2,4-Dichlorophenoxyacetic Acid

Guideline

The interim maximum acceptable concentration (IMAC) for 2,4-dichlorophenoxyacetic acid in drinking water is 0.1 mg/L (100 µg/L).

Identity, Use and Sources in the Environment

2,4-Dichlorophenoxyacetic acid, commonly referred to as 2,4-D, is a systemic chlorophenoxy herbicide used widely in Canada (more than four million kilograms annually)[1] in the control of broadleaf weeds in cereal cropland and on industrial property, lawns, turf, pastures and non-cropland. It is also used to control aquatic weeds. Commercial 2,4-D products are marketed as alkali salts, amine salts and ester formulations.

2,4-D has a molecular weight of 221.0 and a molecular formula of $C_8H_6O_3Cl_2$. It is soluble in organic solvents. The reported solubility of the free acid in water varies considerably and is given as 0.09% or 900 mg/L at 25°C.[2] In a review of the literature concerning the solubility of 2,4-D, Que Hee and Sutherland[3] selected the value of 522 mg/L as the most reliable. The dimethylamine salt is very soluble (300%), whereas the esters are insoluble in water but soluble in organic solvents.[2] 2,4-D has a vapour pressure of 1.05×10^{-2} mmHg at 25°C.[3] The vapour pressures of the various esters are lower, ranging from 1.1×10^{-3} mmHg for the ethyl ester to 2×10^{-6} mmHg for the isooctyl ester.[2]

Impurities may be present in the technical product as a result of the manufacturing process. These include 2,6-dichlorophenoxyacetic acid, the 2- and 4-chlorophenoxyacetic acids, bis-(2,4-dichlorophenoxy)acetic acid, and 2,4-dichlorophenol. During the 1960s, contamination with polychlorinated dibenzodioxins and furans was reported, but the most toxic congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), has not been found.[4] Changes in manufacturing practices and regulations now limit dioxin contamination to less than 0.1 ppm.

2,4-D may enter the environment through effluents and spills arising from its manufacture and transport and through direct application as a weed control agent. Esters of 2,4-D are readily hydrolysed to the free acid, which, in turn, is rapidly degraded in soil under many environmental conditions. 2,4-D is removed from the environment principally by biodegradation through several possible pathways, with the formation of 2,4-dichlorophenol as an intermediate.[5] The half-life of 2,4-D in soil is reported to range from four to seven days in most soil types[6–8] and up to six weeks in acidic soils.[8–10] Factors influencing the biodegradation rate include the concentration and formulation of the herbicide applied, soil type, concentration and acclimatization of micro-organisms, moisture, temperature, pH and oxygen content.[2,11,12] The extent to which 2,4-D will leach to groundwater is inversely related to the organic matter content and directly related to soil pH.[3] 2,4-D was considered a "marginal leacher" by the U.S. Environmental Protection Agency (EPA)[13] and given an intermediate rating by Agriculture Canada.[14]

Residues of 2,4-D may be present in surface waters as a result of direct application, runoff or aerial drift from treated areas or leaching from groundwaters. The herbicide is rapidly biodegraded in water, although some may be degraded by photolysis near the surface. The main degradation product of 2,4-D is 2,4-dichlorophenol.[15] Persistence of 2,4-D in aquatic systems is dependent on water type, nutrient levels, rain, sunlight, temperature, population and previous exposure of micro-organisms and oxygen content.[3,16] Half-lives range from one to several weeks in aerobic conditions and can exceed 80 to 120 days in anaerobic conditions.[17] 2,4-D is not expected to accumulate in bottom sediments and muds.[3] 2,4-D does not bioaccumulate in aquatic or terrestrial organisms (with the exception of some algae) because of its rapid degradation.[2]

Atmospheric contamination by 2,4-D may occur as a result of volatilization and drift from application by spraying. Residues in the atmosphere are predominantly in the form of isopropyl and butyl esters.[17] 2,4-D is removed from the atmosphere by photo-oxidation and rainfall, with a half-life of less than one day.[18]

