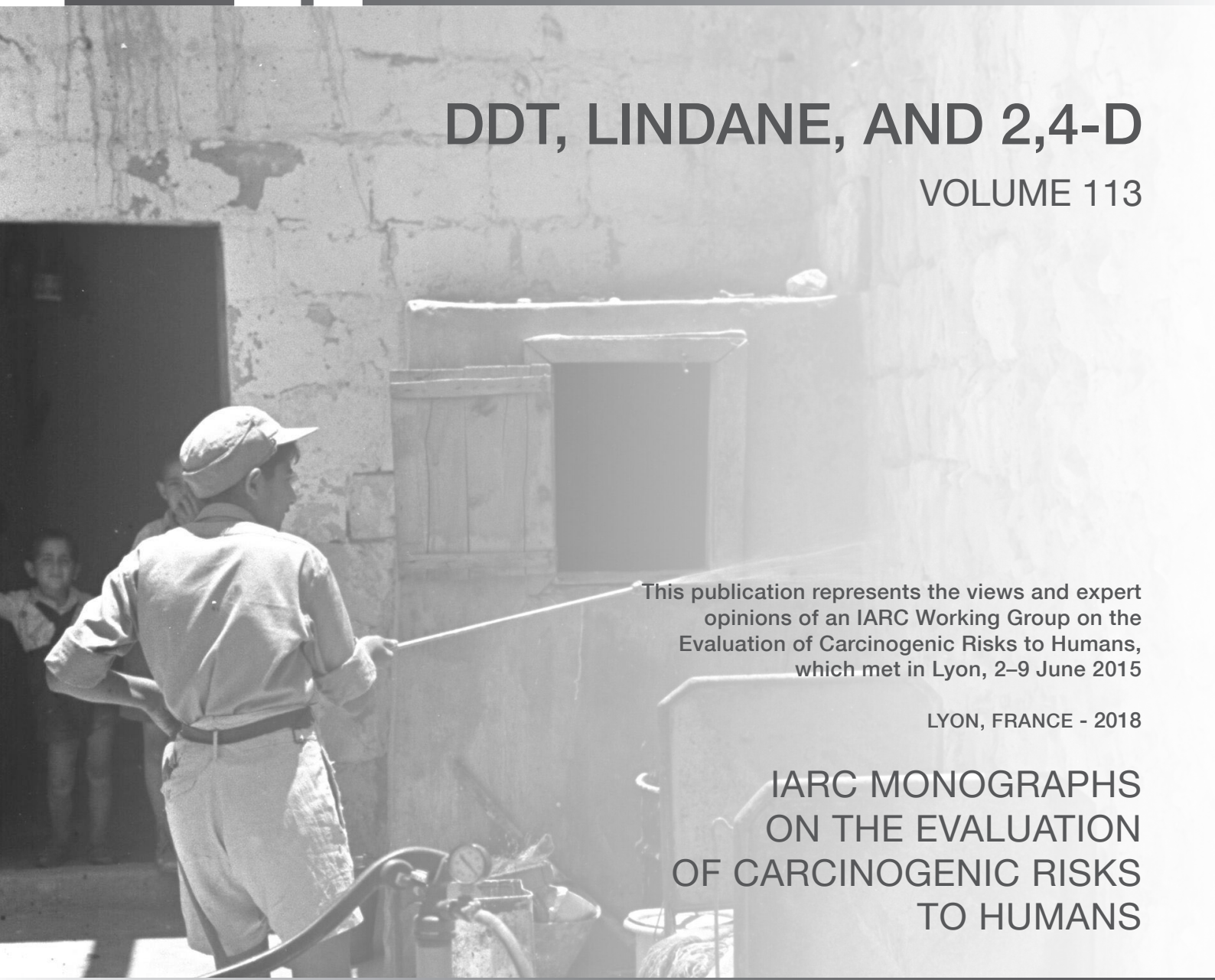
IARC MONOGRAPHS



DDT, LINDANE, AND 2,4-D

VOLUME 113

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, 2–9 June 2015

LYON, FRANCE - 2018

IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

2,4-DICHLOROPHENOXYACETIC ACID

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 94-75-7

*Chem.Abstr.Serv.Name:*2,4-Dichlorophenoxy-acetic acid

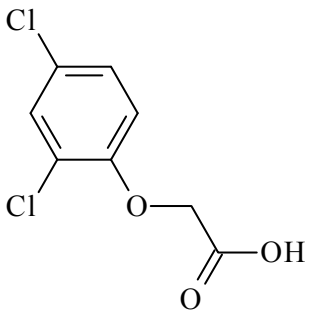
Preferred IUPAC Name: 2-(2,4-Dichloro-phenoxy) acetic acid

Synonyms: 2,4-D; 2,4 dichlorophenoxyacetic acid; 2,4-dichlorophenoxyacetic acid

Trade Names: 2,4-Dichlorophenoxyacetic acid (2,4-D) has been used in many commercial product formulations. Selected trade names include: Hedonal; 2,4-D; Estone; Agrotect; Fernesta; Fernimine; Netagrone; Tributon; Vergemaster; Amoxone; Dicopur; Dormone; Ipaner; Moxone; Phenox; Pielik; Rhodia; Weedone; B-Selektonon.

Additional trade names are available in the PubChem Compound database (NCBI, 2015).

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_8H_6Cl_2O_3$

Relative molecular mass: 221.03

1.1.3 Chemical and physical properties of the pure substance

Description: Colourless crystals or white powder

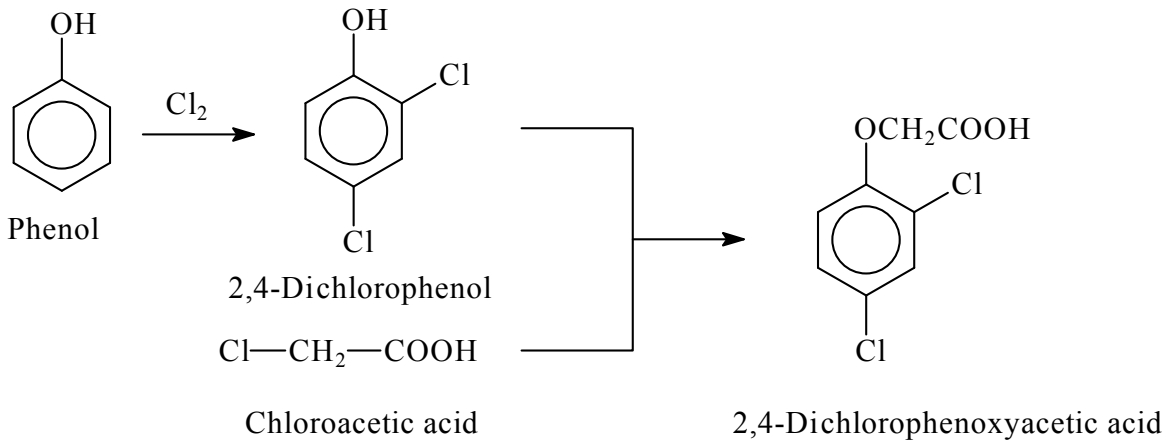
Solubility: Slightly soluble in water (g/100 mL at 25 °C, 0.031). Soluble in organic solvents (ethanol, acetone, dioxane)

Octanol/water partition coefficient: log P_{ow}, 2.81

Conversion factor: 1 ppm = 9.04 mg/m^3, assuming normal temperature (25 °C) and pressure (101 kPa)

See IPCS/ICSC (2015)

Fig. 1.1 Production of 2,4-dichlorophenoxyacetic acid (2,4-D) via 2,4-dichlorophenol



Reprinted from Chemosphere, 92(3), Liu et al. (2013) Formation and contamination of polychlorinated dibenzodioxins/dibenzofurans (PCDD/Fs), polychlorinated biphenyls(PCBs), pentachlorobenzene (PeCBz), hexachlorobenzene (HxCBz), and polychlorophenols in the production of 2,4-D products, pp 304–308, Copyright (2013), with permission from Elsevier

1.1.4 Esters and salts of 2,4-D

Several esters and salts of 2,4-D with various properties have been manufactured and used in herbicide products (NPIC, 2008). In humans, esters and salts of 2,4-D undergo rapid acid or enzymatic hydrolysis in vivo to yield 2,4-D (Garabrant & Philbert, 2002) (see Section 4.1). Esters and salts also undergo hydrolysis to the acid in environmental media at different rates depending on specific conditions of pH, moisture, and other factors (NPIC, 2008). Relevant ester and salt forms of 2,4-D include the following:

- 2,4-D salt (CAS No. 2702-72-9)
- 2,4-D diethanolamine salt (CAS No. 5742-19-8)
- 2,4-D dimethylamine salt (CAS No. 2008-39-1)
- 2,4-D isopropylamine salt (CAS No. 5742-17-6)
- 2,4-D isopropanolamine salt (CAS No. 32341-80-3)
- 2,4-D butoxyethyl ester (CAS No. 1929-73-3)
- 2,4-D butyl ester (CAS No. 94-80-4)
- 2,4-D 2-ethylhexyl ester (CAS No. 1928-43-4)
- 2,4-D isopropyl ester (CAS No. 94-11-1)
- 2,4-D isooctyl ester (CAS No. 25168-26-7)
- 2,4-D choline salt (CAS No. 1048373-72-3)

Physical properties of these 2,4-D salts and esters have been reported elsewhere (NPIC, 2008).

