ppm (feeding), ≥ 99 10 000 ppm (injection)	NR	Negative	Zimmering et a/. (1985)
Gene mutation	Hamster V79 fibroblasts, <i>hprt locus</i>	1 0-1 00 µg/ml	NR	Positive	Pavlica et a/. (1991)
Chromosomal aberration <i>in vitro</i>	Chinese hamster ovary cells	500–920 µg/ml without S9; 1900-5000 µg/ml with S9	NR	Negative Equivocal	Galloway ef a/. (1987)
Chromosomal aberration <i>in vitro</i>	Embryonic bovine kidney and peripheral lymphocytes	1-1 000 ppm	NR	Negative	Bongso & Basrur (1973)
Chromosomal aberration <i>in vitro</i>	Human lymphocytes	0-125-0.35 mmol/litre 0.125-1 .250 mmol/litre	55	Negative Positive"	Mustonen et al. (1986)
Chromosomal aberration <i>in vivo</i>	Human lymphocytes	0.03–0.04 mg/m ³	33.3	Negative	Mustonen et al. (1986)
Chromosomal aberration <i>in vivo</i>	Rat bone marrow	0-350 µg/kg bw intraperitoneally per 4 or 24 h; three replicates	NR	Negative	Turkula & Jalal (1987)
Chromosomal aberration <i>in vivo</i>	Rat bone marrow	0, 17.5, 35, or 70 mg/kg bw per day intraperitoneally twice	NR	Negative"	Adhikari & Grover (1988)
Sister chromatid exchange <i>in vivo</i>	Rat lymphocytes	100 mg/kg bw	NR	Negative	Mustonen et al. (1989)
Sister chromatid exchange <i>in vitro</i>	Chinese hamster ovary cells	50-299 µg/ml without S9 500-4200 µg/ml with S9	NR	Positive Negative	Galloway et al. (1987)
Sister chromatid exchange <i>in vivo</i>	Human lymphocytes	NR	33.3	Negative	Linnainmaa (1983)
Micronucleus formation <i>in vivo</i>	ICR mouse bone marrow	40–400 mg/kg bw	96.1	Negative	Ivett (1990a)
Unscheduled DNA synthesis	Primary Fischer 344 rat hepatocytes	0.969–2890 µg/ml	96.1	Negative	Cifone (1990a)
DNA damage	Human fibroblasts (PM2 DNA)	0-1 00 nmol/litre	NR	Negative	Clausen et a/. (1990)

Table 2 (contd)

End-point	Test system	Concentration	Purity (%)	Results	Reference
DEA salt					
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	500-1 4 000 µg/plate with and without S9	73.8	Negative	Lawlor & Holloway (1990)
Chromosomal aberration in vivo	ICR mouse bone marrow	60-600 mg/kg bw	73.8	Negative	Ivett (1990b)
Unscheduled DNA synthesis	Primary Fischer 344 rat hepatocytes	1 0-500 µg/ml	73.8	Negative	McKeon (1990)
DMA salt					
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	333-1 0 000 µg/ plate	66.2	Negative	Lawlor & Valentine (1990b)
Micronucleus formation in vivo	ICR mouse bone marrow	60-600 mg/kg bw	66.2	Negative	Ivett (1990b)
Unscheduled DNA synthesis	Primary Fischer 344 rat hepatocytes	70.5-1 00 µg/ml	66.2	Negative	Cifone (1990b)
IPA salt					
Reverse mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537	1 0-1 0 000 µg/plate	50.1	Negative	Samson & Gollapudi (1989a)
Gene mutation	Chinese hamster ovary cells, <i>hprt</i> locus	500-3000 µg/ml	50.2	Negative	Linscombe & Lick (1994a)
Chromosomal aberration	Rat lymphocytes	96-6137 µg/ml with and without S9	50.2	Negative	Linscombe & Lick (1994b)
Micronucleus formation in vivo	ICR mouse bone marrow	75-750 mg/kg bw	50.2	Negative	Gollapudi et al. (1990a)
Unscheduled DNA synthesis	Primary Fischer 344 rat hepatocytes	5-500 µg/ml	50.2	Negative	McClintock & Gollapudi (1990)
TIPA salt					
Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537	1000-1 0 000 µg/plate	72.2	Negative	Samson & Gollapudi (1989b)
Gene mutation	Chinese hamster ovary cells, <i>hprt</i> locus	78-5000 µg/ml	70.9	Negative	Linscombe & Lick (1994c)
Chromosomal aberration	Rat lymphocytes	800-5000 µg/ml	70.9	Negative	Linscombe & Lick (1994d)
Micronucleus formation in vivo	ICR mouse bone marrow	75-750 mg/kg bw	70.9	Negative	Gollapudi et al. (1990b)
BEH ester					
Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537, TA1538	5-5000 µg/plate with S9; 1.6-1 667 µg/plate without S9	95.6	Negative	Samson & Gollapudi (1990)
Chromosomal aberration	Rat lymphocytes	87.5-1 400 µg/litre	94.6	Negative	Linscombe & Lick (1994e)
Micronucleus formation in vivo	ICR mouse bone marrow	37.5-375 mg/kg bw	95.6	Negative	Gollapudi et al. (1990c)
Unscheduled DNA synthesis	Primary Fischer 344 rat hepatocytes	5-500 µg/ml	95.6	Negative	McLintock & Gollapudi (1990)

Table 2 (contd)

End-point	Test system	Concentration	Purity (%)	Results	Reference
EH ester					
Reverse mutation	<i>S. typhimurium</i> TA97, TA98, TA100, TA1535, TA1537, TA1538	333–10 000 µg/plate with and without S9	98	Negative	Lawlor & Valentine (1990c)
Micronucleus formation <i>in vivo</i>	ICR mouse bone marrow	50–500 mg/kg bw	98	Negative	Ivett (1990c)
Unscheduled DNA synthesis	Primary Fischer 344 rat hepatocytes	0.5–25 µg/ml	98	Negative	Cifone (1990c)

^a Attributed to 'unidentified clastogens' (i.e. contaminants) by investigators

^b Occupational exposure. Present in urine at 0.09–1.14 mg/litre in nonsmokers and 0.11–1.56 in smokers

^c Positive at two highest doses, but results were similar with dimethyl sulfoxide solvent as a control

(g) *Special studies*

(i) *Dermal and ocular irritation and dermal sensitization*

No skin irritation was observed in rabbits after dermal applications of 2,4-D, the amines DMA, DEA, IPA, and TIPA or the BEH and EH esters for 4 h (Keller *et al.*, 1977; Myer, 1981e; Carreon *et al.*, 1983; Jeffrey, 1987b; Mizell *et al.*, 1989; Schults *et al.*, 1990d; Berdasco, 1992).

2,4-D, the DMA, DEA, IPA, and TIPA salts, and the BEH and EH esters were shown to be severe eye irritants when instilled into the conjunctival sac of rabbits. Consistent eye lesions observed in these studies were corneal opacity, chemosis, redness of the conjunctivae, and ocular discharge. Iridial inflammation was also seen. No evidence of amelioration was evident three days later (Keller *et al.*, 1977; Kirsh, 1983; Carreon *et al.*, 1983; Carreon & Rao, 1986; Jeffrey, 1987a; Berdasco & Mizell, 1989; Schults *et al.*, 1990c)

The sensitization potential of 2,4-D, the DMA, DEA, IPA, and TIPA salts, and the BEH and EH esters has been assessed in guinea-pigs by the Buehler method of dermal induction. No evidence of delayed contact hypersensitivity was seen in any of the studies (Keller *et al.*, 1977; Carreon *et al.*, 1983; Carreon & Rao, 1985; Gargus, 1986; Jeffrey, 1986; Jeffrey & Rao, 1986; Schults *et al.*, 1990e).