Exposure

2,4-D was detected in 52 of 805 samples of raw and treated drinking water from municipal and private supplies in surveys conducted in six Canadian provinces from 1971 to 1986. The maximum concentration found was 29 µg/L (ppb).[19] No residues have been detected in drinking water samples analysed routinely in U.S. market basket surveys (detection limit 5 µg/L).[4] Seventy-eight of 447 samples of surface waters in three Canadian agricultural areas surveyed from 1981 to 1985 had detectable 2,4-D concentrations; mean annual detectable concentrations of the herbicide in these areas ranged from 0.01 to 0.7 µg/L.[20] 2,4-D was detected in 38.5% of 1386 surface water samples from the Canadian prairies tested from 1971 to 1977 (detection limit 0.004 µg/L); mean levels were less than 0.3 µg/L.[21]

Although 2,4-D has low potential for bioaccumulation, human exposure to this herbicide may occur from residues in agricultural food commodities. Based on the maximum limits for pesticide residues established by the Food Directorate, the theoretical maximum daily intake of 2,4-D in food averages 0.87 µg/kg bw for a 60-kg adult. The intake of 2,4-D found in a U.S. total diet study for 1987 was 0.0001 µg/kg bw per day for a 60- to 65-year-old female and less than 0.0001 µg/kg bw per day for six- to 11-month-old children and 14- to 16-year-old males.[22] This is three to four orders of magnitude below the theoretical intake. No residues of 2,4-D ester were detected in a total diet study conducted in Canada during 1976 to 1978 (minimum detectable concentration 500 ppb).[23]

In surveys of ambient air concentrations of 2,4-D in the prairies, where the herbicide was heavily used, 30% of samples from 1966 to 1975 contained less than 0.01 µg/m³; 40% of the samples had between 0.01 and 0.099 µg/m³; 20% contained between 0.1 and 1 µg/m³; and 10% had more than 1 µg/m³.[24] Average intake of airborne 2,4-D is difficult to estimate but is extremely low.

Analytical Methods and Treatment Technology

Residues of 2,4-D and its salts or esters in water are commonly measured by extraction, chemical derivatization, separation by gas–liquid chromatography and electron capture detection. This method is suitable for detection of picogram levels.[4] Electrolytic conductivity detection is also used, with a detection limit of 0.1 µg/L.[20] Other methods used in the determination of 2,4-D residues include high-performance liquid chromatography and thin-layer chromatography.[3]

Common water treatment processes are not effective in removing 2,4-D from water. Activated carbon adsorption, either powdered or granulated, is the method of choice for removing 2,4-D from drinking water supplies. Powdered activated carbon removed 90% of an initial dose of 1.0 mg/L.[25] No information was available on the effectiveness of this treatment at lower concentrations of 2,4-D.

Health Effects

Metabolism

2,4-D, administered orally as the free acid or as the sodium or amine salts, is absorbed rapidly and almost completely in rats, calves, pigs and human volunteers.[26–28] The average half-time for absorption in humans was four hours.[28] Absorption is much slower and less complete for esters of 2,4-D, which are probably hydrolysed to the free acid before absorption.[29] In humans, dermal absorption of the acid was slow.[30] Dermal absorption of 2,4-D acid ranged from 6% on the human forearm to 36% on the rabbit back. Dermal absorption of 2,4-D isooctyl salt was also 6% in humans but up to 50 to 56% in rabbits and monkeys, respectively. Conversely, dermal absorption of 2,4-D amine in aqueous solution was 58% in humans and 12 to 20% in rabbits and rats. Large variations in dermal absorption were noted depending on anatomical site and on the solute carrier.[31,32]

In rats, after absorption by the oral route, 2,4-D acid was distributed throughout the body, with peak concentrations in blood after three hours[33] and in kidneys, liver, spleen and lungs after six hours.[29] After low doses, tissue concentrations were highest in kidneys, liver, blood and lungs. The concentration in brain appeared to be lower than the concentration in other tissues in rats,[26] pigs[29] and humans.[17] High doses (250 mg/kg bw) resulted in increased accumulation in the brain of rats.[34]

In male human volunteers given an oral dose of 5 mg/kg bw, elimination of 2,4-D was fairly rapid, with a half-time for plasma clearance of 11.6 hours and an elimination half-time of 17.7 hours. Eighty-two percent was excreted unchanged in urine, and 13% was excreted as a conjugate.[28] Similar results were obtained in another study on six male volunteers in which the highest concentration in plasma was reached in 7 to 24 hours, with a plasma clearance half-time of 33 hours.[35] Seventy-five percent was excreted unchanged in urine after 96 hours, the rate being somewhat slower than in the previous study.[28] There was considerable individual variation in pharmacokinetics; a half-life of 12 to 22 hours for urinary clearance was reported after occupational exposure via inhalation.[36]