1.2 Production and use

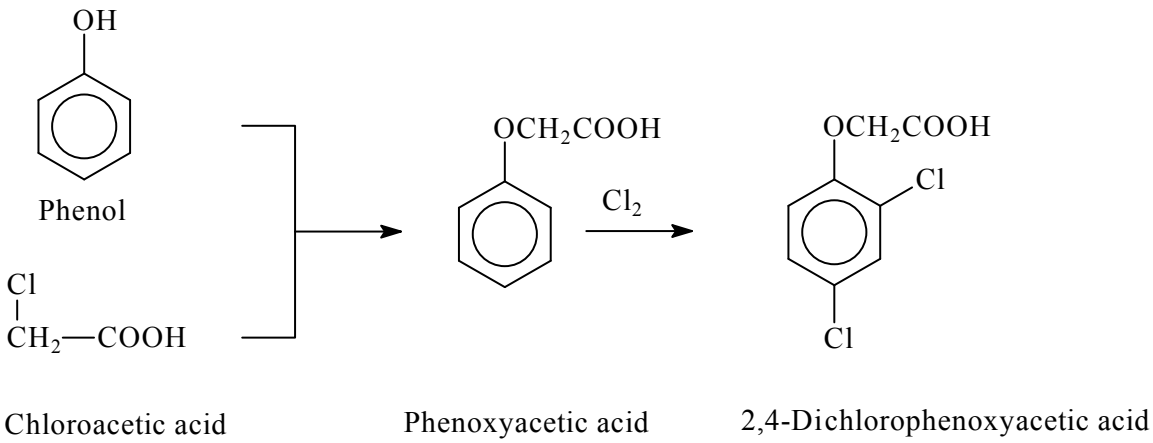
1.2.1 Production

Two processes are currently used for the production of 2,4-D. In the first process, phenol is condensed with chloroacetic acid forming phenoxyacetic acid, which is subsequently chlorinated (Fig. 1.1). In the second process, phenol is chlorinated, generating 2,4-dichlorophenol, which is subsequently condensed with chloroacetic acid (Fig. 1.2).

The butyl ester derivative of 2,4-D is produced by the esterification of the acid with butanol in the presence of a ferric chloride catalyst and chlorine (Liu et al., 2013).

No reliable data on current global production of 2,4-D were available to the Working Group.

Fig. 1.2 Production of 2,4-dichlorophenoxyacetic acid (2,4-D) via phenoxyacetic acid



Reprinted from Chemosphere, 92(3), Liu et al. (2013) Formation and contamination of polychlorinated dibenzodioxins/dibenzofurans (PCDD/Fs), polychlorinated biphenyls(PCBs), pentachlorobenzene (PeCBz), hexachlorobenzene (HxCBz), and polychlorophenols in the production of 2,4-D products, pp 304–308, Copyright (2013), with permission from Elsevier

In 2010, the production of 2,4-D reached 40 000 tonnes in China (Liu et al., 2013).

1.2.2 Use

2,4-D is a synthetic auxin, and was the first chemical that could selectively control dicotyledons or broadleaf plants, but spare most monocotyledons, which include grasses and narrow-leaf crops such as wheat, maize (corn), rice, and similar cereal crops (Song, 2014).

2,4-D was first marketed in 1944 and produced by the American Chemical Paint Company. The derivatives of 2,4-D constitute a series of systematic herbicides that are widely used in broadleaved weeds. 2,4-D is one of the world's most common herbicides because of its general applicability and low cost (Liu et al., 2013)

There are more than 600 products containing 2,4-D currently on the market (Song, 2014). In 2001, the dimethylamine salt and 2-ethylhexyl ester accounted for approximately 90–95% of the total global use of 2,4-D (Charles et al., 2001).

2,4-D is sold in various formulations under a wide variety of brand names and is found, for example, in commercial mixtures of lawn herbicide. 2,4-D can be used alone and is also commonly formulated with other herbicides, for example, dicamba (3,6-dichloro-2-methoxybenzoic acid), mecoprop (methylchlorophenoxypropionic acid, MCPP), mecoprop-P (the (*R*)-(+)-enantiomer of mecoprop), MCPA (2-methyl-4-chlorophenoxyacetic acid), picloram (4-amino-3,5,6 trichloropicolinic acid), and clopyralid (3,6-dichloro pyridine-2-carboxylic acid) (PubChem, 2015). 2,4-D in combination with glyphosate is used as the basis of a herbicide formulation designed for weed control in crops of corn and soybean that have been genetically modified to tolerate 2,4-D and glyphosate via insertion of a bacterial aryloxyalkanoate dioxygenase gene into the plant genome (Wright et al., 2010).

On 18 September 2014, the United States Environmental Protection Agency (EPA) granted registration for a herbicide containing the active ingredients 2,4-D, choline salt, and glyphosate dimethylammonium salt to be used on corn and soybean crops genetically engineered to be resistant to 2,4-D and glyphosate (EPA, 2014).

In the USA, 2,4-D is one of the 10 most commonly used conventional active ingredients of pesticide used in the agricultural sector. Use estimates from 2001 to 2007 ranged from 24 to

35 million pounds [~11 × 10^3 to 16 × 10^3 tonnes]. In the non-agricultural sectors, i.e. home/garden and industry/commercial/government, 2,4-D is the most commonly used active herbicide ingredient, with use estimates between 2001 and 2007 of 8–11 and 16–22 million pounds [~3.6 × 10^3 to 5 × 10^3 and 7 × 10^3 to 10 × 10^3 tonnes], respectively (EPA, 2011). In Canada, 14 tonnes and 87 tonnes of 2,4-D (diverse formulations) were used in British Columbia, and in Ontario respectively, in 2003 (CAREX-CANADA, 2009).

In the USA, application of the herbicide has occurred in pasture and rangelands (24%), lawns by homeowners with fertilizer (12%), spring wheat (8%), winter wheat (7%), lawn/garden without fertilizer (6%), soybean (4%), summer fallow (3%), hay other than alfalfa (3%) and roadways (3%). Other crops on which 2,4-D is used included filberts, sugarcane, barley, seed crops, apples, rye, cherries, oats, millet, rice, soybean, and pears. 2,4-D is also used in forestry, turfgrass management, and in the control of weeds near powerlines, railways, and similar corridors. Rates of application were generally less than 1.7 kg of acid equivalents per hectare, and generally less than 2.2 kg/Ha were applied annually. 2,4-D is predominantly used in the Midwest, Great Plains and Northwestern regions of the USA (EPA, 2005). Low concentrations of 2,4-D are used as plant growth regulators to induce callus formation (Liu et al., 2013). Agricultural use of 2,4-D includes both crop and non-crop applications of primarily liquid formulations, and a variety of application methods ranging from tractor-mounted booms to backpack sprayers. Forestry application ranges from backpack spraying to aerial application. Turf applications may use either liquid spray or granular formulations.

A mixture of roughly equal parts of 2,4-D and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), known as "agent orange", was used by military forces of the USA as a defoliant in the Viet Nam war (Kahn et al., 1988).

1.3 Measurement and analysis

Exposure to humans may occur as a result of ingestion, inhalation, or dermal absorption of 2,4-D, or any of its salts and esters, through occupational exposure during manufacture or use of herbicide products, or via contact with 2,4-D residues in food, water, air, or soil. Measurement methods have been developed for analysis of 2,4-D and its esters and salts in a wide range of biological, personal air, and dermal samples taken during monitoring for exposure, and in food, and environmental media. Some gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been developed as "multi-residue" methods that can provide simultaneous extraction and analysis of other phenoxy acid herbicides (e.g. MCPA, MCPP, dicamba, 2,4,5-T) or even wider ranges of acidic or otherwise difficult-to-analyse pesticides (Raina-Fulton, 2014).