(ii) *Dermal toxicity*

In a series of studies with 2,4-D and its salts and esters, groups of five male and five female New Zealand white rabbits received 15 repeated dermal applications for 6 h/day, on five days per week for 21 days.

When 2,4-D (purity, 96.1%) was applied at 0, 10, 100, or 1000 mg/kg bw per day, no systemic toxicity was seen. 2,4-D was mildly irritating to the skin, but no skin lesions were seen (Schultze, 1990b). Dermal applications of the DEA salt (purity, 73.9%) in distilled water at 0, 15, 150, or 440 mg/kg bw per day induced hepatic toxicity only at the high dose, seen as an elevation in the serum activities of alanine and aspartate transaminases and alkaline phosphatase, increases in absolute and relative liver weights, and corroborative liver lesions consisting of hypertrophy of hepatocytes and the presence of hyaline droplets within the hepatocytes. The DEA salt at 150 and 440 mg/kg bw per day induced dermal toxicity characterized by histopathological lesions of the skin, including acanthosis,

hyperkeratosis, and chronic dermatitis; acute dermatitis, surface exudate, dermal haemorrhage, and vesiculation of the epidermis were also seen at the highest dose. The NOAEL was 150 mg/kg bw per day for systemic toxicity and 15 mg/kg bw per day for dermal toxicity (Siglin, 1991).

The DMA salt (purity, 66.18%) in distilled water at 0, 18, 180, or 540 mg/kg bw per day did not induce systemic toxicity. Dermal toxicity induced by doses of 180 and 540 mg/kg bw per day was characterized by histopathological lesions of the skin, including acanthosis, hyperkeratosis, oedema, superficial crusting (inspissated serum, necrotic cells, and debris on the epidermal surface) and chronic active inflammation. The NOAEL was 540 mg/kg bw per day for systemic toxicity and 18 mg/kg bw per day for dermal toxicity (Schultze, 1990c).

Applications of the IPA salt (purity, 50.2%) in distilled water at 0, 50, 125, or 350 mg/kg bw per day also induced no systemic toxicity. Dermal toxicity at 125 and 350 mg/kg bw per day was characterized by skin lesions including focal and multifocal irritation (inflammation and epidermal hyperplasia). The NOAEL was 350 mg/kg bw per day for systemic toxicity and 50 mg/kg bw per day for dermal toxicity (Mizell, 1990a).

The TIPA salt (purity, 72.2%) was applied in distilled water at 0, 100, 350, or 1000 mg/kg bw per day. No systemic or dermal toxicity was seen (Mizell *et al.*, 1990).

The BEH ester (purity, 94.6%) in corn oil at 0, 50, 150, or 500 mg/kg bw per day did not induce systemic or dermal toxicity (Mizell, 1990b).

The EH ester (purity, 98%) at 0, 16, 160, or 1600 mg/kg bw per day did not induce systemic toxicity, but the two highest doses induced dermal toxicity characterized by histopathological lesions of the skin, including acanthosis, hyperkeratosis, and necrotic cellular debris on the epidermal surface. The NOAEL for dermal toxicity was 16 mg/kg bw per day (Schultze, 1990d).

(iii) Neurotoxicity

No polyneuropathy was seen in male Fischer rats given single intraperitoneal injections of 2,4-D at 100 mg/kg bw per day on six days per week for three weeks or 80 mg/kg bw per day on three days per week for 12 weeks (Toyoshima *et al.*, 1985).

Groups of 10 male and 10 female Fischer 344 rats received 2,4-D in corn oil by gavage as single doses of 0, 15, 75, or 250 mg/kg bw. Neurobehavioural evaluations consisting of a functional observational battery and tests for motor activity were conducted one day before treatment, 6 h after dosing (at the time of peak effect), and on days 8 and 15. Neuropathological examination of the central and peripheral nervous tissues was conducted at termination. The functional observational battery on day 1 showed that animals of each sex at 250 mg/kg bw had increased incidences of incoordination and slight gait abnormalities, described as forepaw flexing or knuckling. The incidence of incoordination had decreased to control levels by day 4 in males and day 5 in females. There were no treatment-related gross or neuropathological alterations. The NOAEL for neurotoxicity was 75 mg/kg bw (Mattsson *et al.*, 1994a).

Groups of male Fischer 344 rats were given oral doses of 2,4-D in corn oil at 20, 40, or 80 mg/kg bw twice weekly for five weeks. Significant increases were observed in grip strength (both fore- and hind-limb) at all doses, but the effect appeared to dissipate with time after cessation of treatment. The effect was confirmed in a separate experiment in which male Fisher rats received 2,4-D at 10, 20, or 40 mg/kg bw per day on five days per week for four weeks. Both fore- and hind-limb grip strengths were

increased in rats receiving 20 or 40 mg/kg bw per day. In animals at 40 mg/kg bw, the increase persisted only two weeks after the end of treatment and was absent six weeks after dosing. The authors suggested that the increase in grip strength was linked to the observed myotonia, a condition characterized by difficulty in relaxing skeletal musculature after forceful contraction (Squibb *et al.*, 1983).

Groups of 15 male and 15 female Fisher 344 rats were fed diets containing 2,4-D at 0, 5, 75, or 150 mg/kg bw per day for 12 months. Functional observational battery and motor activity evaluations were conducted at 3, 6, 9, and 12 months. The NOAEL was 75 mg/kg bw per day on the basis of increased relative forelimb grip strength in animals of each sex (Mattsson *et al.*, 1994b).

Clinical signs indicative of neurotoxicity, such as ataxia, decreased motor activity, myotonia, prostration, lateral recumbency, and impairment or loss of righting reflexes, were observed in pregnant rabbits after oral administration of 2,4-D, the DEA, DMA, IPA, and TIPA salts and the BEH and EH esters at doses equivalent to 30 mg/kg bw per day of the acid on days 6–18 of gestation (Hoberman, 1990; Rodwell, 1991; Breslin *et al.*, 1991; Liberacki *et al.*, 1991; Martin, 1991; Zablotny *et al.*, 1991).

Groups of four adult female mongrel dogs were given gelatin capsules containing single doses of 2,4-D at 0, 25, 50, 75, 100, or 120 mg/kg bw. Clinical neurological examinations, electromyography, and measurements of motor nerve conduction velocity were conducted before treatment and on days 1, 3, 7, 14, 21, and 28 after treatment. Histopathological examination was conducted on two dogs at each dose killed on day 7 and at termination on day 28. Transient, generalized myotonic discharges were observed in the skeletal muscles of dogs given doses ≥ 50 mg/kg bw (Steiss *et al.*, 1987).

Electroencephalographic activity was evaluated in single English pointer dogs given gelatin capsules containing the DMA salt of 2,4-D at 0, 1.3, 8.8, 44, 180, or 220 mg/kg bw. By 24 h after treatment, the dog given 180 mg/kg bw showed mild sedation accompanied by excessive slowing in the electroencephalogram (EEG), with loss of low-voltage activity. In the dog given 220 mg/kg bw, nonspecific alterations in the EEG were suggestive of irritation, and mild seizure activity was detected 7 h after treatment. The EEG had returned to normal 24 h after treatment. No changes in the EEG were seen at the lower doses (Arnold *et al.*, 1991).

Groups of three male and three female English pointer dogs were given gelatin capsules containing the DMA salt of 2,4-D at 0, 1, 1.3, 8.8, 44, 87, 180, or 220 mg/kg bw. An electromyogram (EMG) was taken before and at various times after treatment. Dogs at 8.8, 44, and 87 mg/kg bw developed clinical manifestations of myotonia detectable only with the EMG; however, dogs at 180 and 220 mg/kg bw rapidly developed clinical and EMG manifestations consistent with a diagnosis of myotonia or pseudomyotonia. No changes in the EMG were seen at the lower doses (Beasley *et al.*, 1991).