In rats, orally administered 2,4-D (25 mg/kg bw) was excreted largely unchanged, principally in urine. Clearance was saturable above a dose of 50 mg/kg bw.[37] Excretion of low doses was rapid; in rats, most of the administered dose was eliminated within 24 hours,[26] and up to 99% of a small radioactively labelled dose was

eliminated in 48 hours. A smaller percentage of doses above 50 mg/kg bw was eliminated over an 11-day period.[26] The dimethyl amine salt of 2,4-D was also rapidly absorbed and excreted after a gavage administration to rats, with peak concentration in blood in 20 minutes and 88% excretion within six hours.[27]

Human Health Effects

Symptoms of acute exposure to high doses of 2,4-D have been reported as a result of poisonings from accidental ingestion and occupational exposure during manufacture or application, usually from a combination of high dermal and inhalation exposures. Symptoms include effects on the gastrointestinal tract, such as nausea, vomiting and diarrhoea, direct myotoxic effects such as muscular weakness, stiffness, muscular spasms and partial paralysis, effects on the kidney, pulmonary oedema and effects on the central and peripheral nervous systems.[4]

Most epidemiological studies on 2,4-D conducted to date have dealt with multiple exposures to various chlorophenoxy herbicides, particularly 2,4,5-trichloro-phenoxyacetic acid (2,4,5-T), and to other chemical agents, including other pesticides and synthetic organics. Until recently, most studies have dealt with populations exposed in the 1950s and 1960s, when the trichlorophenol-based chlorophenoxy herbicides 2,4,5-T and silvex were contaminated with polychlorinated dioxins and furans, including TCDD, which has been associated with the induction of soft-tissue sarcomas (STS).[38] Non-trichlorophenol-based herbicides are believed to have been much less contaminated with the more toxic dioxins and have not been shown to contain TCDD.[4]

In a series of population-based case–referent studies conducted in Sweden in the late 1970s and early 1980s, strong associations, with relative risks (RR) in the range 5 to 7, were noted between STS and multiple lymphomas, including Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL), and the use of chloro-phenoxy herbicides (including 2,4,5-T) by agricultural or forestry workers.[39–42] The Swedish studies served to focus attention on STS, NHL and HD as the outcomes of interest in succeeding case–referent and cohort studies.

STS

In a registry-based study (55 cases) to test the reproducibility of their previous findings for STS, Hardell and Eriksson[43] observed a threefold increase in risk for STS (based on nine cases), or about half the risk observed in their previous study,[39] for those exposed to chlorophenoxy herbicides (including 2,4,5-T), when compared to population-based controls. This increase in relative risk was reduced to twofold, or borderline

significance (p = 0.09), when compared with cancer controls; this indicated that, in this study, recall bias on the part of cancer patients may have played a role in the observed elevation of risk. In a population-based case–control study in central Sweden of 237 cases with STS, exposure to phenoxyacetic acids (including 2,4,5-T) did not result in a significant increase in risk (RR 1.34; confidence interval [CI] 0.70 to 2.56). Of the 23 cases and 18 controls who were exposed, only four cases and seven controls were exposed to phenoxyacetic acids other than 2,4,5-T (i.e., assumed to be without TCDD contamination). The relative risk was not significant for this group because of low numbers.[44]

Other case–referent studies on STS conducted in New Zealand,[45,46] Italy[47] and the United States (Kansas[48] and Washington[49]) have not confirmed the association between STS and chlorophenoxy use observed in the original Swedish studies. The Kansas study[48] involved exposure to a number of herbicides including 2,4-D, but little to other chlorophenoxy herbicides, whereas subjects in the other three studies were exposed to several chlorophenoxy herbicides, with significant exposure to 2,4,5-T.