Analysis of 2,4-D acid in urine is the most widely used approach for biomonitoring of human exposure (Baker et al., 2000; Lindh et al., 2008), because excretion of 2,4-D and its acid-hydrolysable conjugates is almost exclusively in the urine. Esters and salts of 2,4-D are rapidly hydrolysed to the acid in exposed humans (see Section 4.1). This is particularly relevant in occupational settings, where exposure to the ester and salt forms are likely to occur. Methods for analysis of 2,4-D in other biological media, including blood and milk, have been developed and applied primarily in studies of toxicology and metabolism in experimental animals (Dickow et al., 2001; Stürtz et al., 2006). Methods of measurement of exposure for 2,4-D acid and its salt and ester forms have included personal and area air samples, dermal patch and bodysuit samples, and hand-wipe samples that are most often used for assessing occupational exposures (NIOSH, 1994; Gardner et al., 2005). Methods for analysis of 2,4-D in air (Waite et al., 2005), water (EPA,

Table 1.1 Representative methods for the analysis of 2,4-D

Sample matrix	Assay procedure	Limit of detection	Reference
Air, workplace	HPLC-UV	15 µg per filter	NIOSH (1994)
Air, ambient	GC-MSD	0.005 ng/m³ based on a 2000 m³ sample volume	Waite et al. (2005)
Ground water	UHPLC-MS/MS	0.0003 µg/L; LOQ, 0.0005 µg/L for 500 mL water samples	McManus et al. (2014)
Drinking-water	GC-ECD	0.055 µg/L	EPA (2000)
Soil	LC-MS/MS	Reporting limit, 0.010 ppm for 20 g of soil sample	Schaner et al. (2007)
Personal exposure (air, hand-wipe, dermal patch)	LC-MS/MS	MDL, 1.1–2.9 µg/L	Gardner et al. (2005)
Urine (human)	LC-MS/MS	0.05 µg/L	Lindh et al. (2008)
Urine (human)	HPLC-MS/MS	0.29 µg/L	Baker et al. (2000)
Plasma (dog)	HPLC-FD	LOQ, 500 µg/L	Dickow et al. (2001)
Serum and milk (rat)	GC-ECD	0.02 ppm [180 µg/L]	Stürtz et al. (2006)
Fruits and vegetables	LC-MS/MS	LOD, not reported; recovery tests performed at 0.01 mg/kg	Shida et al. (2015)
Cereals	LC-MS/MS	LOQ, 0.05 mg/kg	Santilio et al. (2011)
Food (duplicate diet)	GC-MS	MDL, 0.25 ng/g for solid food based on 8 g of homogenized food MDL, 0.20 ng/mL for liquid food based on 30 mL homogenized liquid food	Morgan et al. (2004)
House dust	GC-MS	MDL, 5 ng/g for 0.5 g of dust sample	Colt et al. (2008)

2,4-D, 2,4-dichlorophenoxyacetic acid; ECD, electron capture detector; FD, fluorescence detection; GC, gas chromatography; HPLC, high-performance liquid chromatography; LC, liquid chromatography; LOD, limit of detection; LOQ, limit of quantitation; MDL, method detection limit; MS, mass spectrometry; MS/MS, tandem mass spectrometry; MSD, mass-selective detection; UHPLC, ultra-high performance liquid chromatography

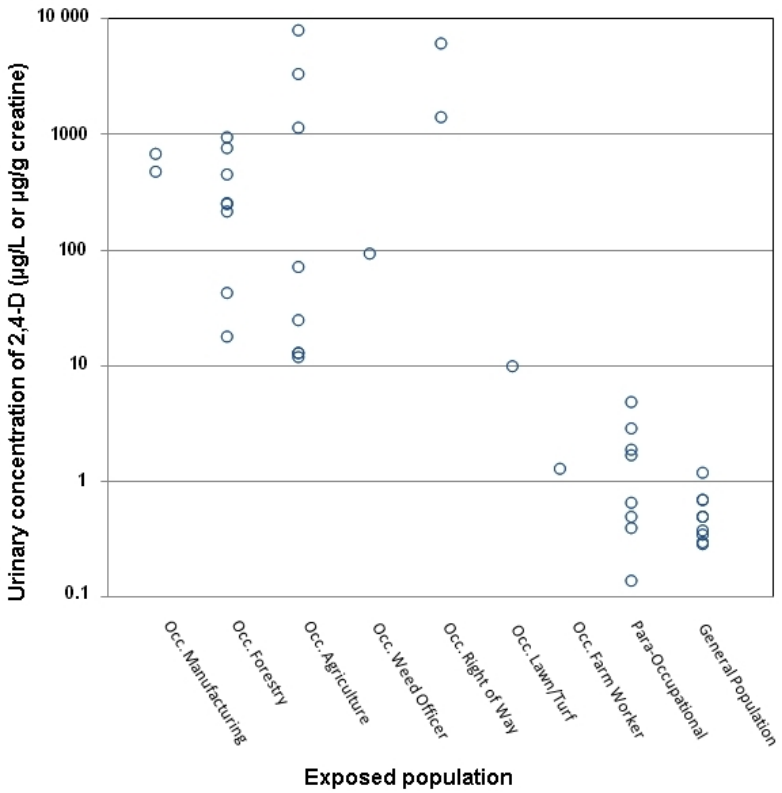
2000; McManus et al., 2014), soil (Schaner et al., 2007), house dust (Colt et al., 2008), and food (Morgan et al., 2004; Santilio et al., 2011; Shida et al., 2015), have primarily (but not exclusively) focused on the acid form of 2,4-D, partly because ester and amine salts of 2,4-D are hydrolysed to the acid at different rates in environmental media, depending on oxygen availability, moisture, and pH levels. In water and aerobic soil and sediment, the half-lives of esters and amines are shorter (in the order of days) than in anaerobic media. 2,4-D undergoes degradation in the outdoor environment, with potentially slower degradation rates in indoor environments (Walters, 1999). Examples of methods of analysis for 2,4-D in a range of media are listed in Table 1.1.

1.4 Occurrence and exposure

2,4-D and its salts and esters do not occur naturally in the environment. Due to widespread production and use of herbicide products containing 2,4-D, there is considerable potential for exposure of humans in occupational and non-occupational settings, as illustrated in Fig 1.3 and Fig. 1.4.

Most of the available data on exposure and environmental occurrence were from North America and Europe. Fewer data were available from other regions of the world. Given the widespread global use of 2,4-D, the lack of data should not be taken as an indicator that human exposures do not occur in other regions.

Fig. 1.3 Urinary concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D)(mean, median, or geometric mean) from studies of occupational or para-occupational exposure, and in the general population



Compiled by the Working Group
Includes multiple subsets of results from several studies: Kolmodin-Hedman & Erne (1980), Draper (1982), Libich et al. (1984), Vural & Burgaz (1984), Knopp (1994), Garry et al. (2001), Hines et al. (2001), Arbuckle et al. (2004, 2005), Curwin et al. (2005a), Alexander et al. (2007), Arcury et al. (2007), Morgan et al. (2008), Bhatti et al. (2010), Thomas et al. (2010a), Zhang et al. (2011), Jurewicz et al. (2012), Rodríguez et al. (2012), Raymer et al. (2014), and CDC (2015)
d, day; occ., occupational

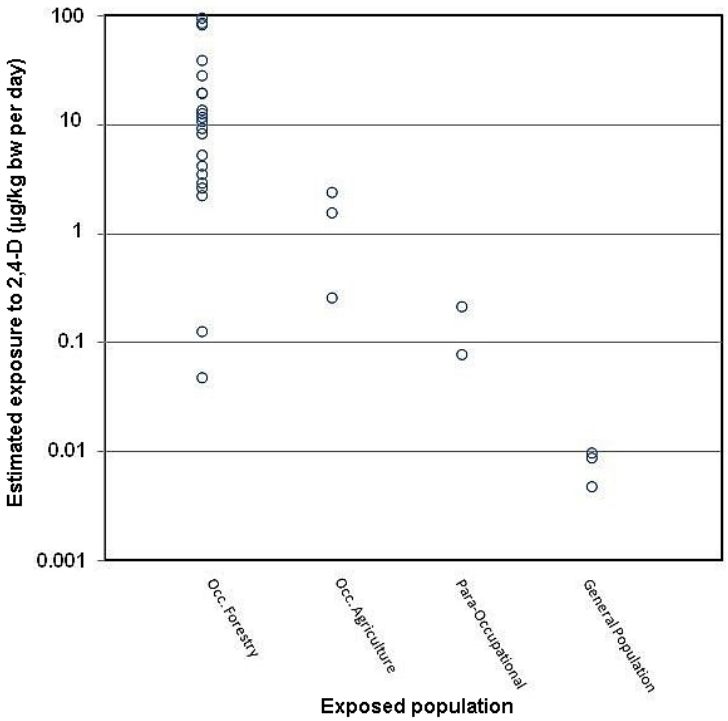
1.4.1 Occupational exposure

Occupational exposure to 2,4-D can result from product manufacturing, agricultural use, forestry, right-of-way, and turf/lawn applications. Indirect or para-occupational exposure may occur in some populations as a result of "take-home" and "drift" pathways. Occupational exposure to 2,4-D typically occurs as a result of dermal absorption and inhalation, although some incidental ingestion may also occur. Some studies cited in a review of dermal absorption of 2,4-D in humans showed that dermal exposure is the primary route of exposure for herbicide-spray applicators (Ross et al., 2005).

(a) Manufacture

In two studies of occupational exposure, workers involved in manufacturing products containing 2,4-D had urinary biomarker concentrations ranging from 35 to 12 693 µg/L, with a mean of 1366 µg/L, in one study as shown in Table 1.2 (Vural & Burgaz, 1984; Knopp, 1994). In one of these studies, values for room air and personal air were 3.2–245 µg/m^3 and

Fig. 1.4 Estimated exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) from studies of occupational or para-occupational exposure, and in the general population



Compiled by the Working Group
Estimates were based on urinary concentrations, except for the general population, for which estimates were derived from residential and dietary measurements. Includes multiple subsets of results from several studies: Lavy et al. (1987), Hines et al. (2001), Alexander et al. (2007), Thomas et al. (2010a), Wilson et al. (2010), Zhang et al. (2011), and Morgan et al. (2014)

23.4–495 µg/m³, respectively (Vural & Burgaz, 1984).