(iv) *Canine malignant lymphoma*

As a model for human non-Hodgkin's lymphoma, the association between exposure to 2,4-D and the development of malignant lymphoma was investigated in pet dogs in a veterinary hospital-based case-control study. Dogs with histopathologically confirmed malignant lymphoma were identified, and two types of controls were selected, comprising dogs diagnosed with other malignancies and dogs in a veterinary hospital for other reasons. The animals were matched by age, year of hospital visit, and hospital. Information on exposure and possible confounders were solicited by mailed questionnaires to the owners. Information was obtained from the questionnaire and/or a telephone interview for 491 cases, 466 nontumour controls, and 479 tumour controls. A modest association was found between malignant lymphoma in dogs and application by their owner of 2,4-D on lawns and/or use of a

commercial lawn-care service, with an odds ratio (OR) of 1.3 (95% confidence interval [CI], 1.1-1.7). The OR was not raised for application of 2,4-D only (OR, 1.3; 95% CI, 0.9-1.8) or for sole use of commercial lawn treatments (OR, 1.3; 95% CI, 0.96-1.7). The OR for application by the owner plus use of a commercial lawn service was 1.9 (95% CI, 0.9-4.1). A positive trend ($p < 0.02$) was found for the frequency of use by the owner (number of applications per year), but not for the duration of use (number of years of application). No significant trends were found for use of commercial lawn-care service. The major weakness of this study was the lack of precise data on exposure to herbicides (Hayes *et al.*, 1991).

3. Observations in humans

Epidemiological studies have suggested an association between exposure to chlorophenoxyacetic acid herbicides, including 2,4-D, and two forms of cancer in humans: soft-tissue sarcomas and non-Hodgkin's lymphoma. The results of these studies are not consistent, however, the associations found are weak, and conflicting conclusions have been reached by the investigators. In addition, most of these studies did not provide information on exposure specifically to 2,4-D, and the risk was related to the general category of phenoxy herbicides, which might include 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and substances contaminated with dioxins, specifically 2,3,7,8-TCDD. While some of the studies have shown a relationship between exposure to 2,4-D and non-Hodgkin's lymphoma, others (including those with positive results) have produced inconsistent findings, raising doubts about whether the relationship is causal.

(a) Case-control studies

(i) Soft-tissue sarcoma

Six case-control studies addressed the association between exposure to phenoxyacetic acid herbicides and chlorophenols and the development of soft-tissue sarcoma in humans. A positive association was reported in patients with exposure to either group of compounds in Sweden (Hardell & Sandstrom, 1979; Eriksson *et al.*, 1981) and in female rice weeders in northern Italy (Vineis *et al.*, 1986). None of these studies, however, reported an OR for exposure to 2,4-D. In contrast, a number of case-control studies in New Zealand and the USA failed to find an association between use of phenoxyacetic acid herbicides and the development of soft-tissue sarcoma (Smith *et al.*, 1983, 1984; Hoar *et al.*, 1986; Woods *et al.*, 1987). The specific findings are described below.

Hardell and Sandstrom (1979) studied 21 living and 31 deceased male patients with soft-tissue sarcoma in northern Sweden who had been exposed to phenoxyacetic acids or chlorophenols; 220 controls were chosen from the general population. The cases of soft-tissue sarcoma were identified from the records of the Department of Oncology of the University Hospital of Umeå between 1970 and 1977. Information on patterns of use of herbicides and chlorophenols was obtained from questionnaires for 36.5% of the cases and 9.2% of the controls who recalled exposure to these compounds. There was a significant ($p < 0.001$), sixfold increase in risk for soft-tissue sarcoma (OR, 5.3; 95% CI, 2.4-11), with 13 cases who had been exposed to phenoxyacetic acids. Of these 13 cases, nine had been exposed to 2,4-D and 2,4,5-T combined, two to 2,4,5-T alone, one to MCPA alone, and one possibly to 2,4-D only. The authors noted that the effects of the individual chemical substances could not be evaluated, as nearly all of the exposed subjects were also exposed to chlorinated dioxins, including 2,3,7,8-TCDD.

Eriksson *et al.* (1981) confirmed the finding of Hardell and Sandstrom of an association between soft-tissue sarcoma and phenoxyacetic acids in southern Sweden, where MCPA and 2,4-D have been

widely used. The study involved 110 cases of soft-tissue sarcoma reported in 1974–78 and 220 controls from the general population. The ORs were 6.8 (95% CI, 2.6–17) for exposure to any phenoxyacetic acid herbicide and 4.2 for exposure to chlorphenoxyacetic acid herbicides other than 2,4,5-T.

Vineis *et al.* (1986) studied cases of soft-tissue sarcoma among female rice weeders in northern Italy, where phenoxyacetic acid herbicides have been used since the beginning of the 1950s. Interviews were carried out with 68 persons (31 women) with histologically confirmed soft-tissue sarcoma and 158 controls (73 women) who had been exposed to 2,4-D, MCPA, and 2,4,5-T. For live women who had been exposed to phenoxyacetic acid herbicides at any time, the OR was 2.7 (90% CI, 0.59–12). For women < 75 years old at the time of interview and who had been exposed in 1950–55, the age-adjusted OR was 15 (90% CI, 1.3–180).

Smith *et al.* (1983, 1984) investigated the association between soft-tissue sarcoma and exposure to phenoxyacetic acid herbicides in New Zealand. The authors selected 82 subjects with soft-tissue sarcoma and 92 controls with other types of cancer from the National Cancer Registry for the years 1976–80. The study failed to show any statistically significant association between use of the herbicides and soft-tissue sarcoma. The OR was 1.3 (90% CI, 0.7–2.5) for those potentially exposed and 1.6 (90% CI, 0.7–3.3) for those probably or definitely exposed for more than one day before the five years prior to cancer registration.

Hoar *et al.* (1986) conducted a population-based case–control study in Kansas, USA, where 2,4-D was the most commonly used herbicide; 2,4,5-T was also used, ‘along with myriad other chemicals’. The study comprised 113 soft-tissue sarcoma cases identified through the University of Kansas Cancer Data Service for the years 1976–82 and 948 controls from the general population of the state. No consistent pattern of excess risk for soft-tissue sarcoma was seen for farmers when compared with non-farmers (OR, 1.0; 95% CI, 0.7–1.6), for herbicide use (OR, 0.9; 95% CI, 0.5–1.6) or for duration and frequency of herbicide use (OR, 1.1; 95% CI, 0.7–1.7).

In a population-based case–control study, Woods *et al.* (1987) evaluated the relationship between occupational exposure of men in Washington State, USA, to phenoxyacetic acid herbicides and chlorinated phenols and the risk of developing soft-tissue sarcoma. The study comprised 128 cases of soft-tissue sarcoma and 694 randomly selected controls without cancer. No statistically significant association was seen with exposure to phenoxyacetic acid herbicides (OR, 0.89; 95% CI, 0.4–1.9).

(ii) *Non-Hodgkin's lymphoma*

The association between exposure to phenoxyacetic acid herbicides and the development of non-Hodgkin's lymphoma has been studied in Sweden, New Zealand, and Kansas, Washington, Nebraska, Iowa, and Minnesota, USA. The overall results of these studies suggest an association, although the evidence is not entirely consistent. Less clear, but still suggestive, is the evidence for a specific association between non-Hodgkin's lymphoma and exposure to 2,4-D. These studies must be interpreted with caution, however, because it is difficult to isolate the specific herbicide (or other factor) that is responsible for the association, which may be due to other chemicals that farmers mix with 2,4-D or with impurities in the 2,4-D that was sold commercially. The association with 2,4-D has not been replicated; and use of 2,4-D may serve as a surrogate for some other, unknown confounding factors. The specific findings are described below.