Results were negative in several cohort studies carried out in order to investigate STS in chemical workers engaged in the production of chlorophenoxy herbicides and in occupational groups whose use of these herbicides has been extensive.[50–54] No cohort was exposed solely or principally to 2,4-D, including the "2,4-D cohort" of 878 chemical workers engaged in the manufacture of 2,4-D at the U.S. facilities of Dow, 75% of whom had also been exposed to 2,4,5-T.[50] Because of the small size of most of the cohorts, not much reliance could be placed on the results, whether positive or negative. In an international historical cohort study, supported by IARC (International Agency for Research on Cancer), on 18 390 production workers or sprayers from 20 cohorts in 10 countries, a statistically non-significant twofold excess risk was noted for mortality from STS. For people first exposed 10 to 19 years before death, this excess was sixfold; however, results were based on only four observed deaths. An additional three cases were still alive, and two cases had been misclassified (not counted), although they had died of what was probably STS, according to the authors. Some effort was made in this study to separate workers potentially exposed to TCDD (n = 11 445, workers exposed to 2,4,5-T or 2,4,5-trichlorophenol) from those who were not (n = 6845). There was no difference in risk between the two groups.[55]

NHL

In a population-based Swedish case–referent study on malignant lymphoma similar to the STS studies, the combined relative risk for HD and NHL was 4.8 (CI 2.9

to 8.1), rising to a relative risk of 7.0 for more than 90 days' total lifetime exposure to a mixture of chlorophenoxy herbicides.[40,56] The relationship between chlorophenoxy exposure and NHL has been examined in four additional case–referent studies in New Zealand and the United States; all studies were conducted similarly to the Swedish studies, but only one specifically examined 2,4-D,[57,58] although an earlier study by the same group found that most chlorophenoxy herbicide use was attributable to 2,4-D.[48] The risk for NHL was not elevated for New Zealand farmers or any individuals who sprayed pesticides, primarily 2,4-D and 2,4,5-T.[59,60] Exposure characterization appeared to be poor in this study and could well have led to random misclassification errors with consequent inability to detect differences in risk. The remaining three studies all suggested a weak link between NHL and chlorophenoxy herbicide use (but not 2,4-D specifically, except in one study in Nebraska). Other chemicals also used concurrently in farming activities may have acted as covariants. In a large study in the state of Washington, with 576 cases of NHL, the relative risk increased from 1.1 (CI 0.8 to 1.4; not significant) for subjects with any past occupational exposure to chlorophenoxy herbicides, primarily 2,4-D and 2,4,5-T, to 1.7 (CI 1.0 to 2.8; borderline significance) for people occupationally exposed to chlorophenoxy herbicides for at least 15 years, and with a minimum 15-year latent period.[49]

In a case–referent study in Kansas (200 cases), farm herbicide use was marginally associated with NHL, with a relative risk of 1.4 (CI 0.9 to 2.6; not significant), but relative risk rose to 2.2 (CI 1.2 to 4.1; significant) for farmers who had used chlorophenoxy herbicides at any time (almost all 2,4-D), and to 6.0 (CI 1.9 to 19.5; significant) for those who used unspecified herbicides more than 20 days per year. The trend to increasing risk with increasing number of days' use per year was highly significant (p = 0.0004).[48] In a second study in Nebraska by the same group, the analysis concentrated on exposure to 2,4-D. The relative risk was reduced to about half the risk observed in the Kansas study for those who mixed or applied 2,4-D, and it was significant only when data were corrected for organophosphate use. For those who used 2,4-D more than 21 days per year, the relative risk of 3 was not significant, but the trend towards increasing risk with increasing number of days' use per year was marginally significant (p = 0.051). There was no trend apparent towards increasing risk as the number of years' exposure increased. The risk was highly significant for those who did not take precautions to reduce exposure by changing clothing soon after exposure or by washing immediately after handling the pesticide. There was no increase in relative risk with the failure to use protective equipment.[57,58] These results are consistent, for the most part, with the results obtained

previously by the same research group in Kansas,[48] but the risk estimates were lower and were non-significant in some cases.

In an incidence study in an agricultural area of northern Italy in which exposure was to a mix of chlorophenoxy herbicides including 2,4-D, 2,4,5-T and MCPA, the incidence of NHL (n = 141 cases) increased from 8.8 per 100 000 over the entire area to 18.2 per 100 000 in the most heavily exposed area (rate ratio 2.2; CI 1.4 to 3.5). Exposure was measured over two years in water and soil, 10 years previously; 2,4-D concentrations in water reached 70 to 460 µg/L in the most heavily contaminated area.[61]