(b) Application

Many studies have been conducted to measure occupational exposure to 2,4-D from agriculture, forestry, right-of-way, and turf application of herbicidal products (Table 1.2). Both external (dermal, air) and biomonitoring methods have been used for exposure assessment of the applicator. Urinary 2,4-D concentrations for forestry applicators ranged from below the limit of detection (LOD) to 1700 µg/L, with means ranging from 17.6 to 454 µg/L for different job tasks (Garry et al., 2001). Estimated mean values for urinary excretion or the absorbed dose ranged from 2.7 to 98 µg/kg bw per day across several studies of forestry-related job tasks (Lavy et al., 1982; Lavy et al., 1987; Zhang et al., 2011). Professional agricultural applicators had urinary concentrations of 2,4-D ranging from not detected (ND) to 2858 µg/L, with values of 58 (geometric mean, GM) and 94 (median) µg/L (Hines et al., 2003; Bhatti et al., 2010). Many studies reported urinary results for farmer applicators, with 2,4-D concentrations ranging from ND to 14 000 µg/L, with GM values ranging from 5.8 to 715 µg/L, and a mean value of 8000 µg/L reported in one study (Kolmodin-Hedman & Erne, 1980; Draper & Street, 1982; Vural & Burgaz, 1984; Grover et al., 1986; Arbuckle et al., 2005; Curwin et al., 2005a; Alexander et al., 2007; Thomas et al.,

Table 1.2 Occupational exposure to 2,4-D

Country, year	Job/process	Media	No. of exposed individuals	Results		Comments/additional data	Reference
				Mean	Range		
Herbicide production							
Germany, 1985–89	2,4-D herbicide production	Urine	41	–	35–12 963 µg/L		Knopp (1994)
		Serum	41	–	3–3537 µg/L		
		Room air	12	–	3.2–245 µg/m^3		
		Personal air	8	–	23.4–495 µg/m^3		
Turkey, 1982	2,4-D herbicide production and application	Urine	15 13	Manufacturing: 1366 µg/L Application: 715 µg/L	60–9510 µg/L ND–1920 µg/L	15 workers manufacturing 2,4-D esters and amine salt; 6 h work shifts, urine collected on Friday; 13 2,4-D applicator crewmen (pilot, flagman, mixer, supervisor) with urine samples collected at end of 3-month application period	Vural & Burgaz (1984)
Forestry workers							
USA, 2002	Forestry backpack applicators	Urine	5 3 1 1	Group A: 768 ± 438 µg/day; 11 ± 5.7 µg/kg bw per day Group B: 951 ± 1089 µg/day; 13 ± 14.1 µg/kg bw per day Mixer/loader: 217 kg per day ± 103 µg/kg per day; 2.7 ± 1.3 µg/kg bw per day Supervisor: 257 ± 117 µg/day; 3.6 ± 1.7 µg/kg per day		Mean estimated total absorbed doses estimated for 5 applicators in group A (without protective clothing), 3 applicators in group B (with standard protective clothing), 1 mixer/loader, 1 supervisor; based on daily 24 h urine samples collected for 6 days	Zhang et al. (2011)

Table 1.2 (continued)

Country, year	Job/process	Media	No. of exposed individuals	Results		Comments/additional data	Reference
				Mean	Range		
USA, year NR	Forestry applicators	Urine	7	Backpack: 454 µg/L	28–1700 µg/L	First void urine collected at end of peak application season	Garry et al. (2001)
			4	Boom spray: 252 µg/L	86–490 µg/L		
			8	Aerial: 42.9 µg/L	ND–97 µg/L		
			5	Skidder: 17.6 µg/L	0.85–58 µg/L		
			15	Controls: 0.5 µg/L	ND–1.8 µg/L		
USA, 1982	Forestry ground workers	Urine, 2,4-D excreted	20	Backpack sprayers: mean, 87.6 (N) and 98 (S) µg/kg per day		24 h urine samples collected; total amount excreted from the application day and 4 following days reported here for normal (N) and special (S) precaution conditions	Lavy et al. (1987)
			20	Injection bar workers: mean, 9.5 (N) and 4.3 (S) µg/kg per day			
			20	Hypohatchet workers: mean, 84.8 (N) and 39.5 (S) µg/kg per day			
			20	Hack/squirt workers: mean, 28.8 (N) and 12.2 (S) µg/kg per day			

Table 1.2 (continued)

Country, year	Job/process	Media	No. of exposed individuals	Results		Comments/additional data	Reference
				Mean	Range		
USA, NR	Aerial crew, forest applications	Urine, 2,4-D excreted	3	Pilots: mean, 19.8 (N) and 8.5 (S) µg/kg per day		24 h urine samples collected; total amount excreted from the application day and the following 5 days, reported here for normal (N) and special (S) precaution conditions	Lavy et al. (1982)
			3	Mechanics: mean, 5.45 (N) and 3.01 (S) µg/kg per day			
			3	Mixer/loaders: mean, 19.6 (N) and 14.0 (S) µg/kg per day			
			3	Supervisors: mean, 2.31 (N) and 0.13 (S) µg/kg per day			
			6	Observers: mean, 0.49 (N) and 0.09 (S) µg/kg per day			
Farmworkers							
USA, 2000–02	Farm applicators	Urine (GM)	68	25 µg/L	1.6–970 µg/L	68 broadcast and hand-spray applicators with 24 h post-application urine; hand-loading, body-loading estimates; air measurements; estimated total absorbed doses for 14 applicators using application day and after 4 days of 24 h urine collection	Thomas et al. (2010a)
		Hand-loading	68	0.39 mg	ND–22 mg		
		Body-loading	68	2.9 mg	0.02–880 mg		
		Personal air	68	0.37 µg/m^3	ND–10 µg/m^3		
USA, 1996	Custom agricultural applicators	Urine (GM)	15	58 nmol/L [12.8 µg/L]	ND–2600 nmol/L [ND–575 µg/L	5–7 24 h urine samples during 6-wk period; estimated amount excreted in 24 h; air, hand-wipe and body-patch samples for 2,4-D 2-ethylhexyl ester	Hines et al. (2001, 2003)
		Hand-loading	15	–	1.3–4300 µg/sample		
		Body patches	15	–	0.3–6200 µg/sample		
		Personal air	15	–	0.06–2.4 µg/m^3		

Table 1.2 (continued)

Country, year	Job/process	Media	No. of exposed individuals	Results		Comments/additional data	Reference
				Mean	Range		
Canada, 1981–82	Farm applicators	Urine, 2,4-D excreted	6	–	215–6258 µg	6 ground-rig spray applicators (one sampled three times); 24 h urine samples collected 4–7 days during/after application; total excreted 2,4-D calculated; hand-wash and dermal-patch samples for estimated dermal exposures	Grover et al. (1986)
		Hand-loading	6	–	10–8840 µg		
		Body-loading	6	–	1.9–1699 mg		
USA, 2000–1	Farm applicators	Urine (GM)	34	71.9 µg/L	1.5–2236	Boom-spray applicators; maximum 24 h urine concentrations during 4-day application and post-application period	Alexander et al. (2007)
USA, 2001	Farm applicators and non-farmers	Urine (GM)	8	Farmers spraying: 2,4-D: 13 µg/L	–	Urine samples collected 1–5 days after application and again 4 wk later	Curwin et al. (2005a)
			14	Farmers not spraying 2,4-D: 0.48 µg/L	–		
			23	Non-farmers: 0.29 µg/L	–		
Canada, 1996	Farm applicators	Urine	43	First 24 h sample: GM, 5.36 µg/L; median, 6.0 µg/L; mean, 27.6 ± 72.5 µg/L	ND–410 µg/L	126 spray applicators using 2,4-D or MCP for first time during growing season; two 24 h urine samples collected from start of application; results reported here for 43 farmers using 2,4-D	Arbuckle et al. (2002, 2005)
			43	Second 24 h sample: GM, 9.9 µg/L; median, 12.0 µg/L; mean, 40.8 ± 91.1 µg/L	ND–514 µg/L		
Sweden, NR	Tractor spray applicators	Urine	4	8000 µg/L	3000–14 000 µg/L	Urine samples during working week and after exposures, personal air samples	Kolmodin-Hedman & Erne (1980)
				24 h excretion: 9 mg	–		
		Air, personal	4	–	100–200 µg/m^3		

Table 1.2 (continued)

Country, year	Job/process	Media	No. of exposed individuals	Results		Comments/additional data	Reference
				Mean	Range		
USA, 2003–4	Lawn turf applicators	Urine	135	Mass excreted during 24 h: median, 14.6 µg	0.1–3658 µg	Sprayers sampled across two herbicide and one insecticide spray seasons; two consecutive 24 h urine samples collected during herbicide spraying; not all sprayers used 2,4-D	Harris et al. (2010)
				Creatinine-adjusted concentrations for samples > LOD: median, 10.2 µg/g	0.2–3001 µg/g		
USA, 1994–95	County noxious weed officers	Urine	31	Mean, 259 ± 432 µg/L; median, 94.1 µg/L	0.07–2858 µg/L	Seasonal county agricultural noxious-weed control applicators; overnight (approx. 12 h) urine samples collected every other week during season	Bhatti et al. (2010)
USA, 1980	Pasture spray application	Urine	2	Crew A driver and sprayer: 1000 and 1300 µg/L respectively at 24 h		2 drivers and 2 sprayers using truck-mounted spray system for pasture land; morning void urine collected for 3 days after application; air samples collected in truck cab; hand rinse; crew A had single application, crew B had multiple applications	Draper & Street (1982)
		Urine	2	Crew B driver and sprayer: 4100 and 2800 µg/L respectively at 24 h			
		Hand loading	–	–	1.2–18 mg		
		Truck cab air	–	–	1.2–2.2 µg/m^3		