Hardell (1981) examined the association between exposure to phenoxyacetic acids or chlorophenols and malignant lymphoma in Sweden. The study comprised 60 hospitalized patients with Hodgkin's

disease, 109 with non-Hodgkin's lymphoma, and 338 controls from the general population. The questionnaire method used was similar to that of Hardell and Sandstrom (1979). A significantly increased risk was found with exposure to phenoxyacetic acid herbicides (OR, 4.8; 95% CI, 2.9-8.1). Although risk estimates were not reported separately for Hodgkin's disease and non-Hodgkin's lymphoma, the authors reported no meaningful difference.

Hoar *et al.* (1986) conducted a population-based case-control study in Kansas, USA, that comprised 121 cases of Hodgkin's disease and 170 of non-Hodgkin's lymphoma identified through the University of Kansas Cancer Data Service for the years 1976-82, and 948 controls from the general population of the State. No association was seen between use of phenoxyacetic acid herbicides and Hodgkin's disease. When the rates of non-Hodgkin's lymphoma for non-farmers were used for comparison, associations of borderline significance were found for farming (OR, 1.4; 95% CI, 0.9-2.1) and for phenoxyacetic acid herbicide use (OR, 2.2; 95% CI, 1.2-4.1). The OR for use of herbicides on wheat, corn, sorghum, or pasture was 1.6 (95% CI, 0.9-2.6). The relative risk (RR) for non-Hodgkin's lymphoma was significantly increased when evaluated by number of days of exposure to herbicides per year and latency. Farmers exposed for more than 20 days per year had a sixfold increase in risk for non-Hodgkin's lymphoma relative to non-farmers (OR, 6.0; 95% CI, 1.9-20). When exposure was restricted to users exposed only to 2,4-D (i.e. eliminating 2,4,5-T), the RR was increased (OR, 2.6; 95% CI, 1.4-5.0). In men exposed only to 2,4-D for > 20 days per year, the OR was 7.6 (95% CI, 1.8-32). The authors had reservations about the accuracy of this determination because of the way in which the questionnaire elicited dates and frequency of herbicide use. Frequent users who mixed or applied the herbicide themselves had an elevated risk (OR, 1.9; 95% CI, 1.1-3.3), and the risk was even higher (OR, 8.0; 95% CI, 2.3-28) for men who mixed or applied the herbicides and who were exposed for more than 20 days per year. An association was also found between the occurrence of non-Hodgkin's lymphoma and failure to use protective equipment, such as rubber gloves and masks (OR, 2.1; 95% CI, 1.0-4.2), in comparison with those who protected themselves (OR, 1.5; 95% CI, 0.7-3.1). The results were difficult to interpret, because the information on exposure was gleaned exclusively from interviews with subjects or their next-of-kin. There is reasonable doubt about whether the next-of-kin would be knowledgeable about the subject's daily weed-control practices or be able to recall with precision such practices 15-20 years later. Furthermore, as no data were collected on the frequency or duration of 2,4-D use, it was not possible to estimate directly an association between the amount of exposure to 2,4-D and non-Hodgkin's lymphoma.

In another population-based case-control study, Woods *et al.* (1987) evaluated the relationship between occupational exposure of men in western Washington State, USA, to phenoxyacetic acid herbicides and chlorinated phenols and the risk of developing non-Hodgkin's lymphoma. The study comprised 576 cases of non-Hodgkin's lymphoma and 694 randomly selected controls with cancer. An association was found between non-Hodgkin's lymphoma and application of herbicides in farming (OR, 1.3; 95% CI, 1.0-1.7) or forestry (OR, 4.8; 95% CI, 1.2-19); however, the forestry sprayers reported combined use of 2,4-D and 2,4,5-T and use of commercial preparations containing other chemicals. The risk for developing non-Hodgkin's lymphoma was also increased for workers potentially exposed to phenoxyacetic acid herbicides in any occupation for a period of 15 years or longer during the 15 years before cancer diagnosis (OR, 1.7, 95% CI, 1.1-2.8). No statistically significant association was seen between non-Hodgkin's lymphoma and exposure to phenoxyacetic acid herbicides (OR, 1.2; 95% CI, 0.8-1.9), even at high levels. Men who reported using 2,4-D specifically had an OR of 0.73 (95% CI, 0.43-1.3), although it was difficult to determine if this OR was controlled for other exposures. In a later report, Woods and Polissar (1989) concluded that phenoxyacetic acid herbicide preparations (e.g. 2,4-D and 2,4,5-T) *per se* do not independently increase the risk but may enhance the risks associated with use of various pesticides and other chemicals in agriculture.

Pearce *et al.* (1986, 1987) and Pearce (1989) studied non-Hodgkin's lymphoma and exposure to phenoxyacetic acid herbicides in New Zealand. In contrast to the USA, where the herbicide evaluated was 2,4-D, the compound used predominantly in New Zealand in 1950–80 was 2,4,5-T. These studies comprised 183 men with non-Hodgkin's lymphoma and 338 male controls obtained from the New Zealand Cancer Registry for the years 1977–81. No excess risk was found (OR, 1.0; 90% CI, 0.7–1.5). When the risk for non-Hodgkin's lymphoma was examined by the number of days of use by year, the trend was not significant, but the risk did increase with use for 10–19 days per year (OR, 2.2; 95% CI, 0.4–13) and then decreased (OR, 1.1; 95% CI, 0.3–4.1) with use for ≥ 20 days per year.

Zahm *et al.* (1990) examined the association between exposure to 2,4-D and the development of non-Hodgkin's lymphoma in eastern Nebraska, USA, in a population-based case-control study that comprised 201 white men with non-Hodgkin's lymphoma and 725 controls. The distinctive feature of this study was that specific information was obtained on the duration and frequency of 2,4-D use. No excess risk for non-Hodgkin's lymphoma was found in subjects with a history of ever having worked or lived on a farm (OR, 0.9; CI, 0.6–1.4), but men who mixed or applied 2,4-D had a 50% increased risk (OR, 1.5; 95% CI, 0.0–2.5). The risk was even higher for farmers who had handled (mixed or applied) 2,4-D for ≥ 21 days per year (OR, 3.3; 95% CI, 0.5–22; $p = 0.051$). No association was seen, however, with the number of years 2,4-D was used on the farm ($p = 0.274$). The risk was also raised with the time that farmers wore their application work clothes before changing into clean clothes: the OR was 1.1 (95% CI, 0.4–3.1) when the clothes were changed immediately after handling and 1.5 (95% CI, 0.8–2.6) for those who changed clothes at the end of the work day or 4.7 (95% CI, 1.1–21) for those who waited until the following day or later to change their clothes. As in the study in Kansas, information on exposure was gleaned exclusively from interviews with subjects or their next-of-kin. The suggestion of an increased risk was based on only three patients with non-Hodgkin's lymphoma who reported use of 2,4-D for ≥ 21 days per year, derived almost entirely from responses of next-of-kin. No trend of increasing risk with increasing days of use was seen when the patients themselves reported on their past exposure.

Cantor *et al.* (1992) studied pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota, USA. The study comprised 622 white men with non-Hodgkin's lymphoma and 1245 white controls. In comparison with the rates for non-farmers, there was a small increase in risk for non-Hodgkin's lymphoma among men who had ever lived or worked on a farm as an adult (OR, 1.2; 95% CI, 1.0–1.5). No significant increase in risk was seen for subjects who had ever handled, mixed, or applied specific herbicides. Use of 2,4-D resulted in similar ORs in the following analyses: for those who had ever mixed or applied 2,4-D (OR, 1.2; 95% CI, 0.9–1.8); for those who had handled 2,4-D with protective equipment (OR, 1.2; 95% CI, 0.9–1.6) or without protective equipment (OR, 1.2; 95% CI, 0.9–1.7); and for those who first used 2,4-D before 1965 in Iowa (OR, 1.2; 95% CI, 0.9–1.9) and Minnesota (OR, 1.4; 95% CI, 0.9–2.3). The authors reported only limited information relevant to the hypothesis of an association between exposure to 2,4-D and cancer. Specific information on the frequency of use of 2,4-D was not reported.