No excess risk was observed for NHL in most of the cohort studies on occupational exposure,[50,51,53,54] although the cohorts generally were too small to provide any conclusive evidence, and all had exposures to chlorophenoxy herbicides in addition to 2,4-D. In the British cohort study, two deaths from NHL were recorded (standard mortality ratio [SMR] 272), more than 10 years after first exposure, and an additional case was still alive.[54] A recent cohort study on farmers in Saskatchewan, where the use of 2,4-D was extensive (constituting 90% and 75% by weight of all herbicides used in the 1960s and 1970s, respectively), showed a trend to increased risk of NHL with increased use of herbicides as measured by the number of hectares sprayed. Because of concomitant exposure to other agents, it was not possible to infer that the association was specifically with 2,4-D.[62] In the IARC study on 20 cohorts with 18 390 workers exposed to chlorophenoxy herbicides, there was no increase in risk of NHL (SMR 95), based on a total of 15 deaths.[55]

HD

Other than the original Swedish studies in which HD was grouped together with NHL, there is little evidence for an increase in risk of HD resulting from exposure to chlorophenoxy herbicides, based on an additional case–referent study[48] and on five cohort studies.[50,51,53–55]

Other endpoints that have been examined by one or more investigators include multiple myeloma, leukaemia, and lung, stomach and prostate cancers. However, except for lung cancer, which is known to have multiple aetiologies, no excess risks were detected for persons exposed to chlorophenoxy herbicides.

Chlorophenoxy herbicides, as a group including 2,4-D, 2,4,5-T and MCPA, have been classified by IARC in Group 2B (possibly carcinogenic to humans).[63] However, based on the studies examined here, it is not possible to ascertain the status of individual chlorophenoxy herbicides with respect to carcinogenicity, as almost all populations studied were exposed to a mixture of chlorophenoxy herbicides. In the only study

in which chlorophenoxy herbicide exposure was clearly to 2,4-D only,[57] the association with NHL was weak.

No particular pattern emerges when the data are analysed by comparing populations exposed to mixtures of 2,4,5-T, which often contained TCDD and other dioxins, with populations exposed to mixtures that did not contain 2,4,5-T or other trichlorophenol-based products. In the IARC multi-cohort study, the risk for STS was slightly increased but could not be attributed to exposure to TCDD.

Of the three endpoints examined most frequently, the associations seen in most studies were weak, usually with less than twofold increases in relative risk, with the exception of the early studies in Sweden.[40,56] The association with NHL appears to be more consistent than that with STS, in three case–control studies and an incidence study; however, this association is not well supported by six cohort studies, including the large IARC study on production workers. It is possible that any association is due to concurrent exposure to some other agent or agents. Farming and exposure to other pesticides and solvents have also been associated with NHL[49,62] and with cancers of the lymphatic system.[64,65]

Genotoxicity

The results of short-term genotoxicity studies conducted to date in humans have been largely negative. For example, *in vivo* SCE tests[66] and *in vitro* and *in vivo* chromosome aberration tests[67] were negative in humans.

In forestry workers engaged in spraying operations with 2,4-D and MCPA, 22 men had detectable levels of 2,4-D in their urine, ranging from 0.24 to 10.02 mg/L. No significant elevations were observed in SCE before, during and after spraying activities. Counts were in the same range as 15 control subjects.[66] Similar results were obtained for the frequency of chromosomal aberrations between 15 controls and 12 workers exposed to 2,4-D and MCPA, as estimated by urinary 2,4-D levels.[67] Other *in vivo* human studies have been reviewed, but results could not be assessed, as workers were exposed to mixtures of 2,4-D and other (non-chlorophenoxy) herbicides.[42]

Health Effects in Experimental Animals

2,4-D is of slight acute toxicity in mammals. The oral LD_{50} for 2,4-D was reported to be 607 and 726 mg/kg bw for male and female F344 rats, respectively, with approximately similar values (536 to 754 mg/kg bw for males and 424 to 840 mg/kg bw for females) for the acid equivalent of the isooctyl, isobutyl, butyl and butoxyethanol esters, and for the sodium and dimethylamine salts.[37] Similar values were obtained for most other species except dogs, in which the oral LD_{50} was 100 mg/kg bw.[4]

In a 13-week subchronic study in Fischer 344 rats, technical (97.5% pure) and purified 2,4-D were administered in the diet at doses of 0, 15, 60, 100 and 150 mg/kg bw per day to groups of 15 animals per sex, dose and compound.[37] Body weights were reduced about 9% at the highest dose in both sexes. Dose-related degenerative changes in the descending proximal renal tubules in male rats and mild cytoplasmic alterations in the renal proximal tubules in both sexes were observed at all dose levels. Relative kidney weight increases were observed in both sexes at 60, 100 and 150 mg/kg bw per day and in males also at 15 mg/kg bw per day. Relative liver weights were increased at the two highest doses, along with slight swelling and staining changes in liver cells, considered non-specific and minor by the authors. There were no significant differences in toxicological response between purified and technical-grade 2,4-D. A no-observed-adverse-effect level (NOAEL) was not established in this study, as effects were noted at the lowest dose level of 15 mg/kg bw per day.