Table 1.2 (continued)

Country, year	Job/process	Media	No. of exposed individuals	Results		Comments/additional data	Reference
				Mean	Range		
Canada, 1979–80	Right-of-way applicators	Urine	12	Roadside gun sprayers: 1.42 ± 1.76 mg/kg	0.04–8.15 mg/kg	Electric right-of-way vehicle or backpack hand-spray applicators; urine collected in morning and afternoon, then combined weekly on Thursdays and daily during air-sampling week	Libich et al. (1984)
		Urine	7	Sprayers in Kapuskasing: 6.16 ± 7.69 mg/kg	0.27–32.74 mg/kg		
		Urine	3	Mist-blower sprayers: 2.55 mg/kg	0.44–5.07 mg/kg		
		Air	12	Roadside gun sprayers: 7.1 ± 4.9 µg/m³	1.0–19.5 µg/m³		
		Air	3	Mist-blower sprayers: 55.2 ± 30.7 µg/m³	16.2–91.3 µg/m³		
United Kingdom, 1983	Mixing/ loading	Dermal exposure	3	Tractor-mounted: 102, 244, 122 mg		3 tractor-mounted and 2 knapsack sprayers with six replicates each; whole-body dermal dosimetry	Abbott et al. (1987)
			2	Knapsack: 13.2, 11 mg			
	Spraying	Dermal exposure	3	Tractor mounted: 33.7, 38.9, 90.2 mg			
			2	Knapsack: 159, 89 mg			

Table 1.2 (continued)

Country, year	Job/process	Media	No. of exposed individuals	Results		Comments/additional data	Reference
				Mean	Range		
Malaysia, NR	Paddy spray applicators	Personal air	NR	Manual sprayers: 0.027 ± 0.019 µg/L Motorized sprayers: 0.038 ± 0.0028 µg/L		Paddy spray applicators using manual or motorized knapsack sprayers; dermal exposures estimated from DREAM model	Baharuddin et al. (2011)
		Dermal exposure	NR	Manual spray with proper PPE: 37.8 ± 22.9 ppm Manual spray without proper PPE: 86.1 ± 53.4 ppm Motorized spray with proper PPE: 21.8 ± 9.3 ppm Motorized spray without proper PPE: 45.7 ± 20.3 ppm			
USA, 2010	Farmworkers	Urine	361	38.2% with 2,4-D levels > LOD (LOD = 210 µg/L) 16% with levels > LLOQ (LLOQ = 50 µg/L) For 60 people with samples > LLOQ: GM, 1.28 (range, 0.52–18.6) µg/L		Farmworkers exposed to multiple chemicals	Raymer et al. (2014)
Thailand, 2006	Farmers	Urine	136	2,4-D detection for 37.5% [75th percentile, 0.66 µg/L (range, ND–598 µg/L)]		Farmers in two communities; 21 reported use of a 2,4-D product but urine collection was not specifically timed to an application; mixed-crop farmers had higher detection rates for 2,4-D	Panuwet et al. (2008)

2,4-D, 2,4-dichlorophenoxyacetic acid; DREAM, dermal exposure assessment method; GM, geometric mean; LLOQ, lower limit of qualification; LOD, limit of detection; MCP, 4-chloro-2-methylphenoxyacetic acid; NC, not calculated; ND, not detected; NR, data not reported; PPE, protective personal equipment

2010a). Urine samples from farmers in Thailand who were not specifically linked to crop application had a 75th percentile concentration of 0.66 µg/L (median levels were < LOD) (Panuwet et al., 2008). Professional lawn-turf applicators had urinary concentrations ranging from 0.2 to 3001 µg/g creatinine, with a median of 10.2 µg/g creatinine; the range of excreted mass during 24 hours ranged from 0.1 to 3658 µg, with a median value of 14.6 µg (Harris et al., 2010). Professional right-of-way pesticide applicators had mean urinary concentrations ranging from 0.04 to 32.74 mg/kg, with means of 1.42 and 6.16 mg/kg for two groups of sprayers (Libich et al., 1984).

Several studies of different occupations reported measurements of 2,4-D in air, hand-wipe, or body-loading samples (Kolmodin-Hedman & Erne, 1980; Draper & Street, 1982; Kolmodin-Hedman et al., 1983; Libich et al., 1984; Grover et al., 1986; Abbott et al., 1987; Knopp, 1994; Hines et al., 2001; Thomas et al., 2010a; Baharuddin et al., 2011).

Exposures in farm applicators appear to have been higher during the 1980s than during the 2000s, but firm conclusions could not be drawn due to the small number of studies available.

(c) Para-occupational exposure

Indirect or para-occupational exposure has been measured in several studies, particularly those involving 2,4-D herbicide spraying on farms, and in farmworker families (Table 1.3). For children of farmers who reported having carried out 2,4-D spray applications, GM urine concentrations ranged from 0.4 to 4.9 µg/L (Arbuckle et al., 2004; Alexander et al., 2007; Rodríguez et al., 2012). For spouses of farmers who reported having carried out 2,4-D spray applications, GM or median urinary concentrations ranged from < LOD to 1.7 µg/L (Arbuckle & Ritter, 2005; Alexander et al., 2007; Jurewicz et al., 2012). In a study of children of farmworkers, the median urinary biomarker concentration was 0.14 µg/L (Arcury et al., 2007). One study measured 2,4-D in house dust at farms where 2,4-D had been applied in the previous 30 days (adjusted GM, 730 ng/g) compared with farms where no spraying had been applied (adjusted GM, 850 ng/g) and with non-farm homes (adjusted GM, 320 ng/g) (Curwin et al., 2005b). [One explanation for the higher concentration of 2,4-D in house dust on farms where no spraying had been carried out compared with farms where spraying was reported to have been undertaken may be that 2,4-D is also widely used on lawns and may be tracked into homes. A second reason is that the ester forms of 2,4-D are often used in agriculture in Iowa, and the study method measured only the acid form.] In the United States Agricultural Health Study (AHS), a longitudinal set of urine samples was collected for 1 year from 30 corn farmers and from 10 non-farmers (controls) (Bakke et al., 2009). For farmers, mean 2,4-D concentrations during pre-planting/off-season, planting, and growing/post-harvest periods were 2.9, 22.9, and 7.8 µg/g creatinine, respectively, while mean 2,4-D concentrations for controls during these periods were 0.5, 1.35, and 0.37 µg/g creatinine, respectively. These data suggested that farmers may be exposed to pesticides at higher levels than controls, even when pesticides are not being actively applied.

1.4.2 Environmental occurrence and exposure in the general population

Exposures of the general population may result from the presence of 2,4-D in house dust, food, air, water, and soil. In some areas, residential exposures may be related to use of 2,4-D on lawns, providing a nearby source for direct exposure and tracking into the home. Exposures may occur through inhalation, dermal absorption, and ingestion (Health Canada, 2010).

Table 1.3 Para-occupational exposure to 2,4-D

Country/year	Number of samples/setting	Media	Results	Comments/additional data	Reference
Canada, 1996	92 children (aged 3–18 yrs) of farm 2,4-D or MCPA spray applicators	Urine	First 24 h sample: mean, 0.9 ± 1.4 (max., 12) µg/L Second 24 h sample: mean, 1.9 ± 10.4 (max., 100) µg/L	9.8–14.1% of samples > LOD; data not reported separately for the children of the 43 2,4-D applicators	Arbuckle et al. (2004)
USA, 2004	60 farmworkers' children (aged 1–6 yrs)	Urine	Median, 0.14 µg/L	41.7% of samples > LOD; no information about 2,4-D use	Arcury et al. (2007)
Nicaragua, 2008	Rural schoolchildren; 208 urine samples from 77 children unrelated to 2,4-D application; 3 samples after parental application of 2,4-D	Urine	Unrelated to application: GM, 0.5 (max., 7.4) µg/L Related to application: GM, 0.4 (max., 0.5) µg/L	Study also included data for parental hours and kg a.i. of 2,4-D used for five periods from pre-conception until 8–10 yrs	Rodríguez et al. (2012)
USA, 2000–1	34 spouses and 53 children (aged 4–17 yrs) of farm applicators of 2,4-D spray	Urine	Children: GM, 4.9 µg/L; range, ND–640 µg/L Spouse: GM, 1.7 µg/L; range, 0.5–24.9 µg/L	Maximum 24 h urine concentrations during 4-day application and post-application period	Alexander et al. (2007)
Canada, 1996	125 spouses of farm applicators of 2,4-D or MCPA spray	Urine	First 24 h: GM, 0.6; median, < 1 µg/L; max., 61 µg/L; mean, 1.32 ± 5.6 µg/L Second 24 h: GM, 0.66; median, < LOD (max., 100) µg/L; and mean, 2.0 ± 9.7 µg/L	7.0–14% of samples > LOD; data not reported separately for the spouses of the 43 2,4-D applicators	Arbuckle & Ritter (2005)
Poland, NR	13 spouses of farm applicators of 2,4-D spray	Urine	Day after application: mean, 3.8 (95% CI, 0.6–8.5) µg/L		Jurewicz et al. (2012)
USA, 2002–03	30 farmers, 10 non-farmers; longitudinal collection of urine samples during 1 yr	Urine	Farmer pre-planting/off-season, planting, growing/post-harvest periods: mean, 2.9, 22.9, and 7.8 µg/g creatinine, respectively Non-farmer pre-planting/off-season, planting, growing/post-harvest: mean, 0.5, 1.35, and 0.37 µg/g creatinine, respectively		Bakke et al. (2009)
USA, 2001	House dust collected from 2 farm homes sprayed with 2,4-D in preceding 30 days; 3 farms with no 2,4-D sprayed; 6 non-farm homes	House dust (adjusted GM)	2,4-D detected in 100% of the farm and non-farm home samples: Farms sprayed with 2,4-D: 730 ng/g No 2,4-D sprayed: 850 ng/g Non-farm homes: 320 ng/g	Dust collected from multiple locations in interiors of homes during each of two visits	Curwin et al. (2005b)