(b) Cohort studies

Cohort studies conducted among workers with occupational exposure to phenoxyacetic acid herbicides have not confirmed the initial hypothesis of an association between exposure to 2,4-D and either soft-tissue sarcoma or non-Hodgkin's lymphoma. While the cohort studies conducted in Sweden, Finland, and the USA failed to show an association (Riihimäki *et al.*, 1983; Wiklund & Holm, 1986; Wiklund *et al.*, 1987; Bond *et al.*, 1988; Wiklund *et al.*, 1988; Wigle *et al.*, 1990), positive results were seen in four further studies (Lynge, 1985; Coggon *et al.*, 1991; Saracci *et al.*, 1991), which, however,

provide conflicting results, each showing an increase in the risk for only one of the two cancers of concern (a different cancer in each cohort). There was an increased risk for soft-tissue sarcoma among Danish workers employed in the manufacture of phenoxyacetic acid herbicides, principally MCPA (Lyngé, 1985), but there was an insignificant increase in risk for soft-tissue sarcoma among workers exposed to multiple phenoxyacetic acid herbicides and chlorophenol (Saracci *et al.*, 1991). A slight increase in the risk for non-Hodgkin's lymphoma was seen among British cohorts exposed to 2,4-D, MCPA, 2,4,5-T, and other phenoxyacetic acids in a manufacturing plant (Coggon *et al.*, 1991). The specific findings are described below.

Lyngé (1985) examined cancer incidence among Danish chemical workers involved in the manufacture of phenoxyacetic acid herbicides in two plants, with 3844 workers in one and 615 in the other. The phenoxyacetic acid herbicides manufactured in these two plants were 2,4-D, dichlorprop, and 2,4,5-T. Cancer cases were identified by linkage with the Danish National Cancer Registry, and the expected numbers of cancer cases were calculated from the incidence rates in the general Danish population. An excess of soft-tissue sarcoma was found, with five cases among male workers and 1.8 expected (relative risk [RR], 2.7; 95% CI, 0.88–6.3); no cases occurred in female workers (0.75 expected). When the latency exceeded 10 years, four cases of soft-tissue sarcoma were observed, with 1.1 expected (RR, 3.7; 95% CI, 1.0–9.4). It should be noted that the chemical plants in which the workers were employed manufactured diverse products, and three of the four patients with soft-tissue sarcoma had been employed for three months or less; only one had been assigned to chlorophenoxyacetic acid operations (0.26 expected). Malignant lymphomas occurred in seven men, with 5.3 cases expected (RR, 1.3; 95% CI, 0.52–2.7), and in one woman, with 1.2 expected (RR, 0.83). None of the seven cases of malignant lymphoma occurred in the department producing phenoxyacetic acid herbicides. The author did not estimate the RR specifically for exposure to 2,4-D.

Wiklund and Holm (1986) and Wiklund *et al.* (1988) studied 354 620 male Swedish agricultural or forestry workers, dividing the cohort into six subcohorts with different presumed exposure to phenoxyacetic acid herbicides. These workers were compared with a reference population of 1 725 845 workers who were not involved in agriculture or forestry. The study did not show a significant excess risk for soft-tissue sarcoma or non-Hodgkin's lymphoma in agricultural or forestry workers in comparison with other groups. Between 1961 and 1979, 331 cases of soft-tissue sarcoma were observed in the study cohort and 1508 in the reference group (RR, 0.9; 95% CI, 0.8–1.0). Non-Hodgkin's lymphoma occurred in 861 men in the study cohort. The RR was not significantly increased in any subcohort, did not differ significantly between the subcohorts, and showed no time-related increase in the total cohort or any subcohort.

Bond *et al.* (1988) investigated the mortality of 878 workers potentially exposed to 2,4-D and its derivatives during their manufacture, formulation, or packaging between 1945 and 1983. Exposure was estimated by establishing an 8-h time-weighted average for each task, and the workers were categorized into three exposure groups: < 0.5, 0.5–4.9, and > 5.0 mg/m³ per year. Special attention was given to deaths from brain neoplasms because of the brain astrocytomas seen in male rats fed 2,4-D in the diet; however, none of the 111 deaths in the cohort was due to a brain neoplasm. There were two deaths from non-Hodgkin's lymphoma (one with generalized lymphosarcoma and the other with reticulum-cell sarcoma) among a subset of workers with potential additional exposure to dioxins (two observed; 0.5 expected; RR, 3.9; 95% CI, 0.4–14). The authors concluded that the results did not support a cause-effect relationship between exposure to 2,4-D and mortality from all causes or from any specific cancer. Bloemen *et al.* (1993) reported the results of four years of additional follow-up, through 1986, for the cohort studied by Bond *et al.* No new deaths from non-Hodgkin's lymphoma were observed.

Wigle *et al.* (1990) studied the mortality of almost 70 000 male farmers in Saskatchewan, Canada, identified in the 1971 Census of Agriculture. No excess mortality was seen for any cause of death, including non-Hodgkin's lymphoma, but a correlation was found between non-Hodgkin's lymphoma and area sprayed with herbicides. Among farmers with < 1000 acres (~ 400 ha), the RR rose with the area sprayed with herbicides: < 100 acres (~ 40 ha), RR, 1.3 (95% CI, 0.7–2.4); 100–249 acres (~ 40–100 ha), RR, 1.9 (95% CI, 1.2–3.3), and > 250 acres (~ 100 ha), RR, 2.2 (95% CI, 1.0–4.6). The authors reported that the chlorophenoxy compound in general use in the area was 2,4-D (90% by weight throughout the 1960s and 75% in the 1970s), but the exposure was not directly related to cases of the disease.

Coggon *et al.* (1991) examined cancer mortality and incidence at four factories in England that produced phenoxyacetic acid herbicides. The four cohorts comprised 2239 men employed during 1964–85 who were exposed not only to 2,4-D, but also to MCPA, 2,4,5-T, and other phenoxyacetic acid herbicides. The subjects were traced through the National Health Service Central Registrar and the National Insurance Index, and their mortality was compared with that in the national population. No cases of soft-tissue sarcoma or Hodgkin's disease were identified, but there were two deaths from non-Hodgkin's lymphoma with 0.87 expected (RR, 2.3; 95% CI, 0.3–8.3), both of which occurred > 10 years after first exposure to phenoxyacetic acid compounds.

Green (1991) studied the mortality of forestry workers who had been employed for six months or more in forestry work at a Canadian public utility during the period of 1950–82. The cohort consisted of 1222 men exposed to 2,4-D and other phenoxyacetic acid herbicides. No overall excess mortality due to soft-tissue sarcoma or non-Hodgkin's lymphoma was seen. The only statistically significant finding was for suicide, with 11 cases observed and 5.2 expected.