2,4-D has been shown to act as a peroxisome proliferator,[68] and it has been suggested[69,70] that peroxisome proliferators may act as epigenetic carcinogens. In an initiation–selection–promotion protocol for induction of liver tumours, five peroxisome proliferators including 2,4-D were tested for their promoting and modulating action in hepatocarcinogenesis. Male Wistar rats, 8 to 12 per group, were given a single intraperitoneal dose of diethylnitrosamine as an initiator. Two weeks later, they were given a necrogenic dose of carbon tetrachloride and submitted to a selection procedure by being fed diets containing 2-acetyl-aminofluorene for a period of two weeks. The animals were then divided into six groups and fed a basal diet (controls) or diets supplemented with 0.05% 2,4-D, or four other compounds, for 28 weeks. Administration of all the compounds except 2,4-D and the control resulted in a 16 to 83% incidence of hepatocellular carcinomas (incidence was 16% for 2,4,5-T). There were no tumours in the 2,4-D or control groups. It was concluded that peroxisome proliferation is not necessarily linked to tumour production under the conditions of this subchronic study.[71]

In a two-year chronic toxicity/oncogenicity study in Fischer 344 rats (60 per sex per dose), animals were administered 2,4-D in the diet at doses equivalent to 0, 1, 5, 15 and 45 mg/kg bw per day. Ten rats per group were sacrificed at the end of one year.[72] There were no treatment-related effects on survival in any group. Body weights were 9% lower in high-dose females than in controls, and a non-significant reduction of 5% was also observed in high-dose males. Food consumption was also reduced in the high-dose group in both sexes (as the reduction in body weight was less than 10%, it was probably due to a reduction in food intake). There were

no dose-related effects on clinical, haematological or biochemical parameters. Kidney and thyroid/parathyroid weights were increased significantly in both males and females at 45 mg/kg bw per day. At a dose of 15 mg/kg bw per day, significant increases were observed in kidney weights in males only and in thyroid to parathyroid weight ratios in females only. No histopathological abnormalities were observed in the thyroid/parathyroid. However, at doses of 5, 15 and 45 mg/kg bw per day, an increased incidence of brown pigment was observed in kidney tubular cells of both males and females. In females, renal transitional epithelial cell hyperplasia, significant only at the highest dose, was also observed. Re-examination of the slides indicated slight pigment also in several animals at the 1 mg/kg bw per day dose level. An increased frequency of renal microcalculi was observed in males at 15 and 45 mg/kg bw per day and in females at 45 mg/kg bw per day. Vacuolization of the cytoplasm of the renal cortex was noted in females at 45 mg/kg bw per day. The NOAEL for non-neoplastic effects in this study was considered to be 1 mg/kg bw per day.

In a two-year study in which B6C3F$_1$ mice (60 per sex per dose) were administered 2,4-D in the diet at doses equivalent to 0, 1, 5, 15 and 45 mg/kg bw per day, the only evidence of an effect was an increase in cytoplasmic homogeneity of renal tubular epithelium in male mice at 5 mg/kg bw per day and above. There were no treatment-related effects on survival, body or organ weights, clinical findings or gross pathology. The NOAEL for non-neoplastic effects was 1 mg/kg bw per day.[73]