a.i., active ingredient; 2,4-D, 2,4-dichlorophenoxyacetic acid; GM, geometric mean; LOD, limit of detection; max., maximum; ND, not detected; NR, data not reported; yr, year

(a) Water

2,4-D may occur in water as a result of direct aquatic uses; from agricultural, forestry, right-of-way, or turf land applications; through application-spray drift; or from atmospheric deposition. Concentrations of 2,4-D in water have been measured for drinking-water supplies, surface water, ground water, and for specific application catchment areas (Table 1.4). In a study of drinking-water supplies in Mexico, 2,4-D concentrations for samples above the detection limit ranged from 0.005 to 0.0038 µg/L (Félix-Cañedo et al., 2013). Detection rates for 2,4-D in surface waters varied widely, with overall concentrations ranging from ND to 14.4 µg/L, and central measures typically < 0.05 µg/L (Phillips & Bode, 2004; Konstantinou et al., 2006; Woudneh et al., 2007; Aulagnier et al., 2008; Loos et al., 2009; Loos et al., 2010b; Glozier et al., 2012; Herrero-Hernández et al., 2013; Tagert et al., 2014). 2,4-D in surface water was found to be associated with agricultural use and, in some cases, was found in urban areas probably as a result of lawn and other turf uses. In a study of ground-water samples from 23 European countries, 2,4-D was detected in only 3.7% of the samples, with a maximum value of 0.012 µg/L (Loos et al., 2010a). Under the United States Geological Survey National Water Quality Assessment Program in 1992–2001, 2,4-D was detected at a frequency of 13% in 1465 samples collected from 62 agricultural surface-water sites, and 13% in 523 samples collected from 19 urban surface-water sites, based on a detection limit of 0.08 µg/L (USGS, 2006). Concentrations at the 90th percentile were 0.11 and 0.16 µg/L, respectively. In ground-water samples in Ireland, the mean 2,4-D concentration was 0.001 µg/L (range, 0.002–0.007 µg/L) (McManus et al., 2014). In a study of surface-water inflow and outflow from a managed turf golf course, the outflow 2,4-D concentration (median, 0.85 µg/L) was significantly higher than the inflow concentration (median, 0.31 µg/L) (King & Balogh, 2010).

(b) Soil

2,4-D is likely to be found in soil in areas where it is applied as an herbicide; however, dissipation/degradation rates have been found to be relatively rapid for most soil types and conditions. While there are many studies published measuring dissipation rates in soils, no publications were found reporting on broad surveys of 2,4-D in soil relevant for assessing the potential for human exposure. One study did report 2,4-D concentrations in soil in 134 home yards in North Carolina and Ohio, USA (Morgan et al., 2008). While most measurements were below the limit of detection, the 95th percentile values were 1.8–4.6 ng/g, with maximum values ranging from 13.3 to 30.5 ng/g.

(c) Residential dust

2,4-D is frequently detected in house dust, with overall concentrations ranging up to 21 700 ng/g, and ranges of GMs, medians, or means from 47.5 to 1035 ng/g (Hartge et al., 2005; Ward et al., 2006; Morgan et al., 2008; Metayer et al., 2013; Deziel et al., 2015; Table 1.5). Surface loading values in homes after lawn applications ranged from 0.05 to 228 µg/m^2 in one study (Nishioka et al., 2001).

(d) Air

2,4-D may occur in outdoor air as a result of application-spray drift, volatilization of applied herbicides, and atmospheric suspension of 2,4-D containing soil and dust. 2,4-D in indoor air may result from tracking-in of 2,4-D in dusts and soils, and from direct intrusion of outdoor air. Concentrations of 2,4-D in outdoor air have been measured in agricultural areas, and in residential indoor and outdoor air (Table 1.6). Several studies in the Canadian prairies measured 2,4-D in outdoor air, with overall ranges of ND–2.73 ng/m^3, and with mean values ranging from 0.059 to 0.44 ng/m^3 (Waite et al., 2005; Yao et al., 2006; Aulagnier et al., 2008). The highest

Table 1.4 Concentration of 2,4-D in water

Country/year of sampling	Number of samples/setting	Results	Comments	Reference
Europe				
Greece, 1988–2000	2,4-D measurement data compiled from literature for 8 rivers	Range of minimum concentrations, ND–0.040 µg/L; range of maximum concentrations, 0.012–1.2 µg/L	2,4-D was detected at least once in 7 out of 8 rivers	Konstantinou et al. (2006)
Ireland, 2012	42 ground-water samples collected from 7 locations	2,4-D: mean, 0.001 (range, 0.002–0.007) µg/L DCP: mean, 0.001 (range, 0.001–0.004) µg/L PAC: mean, 0.456 (range, 0.015–4.15[a]) µg/L	PAC is a transformation product or impurity of 2,4-D and MCPA DCP is a transformation product of 2,4-D	McManus et al. (2014)
Spain, 2011	7 surface-water samples from Ebro river and tributaries; 32 ground-water samples from 3 areas of the La Rioja vineyard region	Rioja Alta: surface water, mean, 0.045 (range, 0.023–0.068) µg/L; ground water, mean, 0.128 (range, 0.046–0.177) µg/L Rioja Baja: surface water, mean, 0.022 (range, 0.020–0.024) µg/L; ground water, mean, 0.031 (range, 0.026–0.034) µg/L Rioja Alavesa: ground water, mean, 0.048 (range, 0.034–0.067) µg/L	2,4-D was detected in 33% of the water samples	Herrero-Hernández et al. (2013)
Europe	122 surface water samples from > 100 European rivers in 27 countries	Detection in 52% of samples; median, 0.003 µg/L; mean, 0.022 µg/L; max., 1.221 µg/L		Loos et al. (2009)
Europe, 2008	164 ground water samples from 23 European Countries	Detection in 3.7% of samples; max., 0.012 µg/L		Loos et al. (2010a)
Europe, 2007	73 Danube River and 23 tributary river surface water samples across 10 countries	Detection in 94% of Danube River samples; median, 0.01 (max., 0.055) µg/L Detection in 72% of tributary rivers; median, 0.003 (max., 0.188) µg/L		Loos et al. (2010b)
Central America				
Mexico, 2008–9	Drinking-water samples from 7 wells, 4 dams, and 15 mixing tanks for surface and ground-water sources supplying 60% of Mexico City water	In mixed water: range, 0.005–0.038 µg/L	2,4-D was found in 20% of the mixed water; 2,4-D was not detected in well and ground-water samples	Félix-Cañedo et al. (2013)

Table 1.4 (continued)

Country/year of sampling	Number of samples/setting	Results	Comments	Reference
North America				
Canada, 2003–5	Surface water collected from 2 reference, 5 agricultural, 2 urban, and 5 mixed agricultural/urban sites	Agricultural sites: range of means, 0–0.044 (overall range, 0–0.345) µg/L Urban sites: range of means, 0.005–0.020 (overall range, 0.002–0.063) µg/L Mixed agricultural/urban sites: range of means, 0.008–0.357 (overall range, 0.002–1.23) µg/L	2,4-D not detected at reference sites	Woudneh et al. (2007)
Canada, 2004	Monthly precipitation samples collected over 5 months at an agricultural site in the Yamaska River Basin, Quebec	2,4-D was detected in one (June) out of 5 monthly samples, at a concentration of 0.007 µg/L		Aulagnier et al. (2008)
Canada, 2007	National survey of 19 sites in 16 urban river watersheds across Canada, including Pacific, prairies, Ontario, Quebec, and Atlantic groupings	2,4-D detected in > 80% of prairie and urban river samples; across all urban samples; mean, 0.172 µg/L; max., > 0.8 µg/L	2,4-D concentrations increased from upstream to downstream across urban sites; highest 2,4-D concentrations were found in summer; 2,4-D concentrations were significantly 2–3 times higher after rain	Glozier et al. (2012)
USA, 2000–1	Surface-water samples from Kisco and Middle Branch of Croton Rivers	Kisco river: 64% of samples > LOD = 0.08 µg/L; 32% > 0.1 µg/L; max., 24 µg/L Middle Branch Croton River: 50% of samples > LOD; 13% > 0.1 µg/L; max., 0.39 µg/L	Highest 2,4-D concentrations measured during stormflow conditions	Phillips & Bode (2004)
USA, 1992–2001	1465 samples from 62 surface-water sites in agricultural areas, 523 samples from 19 surface-water sites in urban areas	Detection frequency of 13% in water from agricultural areas, and 13% in water from urban areas Concentrations at 90th percentile: 0.11 µg/L in water from agricultural areas; and 0.16 µg/L in water from urban areas	Based on LOD of 0.08 µg/L in the USGS National Water Quality Assessment Program	USGS (2006)
USA, 2003–8	Surface water inflow and outflow from a managed turf golf course	Inflow: median, 0.31 µg/L Outflow: median, 0.85 (max., 67.1) µg/L	Outflow concentration was significantly higher than inflow	King & Balogh (2010)
USA, 2002–3	Surface-water samples collected from 7 sites in the upper Pearl River basin	Median, 0.17 (range, 0.10–14.4) µg/L		Tagert et al. (2014)