Saracci *et al.* (1991) surveyed a population of 16 863 male and 1527 female production workers or sprayers in 10 countries, identified through the International Registry of Workers Exposed to Phenoxy Herbicides and their Contaminants, established by the International Agency for Research on Cancer and the US National Institute of Environmental Health Sciences. The cohorts of Lynge (1985), Coggon *et al.* (1991), and Green (1991), described above, were included. The workers were thus exposed to 2,4-D, dichlorprop, 2,4,5-T, MCPA, other phenoxyacetic acids, and a number of chlorinated phenols. There was no overall increase in mortality from any cause. Four deaths due to soft-tissue sarcoma were seen, with 2.0 expected (RR, 2.0; 95% CI, 0.53–5.0); three occurred in sprayers (RR, 8.8; 95% CI, 1.8–26), all occurred 10–19 years after first exposure, and two of the cases arose after exposure of less than one year. Since the workers were exposed to a number of chlorophenoxyacetic acid herbicides and chlorinated phenols, it could not be determined which, if any, of these chemicals was responsible for the reported increase in risk for soft-tissue sarcoma.

Riihimäki *et al.* (1983) studied 1926 Finnish farm workers who had been exposed to phenoxyacetic acid herbicides for at least two weeks between 1951 and 1971. There was no excess cancer risk, and no cases of soft-tissue sarcoma or non-Hodgkin's lymphoma were observed.

Wiklund *et al.* (1987) studied cases of non-Hodgkin's lymphoma and Hodgkin's disease among 20 245 Swedish pesticide applicators, 72% of whom were estimated to have been exposed to phenoxyacetic acid herbicides. The most commonly used pesticide was MCPA, but 2,4-D was also used. Lymphomas did not occur in excess: 11 cases of Hodgkin's disease (RR, 1.2; 95% CI, 0.6–2.2) and 21 cases of non-Hodgkin's lymphoma (RR, 1.0; 95% CI, 0.63–1.5) were observed, with 9.1 and 21 expected, respectively.

Asp *et al.* (1994) conducted an 18-year follow-up for cancer mortality and morbidity in a cohort of 1909 men who had sprayed chlorophenoxyacetic acid herbicides (a mixture of 2,4-D and 2,4,5-T) in 1955-71. Overall mortality from cancer was slightly less than that in the general population (SMR, 0.83; 95% CI, 0.65-1.0), and none of the deaths was due to soft-tissue sarcoma or non-Hodgkin's lymphoma. One case of non-Hodgkin's lymphoma was found, with 2.8 expected; no cases of soft-tissue sarcoma were seen.

(c) *Overall assessments of epidemiological studies*

Over the past eight years, a number of scientific panels, convened under the auspices of various groups, have evaluated the epidemiological studies that addressed the possible association between use of phenoxyacetic acid herbicides, 2,4-D in particular, and the occurrence of soft-tissue sarcoma, non-Hodgkin's lymphoma, and Hodgkin's disease. Their conclusions are summarized below.

A working group convened by the International Agency for Research on Cancer (IARC, 1987) concluded that there was limited evidence that chlorophenoxy herbicides are carcinogenic to humans. 2,4-D could not be clearly distinguished from other chlorophenoxy herbicides, some of which contain dioxins.

The Ontario Pesticide Advisory Committee of the Ontario Ministry of the Environment (Anders *et al.*, 1987), using IARC terminology, concluded that '...there is limited evidence of carcinogenicity in man from exposure to phenoxyacetic acid herbicides. In terms of exposure to 2,4-D specifically, the evidence must still be regarded as inadequate to classify it as a carcinogen.'

A panel at the Harvard School of Public Health (1990; Ibrahim *et al.*, 1991) concluded: 'Although a cause-effect relationship is far from being established, the epidemiological evidence for an association between exposure to 2,4-D and non-Hodgkin's lymphoma is suggestive and requires further investigation. There is little evidence of an association between use of 2,4-D and soft-tissue sarcoma or Hodgkin's disease, and no evidence of an association between 2,4-D use and any other form of cancer.'

Munro *et al.* (1992) concluded: 'The case-control epidemiological studies that have been the source of the cancer risk hypothesis are inconclusive. Problems in assessing exposure based on patient's memories make these studies difficult to interpret. Cohort studies of exposed workers do not generally support the specific hypothesis that 2,4-D causes cancer. Taken together, the epidemiological studies provide, at best, only weak evidence of an association between 2,4-D and the risk of cancer.'

The Joint Committee of the Science Advisory Board/Scientific Advisory Panel (US Environmental Protection Agency, 1994) concluded '...that while there is some evidence that non-Hodgkin's lymphoma may occur in excess in populations which are likely to be exposed to 2,4-D, the data are not sufficient to conclude that there is a cause and effect relationship between exposure to 2,4-D and non-Hodgkin's lymphoma. The data are, however, sufficient to require continued examination of the issue through further studies.'

Comments

2,4-D was rapidly absorbed, distributed, and excreted after oral administration to mice, rats, and goats. At least 86-94% of an oral dose was absorbed from the gastrointestinal tract in rats. Once absorbed, 2,4-D was widely distributed throughout the body but did not accumulate because of its rapid

clearance from the plasma and rapid urinary excretion. 2,4-D was excreted rapidly and almost exclusively (85–94%) in urine by 48 h after treatment, primarily as unchanged 2,4-D. No metabolites have been reported other than conjugates. Pharmacokinetic studies with salts and esters of 2,4-D have shown that the salts dissociate and esters are rapidly hydrolysed to 2,4-D, after which their fate was indistinguishable from that of the acid. The similarity in the fate of 2,4-D and its salts and esters explains their similar toxicity.

In humans who ingested 2,4-D, it was quickly absorbed and excreted rapidly in the urine; about 73% of the administered dose was found in the urine after 48 h. No metabolites were detected.

After dermal applications of 2,4-D to volunteers, $\leq 5.8\%$ of the dose was absorbed within 120 h. When the acid and its dimethylamine (DMA) salt were applied, about 4.5% of the acid and 1.8% of the salt were absorbed, and, of this, about 85% of the acid and 77% of the salt were recovered in the urine 96 h after application.

2,4-D, its amine salts, and its esters are slightly toxic when administered orally or dermally, the oral LD₅₀ values being 400–2000 mg/kg bw and the dermal LD₅₀ value generally exceeding 2000 mg/kg bw. In rats exposed to 2,4-D at the maximum attainable concentration (up to 5.39 mg/litre) by inhalation for 4 h, no deaths were seen. While 2,4-D and its amine salts and esters do not induce dermal irritation in rabbits or dermal sensitization in guinea-pigs, they cause severe eye irritation in rabbits. WHO has classified 2,4-D as ‘moderately hazardous’ (WHO, 1996).

In mice fed diets that provided 2,4-D at doses of 0, 5, 15, 45, or 90 mg/kg bw per day for three months, renal lesions were observed in animals of each sex at all doses. An NOAEL was not identified.

In mice fed diets providing 2,4-D at doses of 0, 1, 15, 100, or 300 mg/kg bw per day for 90 days, treatment-related changes were observed in animals of each sex at doses ≥ 100 mg/kg bw per day. These effects included decreases in glucose level in females, decreases in thyroxine activity in males, and increases in absolute and/or relative kidney weights in males. The NOAEL was 15 mg/kg bw per day.

In rats fed diets providing 2,4-D at doses of 0, 1, 5, 15, or 45 mg/kg bw per day for 90 days, renal lesions were observed at doses ≥ 5 mg/kg bw per day. The NOAEL was 1 mg/kg bw per day.

In rats fed diets providing 2,4-D at doses of 0, 1, 15, 100, or 300 mg/kg bw per day for 90 days, treatment-related changes were observed in animals of each sex at doses ≥ 100 mg/kg bw per day. These effects included decreases in body-weight gain, haematological and clinical chemical alterations, changes in organ weights, and histopathological lesions in the adrenals, liver, and kidneys. The NOAEL was 15 mg/kg bw per day.