2,4-D did not exhibit any carcinogenicity in three long-term studies in rats and mice;[74,75] however, these studies were inadequate for the evaluation of carcino-genicity.[42,75] In the recent bioassay conducted in B6C3F$_1$ mice,[73] there were no oncogenic effects at any dose; however, the maximum tolerated dose (MTD) was unlikely to have been reached, thus precluding an assessment of oncogenicity. In the two-year oncogenicity bioassay in Fischer 344 rats,[72] an increase in astrocytomas of the brain was observed in males in the highest dose group of 45 mg/kg bw per day (6 tumours), as well as a significant positive dose-related trend for this effect. The number of tumours observed in males was 1, 0, 0, 2 and 6 for doses of 0, 1, 5, 15 and 45 mg/kg bw per day, respectively, with an incidence of 3.6% in the high-dose group (2.8% if only the four tumours from the standard number of brain sections is considered). There was no reduction in tumour latency; tumours were seen in males only; no pre-neoplastic lesions were observed in any group; all tumours were solitary; and there was no trend towards increased anaplasia in treated animals. The systemic toxicity noted in the study supports the conclusion that an MTD was reached (although the U.S. EPA concluded that it was not). New bioassays have been instituted to clarify the status of 2,4-D with respect to its carcinogenicity to animals.

Because dogs are subject to canine malignant lymphoma, which is aetiologically, histologically and behaviourally similar to NHL, a canine hospital-based case–control study was carried out by the U.S. National Cancer Institute (NCI) in Minnesota, Indiana and Colorado on 491 cases, 466 non-tumour controls and 479 tumour controls, in order to investigate the risk associated with the use of chemicals around the home, including 2,4-D. There was a modest increase in risk (odds ratio [OR] 1.3; CI 1.04 to 1.67) among dogs whose owners applied 2,4-D to their lawns, and the odds ratio rose to a twofold excess with four or more yearly applications (p for trend <0.02). A wide variety of household factors were taken into consideration as potential confounders.[76] These results were consistent with findings in humans.

The results of short-term genotoxicity studies con-ducted to date in animals have been largely negative. 2,4-D was not mutagenic in a number of microbial assays on *Salmonella typhimurium* (various strains), *Bacillus subtilis* and *Escherichia coli*. These studies have been reviewed in several publications.[4,63,74,75,77] Gene conversion tests in the yeast *Saccharomyces cerevisiae* were positive at low pH but not at neutral pH.[78] Positive results were obtained in mutation and chromosome aberration tests in plants, possibly as a result of cytotoxicity.[63,75]

Results in mammalian cells have generally been negative overall, particularly in *in vivo* tests. *In vitro* tests have given mixed results. An unscheduled DNA synthesis test gave negative results in rat hepatocytes[79] and one of two tests on human fibroblast cells,[80] with a positive result in the other.[81] Sister chromatid exchange (SCE) tests *in vitro* gave negative results in Chinese hamster ovary cells[70] and positive results in human lymphocyte cells.[82] *In vivo* SCE tests were negative in the mouse,[83] rat lymphocytes[70,84] and hamster.[70] Chromosome aberration tests *in vivo* were positive in rats at 35 and 70 mg/kg bw but negative at 17.5 mg/kg bw. A dose–response relationship was observed.[85]

Reproduction, as measured by fertility and litter size, was not affected by doses of 2,4-D up to 1500 μg/g of diet (about 75 mg/kg bw) in a three-generation study in rats. However, the number of pups surviving to weaning was sharply reduced at 75 mg/kg bw but was unaffected by a dose of 25 mg/kg bw.[86] In a two-generation rat reproduction study submitted by the Industry Task Force, no definite treatment-related reproductive effects were apparent. However, the study was flawed by a possible infection in some animals. A NOAEL was not established.[87]

In rats orally dosed with 2,4-D on days 6 to 15 of gestation, there were no effects on fertility, gestation, viability of pups and neonatal growth at any dose up to 87.5 mg/kg bw, but administration of the isooctyl or propylene glycol butyl ether esters at 75 or 87.5 mg/kg bw resulted in decreased viability of offspring.[88] Abnormal spermatogenesis and reductions in testis and prostate weights have been reported in rats administered 87.5 mg/kg bw of 2,4-D butyl ester, but no effects were seen at 37.5 mg/kg bw.[89] It was suggested that toxicity could have been caused by the surfactant contained in the formulation used.[4]

2,4-D given in the drinking water of rats at a concentration of 1000 mg/L during pregnancy and for an additional 10 months did not cause any effects on repro-duction, and there were no malformations observed in the young. The second-generation rats given 2,4-D at 1000 mg/L in drinking water for two years showed retarded growth and increased mortality.[74,90]

No consistent effects on foetal mortality, viability, or abnormalities were observed in hamsters administered technical 2,4-D (without detectable dioxin contamination) by gavage at doses of 0, 20, 40, 60 or 100 mg/kg bw on days 6 to 10 of gestation.[91]