[a] Extrapolated concentration

2,4-D, 2,4-dichlorophenoxyacetic acid; DCP, 2,4-dichlorophenol; LOD, limit of detection; max., maximum; MCPA, 4-chloro-2-methylphenoxy acetic acid; ND, not detected; PAC, phenoxyacetic acid

Table 1.5 Concentrations of 2,4-D in residential dust

Country/year of sampling	Number of samples/setting	Results	Comments	Reference
USA, 2001–7	House dust collected from 277 homes of children with leukaemia, and 306 control homes	2,4-D was detected in 98% of homes; median, 102 ng/g; 75th percentile, 419 ng/g		Deziel et al., 2015
USA, 2001–7	House dust collected in 333 control homes in a case–control study	2,4-D was detected in > 92% of homes; mean, 831 ± 6041 ng/g		Metayer et al. (2013)
USA, 1998–2000	House dust from 112 home subset of NHL case–control study	2,4-D detected in 95% of homes; GM, 1035 ng/g	Total crop acreage within 750 m of home was significantly associated with increased 2,4-D concentration	Ward et al. (2006)
USA, 2000–6	House dust from 66 homes in NC and 62 homes in OH	OH: median, 156 (range, < LOD–21 700) ng/g NC: median, 47.5 (range, < LOD–7390) ng/g		Morgan et al. (2008)
USA, 1998–2000	House dust from 510 control homes in a NHL case–control study	For control homes: 110 homes < LOD; 161 homes < 500; 59 homes, 500–599; 162 homes, 1000–9999; and 18 homes, > 10 000 ng/g		Hartge et al. (2005)
USA, NR	House indoor-air and surface-wipe and vacuum samples collected at 11 occupied and 2 unoccupied homes during week before application and week after application of 2,4-D	Mean 2,4-D concentrations on particles in air ranged from approx. 1 to 10 ng/m^3, with differences between particle size and collection period; 2,4-D surface loadings ranged from 0.05 to 228 µg/m^2 for carpets, with lower values for bare floors, tables, and window sills	Exposures to young children were estimated to be: median, 1.37 (max., 1.94) µg/day pre-application and 2.42 (max., 8.87) µg/day post-application; track-in factors were important	Nishioka et al. (2001)

2,4-D, 2,4-dichlorophenoxyacetic acid; GM, geometric mean; LOD, limit of detection; OH, Ohio; NC, North Carolina; ND, not detected; NHL, non-Hodgkin lymphoma

Table 1.6 Concentrations of 2,4-D in air

Country/year of sampling	Number of samples/setting	Results	Comments	Reference
Canada, 2004	Weekly and monthly outdoor air samples over 5 months at an agricultural site in the Yamaska River basin	Detection in 38% of the May–June weekly air samples; mean, 0.44 (range, < LOD–1.31) ng/m^3	2,4-D not detected in any of the monthly air samples collected July–September	Aulagnier et al. (2008)
Canada, 2003	Weekly outdoor air samples collected at 8 sites in agricultural and receptor regions over 1 or 3 months	At three prairie sites, means ranged from 0.059 to 0.331 (overall range, ND–1.46) ng/m^3	Highest 2,4-D concentrations were found during typical weeks of application	Yao et al. (2006)
Canada, 2002	6 weekly outdoor air samples at 4 sites on a 500-km north–south transect in Saskatchewan	Across all sites: mean, 0.35 ng/m^3, median, 0.15 ng/m^3; max., 2.73 ng/m^3	Highest 2,4-D concentrations were found during typical weeks of application	Waite et al. (2005)
France, 2001	4 outdoor air samples collected at an urban site, and 5 air samples collected at a rural site	Urban site: range, ND–11 ng/m^3 Rural site: range, ND–37 ng/m^3	Concentrations in gas plus particle phase reported	Baraud et al. (2003)
Netherlands, 2000–1	18 sites nationwide, air and precipitation samples collected once during each 4-week period for 2 yrs; weekly samples collected at three sites	2,4-D was not detected in any air samples Detection in 9% and 31% of precipitation samples in 2000 and 2001 respectively, with means of 0.8 and 1.9 ng/L	Deposition amounts to soil and surface waters were estimated	Duyzer & Vonk (2003)
USA, 2000–1	Home indoor and outdoor air at 66 homes in North Carolina and 67 homes in Ohio	North Carolina, indoor air: 75th percentile, 0.8 ng/m^3; max., 3.7 ng/m^3 Ohio, indoor air: 75th percentile, 0.8 ng/m^3; max., 2.0 ng/m^3 North Carolina, outdoor air: 75th percentile, < LOD; max., 1.7 ng/m^3 Ohio, outdoor air: 75th percentile, 0.3 ng/m^3; max., 3.2 ng/m^3		Morgan et al. (2008)
USA, year NR	Home indoor air collected at 11 occupied and 2 unoccupied homes during pre-application and post-application week	Mean indoor 2,4-D concentrations associated with $PM_{2.5}$ particles ranged from approximately 0.7–3.9 ng/m^3 during application and 0.8–1.5 ng/m^3 3 days after application; mean indoor 2,4-D concentrations associated with PM_{10} particles ranged from approximately 4.2–9.6 ng/m^3 during application, and 1.8–3.4 ng/m^3 3 days after application	Homeowner and pet track-in were significant factors for intrusion; resuspension of floor dust was the major source of 2,4-D in air	Nishioka et al. (2001)

2,4-D, 2,4-dichlorophenoxyacetic acid; LOD, limit of detection; max., maximum; ND, not detected; NR, not reported; $PM_{12.5}$, particulate matter with a diameter of ≤ 2.5 μm; PM_{10}, particulate matter with a diameter of ≤ 10 μm

concentrations were observed during weeks when 2,4-D was typically applied. In France, outdoor air concentrations ranged from ND to 11 ng/m^3 in an urban location, and ND to 37 ng/m^3 in a rural location (Baraud et al., 2003). In a 2-year nationwide monitoring campaign in the Netherlands, 2,4-D was not detected in air, but was detected in precipitation, with mean concentrations of 0.8 and 1.9 ng/L in 2000 and 2001, respectively (Duyzer & Vonk, 2003). In two states in the USA, 75th percentile concentrations of 2,4-D in indoor residential air were each 0.8 ng/m^3, with maximum concentrations ranging from 2.0 to 3.7 ng/m^3 (Morgan et al., 2008). In the same study, outdoor residential air concentrations of 2,4-D at the 75th percentile ranged from ND to 0.3 ng/m^3, with maximum values ranging from 1.7 to 3.2 ng/m^3. In other homes with lawn-turf applications, 2,4-D concentrations associated with indoor particulate matter of aerodynamic diameter < 2.5 μm ($PM_{2.5}$) ranged from approximately 0.7 to 3.9 ng/m^3 during application, and 0.8 to 1.5 ng/m^3 3 days after application. Mean indoor 2,4-D concentrations associated with particulate matter of aerodynamic diameter < 10 μm (PM_{10}) ranged from approximately 4.2–9.6 ng/m^3 during application, and 1.8–3.4 ng/m^3 at 3 days after application (Nishioka et al., 2001).

(e) Food

2,4-D residues may be found in some food commodities as a result of use of 2,4-D in agriculture. In 2015, the European Food Safety Authority (EFSA) reported the results of the control activities related to pesticide residues in food carried out in 2013 in the European Union member states, Norway and Iceland (EFSA, 2015). As part of this monitoring programme, 2,4-D was analysed in 2756 food samples and found to be above the LOQ for a single result; the measured concentration of 2,4-D in one lettuce sample was 0.075 mg/kg, and thus higher than the maximum residue level (MRL) of 0.05 mg/kg. EFSA reported results from residue trials for several plant commodities, with all results being less than the proposed MRLs (EFSA, 2011).

Duplicate diet studies provide the most relevant information regarding potential human exposures, accounting for the wide variety of combined foods consumed, and also processing and cooking factors. However, only one duplicate diet study measuring 2,4-D was available (Morgan et al., 2008; Table 1.7). In this study, 2,4-D was not detected in more than half of the duplicate diet samples for children and adult caregivers. The 75th percentile levels for solid foods ranged from 0.4 to 0.9 ng/g, with maximum levels ranging from 3.7 to 20.2 ng/g. The maximum levels for liquid foods/beverages ranged from 0.2 to 0.8 ng/g.