In six studies of toxicity, rats fed diets containing the diethanolamine (DEA), DMA, isopropylamine (IPA), or triisopropanolamine (TIPA) salts or the butoxyethylhexyl (BEH) or 2-ethylhexyl (EH) esters at acid-equivalent doses of 0, 1, 15, 100, or 300 mg/kg bw per day for 13 weeks, the results demonstrated the comparable toxicity of the acid, salts, and esters. The NOAEL was 15 mg acid-equivalent per kg bw per day for all six compounds.

Dogs were given gelatin capsules containing 2,4-D at 0, 0.03, 1, 3, or 10 mg/kg bw per day or diets containing 2,4-D, the DMA salt, or the EH ester at acid-equivalent doses of 0, 0.5, 1, 3.75, or 7.5 mg/kg bw per day for 13 weeks. Treatment-related findings were observed in the three studies at doses ≥ 3.0 mg/kg bw per day. The NOAEL was 1.0 mg acid-equivalent per kg bw per day in all three studies.

In a two-year study of toxicity and carcinogenicity, mice were fed diets providing 2,4-D at doses of 1, 15, or 45 mg/kg bw per day. Increases in absolute and/or relative kidney weights and renal lesions were observed at 15 and 45 mg/kg bw per day. There was no evidence of carcinogenicity. The NOAEL was 1 mg/kg bw per day.

In another two-year study of toxicity and carcinogenicity, mice were fed diets providing 2,4-D at doses of 0, 5, 62.5, or 125 mg/kg bw per day (males) or 0, 5, 150, or 300 mg/kg bw per day (females). Dose-related increases in absolute and/or relative kidney weights and renal lesions were seen in animals

of each sex at doses ≥ 62 mg/kg bw per day. There was no evidence of carcinogenicity. The NOAEL was 5 mg/kg bw per day.

In another two-year study, rats received diets providing 2,4-D at doses of 0, 1, 5, 15, or 45 mg/kg bw per day. Renal lesions were seen in animals of each sex at doses ≥ 5 mg/kg bw per day. There was no evidence of carcinogenicity. The NOAEL was 1 mg/kg bw per day.

In a further two-year study, rats were fed diets providing 2,4-D at doses of 0, 5, 75, or 150 mg/kg bw per day. Treatment-related effects were observed in animals of each sex at doses ≥ 75 mg/kg bw per day. The effects included decreases in body-weight gains and food consumption, increases in serum alanine and aspartate aminotransferase activities, decreased thyroxine concentrations, increases in absolute and relative thyroid weights, and histopathological lesions in the eyes, kidneys, liver, lungs, and mesenteric fat. There was no evidence of carcinogenicity. The NOAEL was 75 mg/kg bw per day in males and 5 mg/kg bw per day in females.

Dogs were fed diets providing 2,4-D at doses of 0, 1, 5, or 7.5 mg/kg bw per day for 52 weeks. At 5 and 7.5 mg/kg bw per day, body-weight gains were decreased, increases were seen in blood urea nitrogen, creatinine, alanine aminotransferase activity, and cholesterol, and histopathological lesions were seen in the kidneys and liver. The NOAEL was 1 mg/kg bw per day.

In a two-generation study of reproductive toxicity, rats received dietary doses of 2,4-D of 0, 5, 20, or 80 mg/kg bw per day. Reduced body weights of F_1 dams and renal lesions in F_0 and F_1 adults were observed at 20 and 80 mg/kg bw per day. The NOAEL for parental and reproductive toxicity was 5 mg/kg bw per day.

In order to evaluate the dermal toxicity of 2,4-D and its salts and esters, rabbits received 15 dermal applications of the acid, the DEA, DMA, IPA, or TIPA salt, or the BEH or EH ester at acid-equivalent doses of 0, 10, 100, or 1000 mg/kg bw per day for 6 h per day on five days per week for 21 days. No systemic toxicity was seen at any dose, and no dermal toxicity was seen with the acid, the TIPA salt, or the BEH ester. Dermal lesions were observed in rabbits treated with the DEA, DMA, or IPA salt or the EH ester at doses ≥ 100 mg/kg bw per day. The lesions were characterized as acanthosis, hyperkeratosis, oedema, inflammation, and epidermal hyperplasia. The NOAEL was 10 mg acid-equivalent per kg bw per day for dermal toxicity and 1000 mg acid-equivalent per kg bw per day (the highest dose tested) for systemic toxicity.

In a study of developmental toxicity, pregnant SpragueDawley rats were given 2,4-D in corn oil by gavage at doses of 12.5, 25, 50, 75, or 88 mg/kg bw per day during days 6–15 of gestation. There was no maternal toxicity. Fetotoxicity was manifested as decreased fetal body weights at doses ≥ 50 mg/kg bw per day. The NOAELs were 88 mg/kg bw per day for maternal toxicity and 25 mg/kg bw per day for developmental toxicity.

In a further study, pregnant Fischer 344 rats received 2,4D in corn oil by gavage at doses of 8, 25, or 75 mg/kg bw per day during days 6–15 of gestation. Decreased body-weight gain of dams at the high dose during the treatment period and increased incidences of skeletal variations (7th cervical and 14th rudimentary ribs and missing sternbrae) were observed at 75 mg/kg bw per day. The NOAEL was 25 mg/kg bw per day for both maternal and developmental toxicity.

The developmental toxicity of the DEA, DMA, IPA, and TIPA salts and the BEH and EH esters was evaluated in pregnant rats after oral administration during days 6–15 of gestation. The acid-equivalent doses tested were 11, 55, or 110 mg/kg bw per day for DEA; 12.5, 50, or 100 mg/kg bw per day for the DMA salt; 9, 25, or 74 mg/kg bw per day for the IPA salt; 12, 37, or 120 mg/kg bw per day for the TIPA salt; 17, 50, or 120 mg/kg bw per day for the BEH ester; and 10, 30, or 90 mg/kg bw per day for the EH ester. The maternal and developmental toxicity of the salts and esters of 2,4-D was comparable to that of the acid. Maternal toxicity, as evidenced by reduced body-weight gain during treatment, was seen in all dams at the high dose of each compound; in addition, mortality, clinical signs, and reduced food consumption were seen in dams given 120 mg/kg bw per day TIPA salt. Although embryo- and

fetotoxicity and teratogenicity were observed with the high dose of the TIPA salt, this may be attributed to maternal toxicity; none of the other compounds had such effects. No external gross or visceral anomalies (malformations or variations) were observed in any of the fetuses, but skeletal variations were seen at the high dose of each compound except the IPA salt which were similar to those seen in the fetuses of dams given the acid. The overall NOAELs were approximately 10 mg acid-equivalent per kg bw per day for maternal toxicity and 50 mg acid-equivalent per kg bw per day for developmental toxicity.

In a study of developmental toxicity, pregnant rabbits were given 2,4-D orally at 0, 10, 30, or 90 mg/kg bw per day during days 6–18 of gestation. Maternal toxicity, which included clinical signs, abortions, and reduced body-weight gain during and after the treatment period, was seen only at the high dose. No gross, visceral, or skeletal malformations or variations were seen in fetuses at any dose. The NOAELs were 30 mg/kg bw per day for maternal toxicity and 90 mg/kg bw per day (the highest dose tested) for developmental toxicity.

The developmental toxicity of the DEA, DMA, IPA, and TIPA salts and the BEH and EH esters was evaluated in rabbits after oral administration during days 6–18 of gestation. The acid-equivalent doses tested were 10, 30, or 60 mg/kg bw per day for the DEA salt; 10, 30, or 90 mg/kg bw per day for the DMA salt; 10, 30, or 75 mg/kg bw per day for the IPA salt; and 10, 30, or 75 mg/kg bw per day for the TIPA salt and for the BEH and EH esters. Unlike 2,4-D, which produced maternal toxicity only at the high dose, most of the amine salts and esters were maternally toxic at the middle and high doses, as evidenced by mortality, clinical signs of neurotoxicity, abortions, and decreases in body-weight gain. No gross, visceral, or skeletal malformations or variations were seen in fetuses at any dose. The overall NOAELs were approximately 10 mg acid-equivalent per kg bw per day for maternal toxicity and 90 mg acid-equivalent per kg bw per day (the highest dose tested) for developmental toxicity.