Administration of 2,4-D and its butyl, isooctyl and butoxyethanol esters to rats on days 6 to 15 of gestation caused reduced foetal weights and increased the frequency of minor skeletal malformations from 5 to 10% in controls to 50 to 70% at doses of 100 mg/kg bw or higher; this effect was noted only at 300 mg/kg for the dimethylamine salt. There were no lasting effects on survival, weight gain and reproductive ability of progeny.[92] Embryotoxicity and foetotoxicity including reduced foetal body weight, subcutaneous oedema, delayed ossification and wavy ribs were observed in rats administered 2,4-D or its isooctyl and propylene glycol butyl ether esters at doses of 50 to 87.5 mg/kg bw on days 6 to 15 of gestation. The NOAEL was 25 mg/kg bw. 2,4-D and its esters were not teratogenic at any dose.[88] Except for an increase in rib buds at 87.5 mg/kg bw caused by the propylene glycol butyl ether and isooctyl esters of 2,4-D, no effects were observed in a repeat experiment using the same dosing regimen.[93]

In mice, embryotoxicity and foetotoxicity (reduced foetal weight, increased foetal mortality) were observed at a dose of 1 mmol/kg bw per day (221 mg/kg bw) of 2,4-D and the isopropyl and isooctyl ester of 2,4-D. Teratogenic effects (cleft palate) were observed at doses of 124 mg/kg bw or greater for 2,4-D but not for the esters.[94]

Classification and Assessment

Although a number of analytical epidemiology studies have been conducted on 2,4-D and related chlorophenoxy compounds, the evidence for their carcinogenicity remains inconclusive. These studies have almost all dealt with multiple exposures to mixtures of chlorophenoxy herbicides, other pesticides and other organic compounds. Most studies have focused on three endpoints — STS, NHL and HD. Several case–control studies were suggestive of a causal association between farming, chlorophenoxy herbicide use and NHL but were not consistent with results from other studies, particularly occupational cohort studies. The association with STS, commonly attributed to dioxins, is at best weak and inconsistent.

Oral administration of 2,4-D did not induce tumour formation in male or female mice or in female rats in two chronic bioassays. A slight dose-related increase in brain tumours was noted in male rats at the highest dose administered. The results were difficult to interpret, and the studies were considered to be limited for several reasons. Currently available evidence suggests that 2,4-D is non-genotoxic. 2,4-D has therefore been classified in Group III (possibly carcinogenic to humans), according to the classification scheme adopted for drinking water.

For compounds classified in Group III, the acceptable daily intake (ADI) is derived on the basis of division of a NOAEL by appropriate uncertainty factors. A provisional ADI for 2,4-D was established, based on kidney toxicity in rats, as follows:

$$\text{ADI} = \frac{1 \text{ mg/kg bw per day}}{100} = 0.01 \text{ mg/kg bw per day}$$

where:

- 1 mg/kg bw per day is the NOAEL observed in a two-year dietary toxicity/oncogenicity study in rats, in which tubular cell pigmentation in the kidney was observed at the next highest dose of 5 mg/kg bw[72]
- 100 is the uncertainty factor ($\times 10$ for interspecies variation; $\times 10$ for intraspecies variation).

Rationale

For compounds in Group III (possibly carcinogenic to humans), an interim maximum acceptable concen-tration (IMAC) for 2,4-D in drinking water may be derived as follows:

$$\text{IMAC} = \frac{0.01 \text{ mg/kg bw per day} \times 70 \text{ kg bw} \times 0.20}{1.5 \text{ L/d}} \approx 0.09 \text{ mg/L}$$

where:

- 0.01 mg/kg bw per day is the ADI, as derived above
- 70 kg is the average body weight of an adult
- 0.20 is the proportion of total daily intake of 2,4-D allocated to drinking water (the theoretical maximum food intake from residues in food is less than 10% of the ADI)
- 1.5 L/d is the average daily consumption of drinking water for an adult.

The current guideline of 0.1 mg/L will be retained for 2,4-D in drinking water in view of the small difference between the old and the new calculations, but it will be changed from a maximum acceptable concentration (MAC) to an interim value (IMAC) until new studies that clarify the status of 2,4-D with respect to carcinogenicity are available and a re-evaluation is completed.

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