In summary, of the four salts tested for developmental toxicity, only the TIPA salt had developmental toxicity in rats and only at a maternally toxic dose; no developmental toxicity was seen in rabbits with this or the other salts. Consequently, the Meeting concluded that the developmental toxicity of the TIPA salt is of little concern.

The genotoxic potential of 2,4-D has been adequately evaluated in a range of assays *in vivo* and *in vitro*. Overall, the responses observed indicate that 2,4-D is not genotoxic, although conflicting results were obtained for mutation in *Drosophila*. In a more limited range of assays, the DEA, DMA, IPA, and TIPA salts and the BEH and EH esters were also not genotoxic *in vivo* or *in vitro*. The Meeting concluded that 2,4-D and its salts and esters are not genotoxic.

In rats given single doses of 2,4-D at 0, 15, 75, or 250 mg/kg bw by gavage, there were no treatment-related gross or neuropathological changes at any dose. Animals of each sex at the highest dose exhibited incoordination and gait abnormalities on day 1, but the signs had disappeared by day 5. The NOAEL was 75 mg/kg bw. When rats were fed diets containing 2,4-D at doses of 0, 5, 75, or 150 mg/kg bw per day for 12 months, neurotoxicity, manifested as increased relative forelimb grip strength, was seen in animals of each sex at 150 mg/kg bw per day. The NOAEL was 75 mg/kg bw per day.

Epidemiological studies have suggested an association between the development of soft-tissue sarcoma and non-Hodgkin's lymphoma and exposure to chlorophenoxy herbicides, including 2,4-D. The results of these studies are not, however, consistent; the associations found are weak, and conflicting conclusions have been reached by the investigators. Most of the studies did not provide information on exposure specifically to 2,4-D, and the risk was related to the general category of phenoxyacetic acid herbicides, a group that includes 2,4,5-T, which can be contaminated with dioxins. Case-control studies provide little evidence of an association between the use of 2,4-D and soft-tissue sarcomas. Although some case-control studies have shown a relationship with non-Hodgkin's lymphoma, others (even the positive studies) have produced inconsistent results, raising doubt about

the causality of the relationship. Cohort studies of exposed workers have not confirmed the hypothesis that 2,4-D causes either neoplasm.

The Meeting was informed of the on-going 'Agricultural Health Study' initiated in North Carolina and Iowa, USA, and of a study of pesticide applicators in Finland. The Agricultural Health Study addresses both cancer and non-cancer risks in men and women directly exposed to pesticides and other agricultural agents, including neurotoxicity, reproductive effects, immunological effects, kidney disease, non-malignant respiratory disease, and the growth and development of their children.

The Meeting concluded that the toxicity of the salts and esters of 2,4-D was comparable to that of the acid. An ADI was therefore established for the sum of 2,4-D and its salts and esters, expressed as 2,4-D. An ADI of 0–0.01 mg/kg bw was established on the basis of the NOAEL of 1 mg/kg bw per day in the one-year study of toxicity in dogs and the two-year study in rats and using a safety factor of 100.

Toxicological evaluation

Levels that cause no toxic effect

Mouse:	15 mg/kg bw per day (13-week study of toxicity) 5 mg/kg bw per day (two-year study of toxicity and carcinogenicity)
Rat:	1 mg/kg bw per day (two-year study of toxicity and carcinogenicity) 5 mg/kg bw per day (two-generation study of reproductive toxicity) 10 mg acid-equivalent/kg bw per day (maternal toxicity in a series of studies of developmental toxicity with salts and esters) 15 mg acid-equivalent/kg bw per day (series of 13-week studies of toxicity with salts and esters) 25 mg/kg bw per day (maternal and developmental toxicity in a study of developmental toxicity)
Rabbit:	10 mg acid-equivalent/kg bw per day (maternal toxicity in a series of studies of developmental toxicity with salts and esters) 30 mg/kg bw per day (maternal toxicity in a study of developmental toxicity) 90 mg/kg bw per day (highest dose tested in studies of developmental toxicity with the acid and its salts and esters)
Dog:	1 mg/kg bw per day (13-week and one-year studies of toxicity)

Estimate of acceptable daily intake for humans

0–0.01 mg/kg bw (sum of 2,4-D and its salts and esters expressed as 2,4-D)

Studies that would provide information useful for continued evaluation of the compound

1. Follow-up on the Agricultural Health Study in North Carolina and Iowa in the USA
2. Follow-up on the study of pesticide applicators in Finland

Toxicological criteria for estimating guidance values for dietary and non-dietary exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) and its amine salts and esters

Exposure	Relevant route, study type, species	Results, remarks
Short-term (1-7 days)	Oral, toxicity, rat, acid, salts, and esters	LD ₅₀ = 400–2000 mg/kg bw
	Dermal, toxicity, rabbit, acid, salts, and esters	LD ₅₀ > 2000 mg/kg bw
	Inhalation, toxicity, rat, acid, salts, and esters	LC ₅₀ > 0.84–5.4 mg/litre
	Dermal, irritation, rabbit, acid, salts, and esters	Not irritating
	Ocular, irritation, rabbit, acid, salts, and esters	Severely irritating
	Dermal, sensitization, guinea-pig, acid, salts, and esters	Not sensitizing
	Oral, single dose, neurotoxicity, rat, acid	NOAEL = 75 mg/kg bw
Medium-term (1-26 weeks)	Dietary, 3 months, toxicity, mouse	NOAEL = 15 mg/kg bw per day, renal toxicity
	Dietary, 3 months, toxicity, rat	NOAEL = 1 mg/kg bw per day, renal lesions
	Dietary, 3 months, toxicity, rat, salts and esters	NOAEL = 15 mg acid-equivalent / kg bw per day, renal toxicity
	Dietary or capsule, 3 months, toxicity, dog	NOAEL = 1 mg acid-equivalent/kg bw per day, reduced body-weight gain and other systemic toxicity
	Dermal, 21 days, repeated dose, rabbit, acid, salts and esters	NOAEL = 1000 mg acid-equivalent/kg bw per day, highest dose tested
	Dietary, 2 generations reproductive toxicity, rat	NOAEL = 5 mg/kg bw per day, reduced body weights in F ₀ and F ₁ adults
	Oral (gavage), developmental toxicity, rat	NOAEL = 25 mg/kg bw per day, maternal and developmental toxicity
	Oral (gavage), developmental toxicity, rat, salts and esters	NOAEL = 10 mg acid-equivalent/kg bw per day for maternal toxicity; 50 mg acid-equivalent /kg bw per day for developmental toxicity
	Oral (gavage), developmental toxicity, rabbit	NOAEL = 30 mg/kg bw per day for maternal toxicity; > 90 mg/kg bw per day for developmental toxicity
Long-term (2 1 year)	Oral (gavage), developmental toxicity, rabbit, salts and esters	NOAEL = 10 mg acid-equivalent/kg bw per day for maternal toxicity; > 90 mg acid-equivalent/kg bw per day (highest dose tested) for developmental toxicity
	Dietary, 2 years, toxicity and carcinogenicity, mouse	NOAEL = 5 mg/kg bw per day, renal effects; no evidence of carcinogenicity
	Dietary, 2 years, toxicity and carcinogenicity, rat	NOAEL = 1 mg/kg bw per day, renal lesions; no evidence of carcinogenicity
	Dietary, 1 year, toxicity, dog	NOAEL = 1 mg/kg bw per day, changes in serum chemistry and lesions in kidneys and liver

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