(f) Biological markers

Several studies have examined exposures to 2,4-D in the general population through measurement of 2,4-D in the urine (Table 1.8). The National Health and Nutrition Examination Survey (NHANES) in the USA provides representative population biomonitoring data for 2,4-D for several age groups, with GM values ranging from 0.288 to 0.385 μg/L, and 95th percentile values ranging from 1.12 to 2.08 μg/L (CDC, 2015). A study of children in Thailand found overall concentrations of 2,4-D in the urine ranging from 0.09 to 1.87 μg/g creatinine, and GMs for different groups ranging from 0.17 to 0.21 μg/g creatinine (Panuwet et al., 2009). In a study of children in Puerto Rico, the frequency at which 2,4-D in the urine was found to be greater than the LOD was 11.8%, and the maximum value was 0.9 μg/L (Lewis et al., 2014). For children in two states in the USA, the overall 2,4-D concentration in urine was < LOD – 12.5 μg/L, with median values of 1.2 and 0.5 μg/L in the two states (Morgan et al., 2008). For homeowner lawn/garden applicators and bystanders living in the home, the amount of 2,4-D excreted after

Table 1.7 Concentrations of 2,4-D in food

Country/year of sampling	Number of samples/setting	Results	Comments	Reference
Europe, 2013	2756 food samples analysed for 2,4-D	Only one food (lettuce) had a 2,4-D concentration of > LOQ (the concentration was 0.075 mg/kg)		EFSA (2015)
Europe, NR	Residue trial results for 13 plant commodity types	Median and maximum residue values of less than the proposed MRL of 0.05 mg/kg for food commodities, and greater than proposed MRLs ranging from 0.05 to 50 mg/kg for grass, straw, and maize forage commodities		EFSA (2011)
USA, 2000–1	Solid-food duplicate-diet samples collected from children and adult caregivers at 66 homes in North Carolina, and 69 homes in Ohio	North Carolina, child: 75th percentile, 0.9 ng/g; max., 4.4 ng/g Ohio, child: 75th percentile, 0.4 ng/g; max., 20.2 ng/g North Carolina, adult: 75th percentile, 0.9 ng/g; max., 4.0 ng/g Ohio, adult: 75th percentile, 0.6 ng/g; max., 3.7 ng/g	2,4-D detected in < 10% of liquid-food duplicate-diet samples with maximum values ranging from 0.2 to 0.8 ng/g	Morgan et al. (2008)

2,4-D, 2,4-dichlorophenoxyacetic acid; LOD, limit of detection; LOQ, limit of quantification; max., maximum; MRL, maximum residue limit; ND, not detected

application ranged from ND to 7.1 µg/kg bw (Harris et al., 1992).

1.4.4 Exposure assessment to 2,4-D in epidemiological studies

The key epidemiological studies evaluated for 2,4-D in this *Monograph* can be categorized as occupational studies of applicators in agriculture settings and farmworkers and one study in the general population. For classification of exposure to 2,4-D, cohort and case–control studies of farmers relied on questionnaire-based approaches to collect information regarding pesticide use, work practices, and other important agricultural and lifestyle factors such as smoking. Two studies re-analysed several of the case–control studies, applying techniques to consider the use of multiple pesticides by individuals working in agriculture. A study of farmworkers combined extant geographically based data on pesticide application for specific crops, locations, and dates with union-based records of work history and location information for individuals. The study of the general population examined exposure–outcome relationships using two approaches: (a) questionnaire-based collection of information about residential use of herbicide; and (b) measurement of 2,4-D in house dust as a surrogate indicator of exposure.

There were no epidemiological studies on cancer that relied on measurement of 2,4-D biomarkers for exposure categorization. For 2,4-D, however, the elimination half-life after exposure in humans is short, with ranges of 10–28 hours after an oral dose (Sauerhoff et al., 1977) and 18–68 hours after a dermal dose of 2,4-D, and 18–87 hours for 2,4-D dimethyl amine (Harris & Solomon, 1992). Thus, it is usually impractical to collect adequate numbers of samples to represent long-term exposures in epidemiological cohorts, or to implement collection for large numbers of participants at key time-points such as after herbicide applications. Biomarker measurement therefore has not been the primary means of classifying 2,4-D exposure for research in cancer

Table 1.8 Exposure to 2,4-D in the general population

Country/year of sampling	Subjects/setting	No. of subjects	Age (yrs)	Medium	Results	Comments	Reference
USA, 2009–10	NHANES general population biomonitoring survey	386	6–11	Urine (μg/L)	GM: 0.385; and 95th percentile, 1.59	Representative population sample for USA; data also available for earlier time periods	CDC (2015)
		401	12–19		0.301 and 1.12		
		1309	20–59		0.288 and 1.33		
		651	60–older		0.349 and 2.08		
USA, 2000–1	Children and their adult caregivers in North Carolina and Ohio	66 (North Carolina)	2–5	Urine (μg/L)	Median, 0.5 (range, < LOD–3.3)	Indoor air, outdoor air, house dust, soil, hand wipe, and food data also available from this study	Morgan et al. (2008)
		66 (North Carolina)	Adults		0.7 (< LOD–5.1)		
		69 (Ohio)	2–5		1.2 (< LOD–12.5)		
		69 (Ohio)	Adults		0.7 (< LOD–8.1)		
USA, NR	Homeowner lawn/ garden applicators and bystanders living in home	24 (applicators)	NR	Urine (total amount 2,4-D secreted) (μg/kg bw)	Range, < LOD–7.1	Samples collected for 96 h after application	Harris et al. (1992)
		24 (bystanders)	NR		No measurable levels		
Puerto Rico, 2010–12	Pregnant women	152	18–40	Urine (μg/L)	> LOD in 11.8% of samples; 95th percentile, 0.6 Max. value, 0.9	Spot urine samples at approx. 20, 24, and 28 weeks of gestation ORs for 2,4-D detection were significantly elevated for consumption of collard greens and spinach in previous 48 h	Lewis et al. (2014)
Thailand, NR	Children from parents with different occupations:			Urine (μg/g creatinine)		Urine first morning void samples collected No significant differences in urine 2,4-D for agricultural vs non-agricultural families, or boys vs girls	Panuwet et al. (2009)
	Farmer	60	12–13		GM, 0.21 (range, 0.13–1.08)		
	Merchant and trader	39	12–13		0.21 (0.09–0.43)		
	Government and company employee	52	12–13		0.17 (0.10–0.38)		
	Labourer	56	12–13		0.19 (0.12–1.87)		

2,4-D, 2,4-dichlorophenoxyacetic acid; GM, geometric mean; LOD, limit of detection; max., maximum; ND, not detected; NR, data not reported; OR, odds ratio; vs, versus; yr, year

epidemiology. A recent review summarized relevant studies of 2,4-D biomarker measurement, and separately summarized exposure metrics in more recent epidemiological investigations of 2,4-D and cancer (Burns & Swaen, 2012).

This section provides an assessment of the strengths and weaknesses of the exposure assessment and assignment methods used in the key epidemiological studies that were evaluated by the Working Group. The detailed discussions of limitations in exposure assessment in the epidemiological investigations described here should not be construed to suggest that these studies are inferior to others in the literature. In fact, in many ways the studies described here have improved on pesticide exposure assessment in this discipline when compared with many studies that relied on less specific exposure-classification categories, such as "farmer" or any non-specific pesticide use.

(a) Studies of occupational exposure

Several studies were based on reported application of 2,4-D in agricultural settings. Exposure assessment in these studies relied on questionnaire-based approaches for reporting use of 2,4-D, together with reporting of factors potentially affecting exposures. Two cohort studies included farm applicators who reported their lifetime use of 2,4-D (Alavanja et al., 2004; Koutros et al., 2013). Three case–control studies included participants who reported using 2,4-D (Brown et al., 1990; Zahm et al., 1990; McDuffie et al., 2001). Three studies performed additional analyses of data from case–control studies to examine joint exposures to multiple pesticides and pesticide classes (Cantor et al., 1992; De Roos et al., 2003; Hohenadel et al., 2011). One study performed additional analyses of data from case–control studies to examine whether reported use of the insect repellent *N*,*N*-diethyl-*meta*-toluamide (DEET) might result in increased penetration of 2,4-D through gloves, and thus increase exposure to 2,4-D (McDuffie et al., 2005). Another case–control study examined lympho-haematopoietic cancer in farmworkers, with proxy data on pesticide-use locations and amounts combined with work-location history (Mills et al., 2005).

The two cohort-based studies were from the AHS, which collected detailed information on pesticide use and factors potentially affecting exposure (e.g. spraying techniques, personal protective equipment, etc.). Based on the detailed use information, a semi-quantitative exposure-assessment method was developed in which estimated intensity was combined with years and annual frequency of use (Dosemeci et al., 2002). The intensity score was based on development of an a-priori exposure-intensity algorithm. Several validity evaluations of the exposure assessment process have been carried out. These included: (i) assessment of the reliability of reporting agricultural factors by requiring completion of the enrolment questionnaires twice, approximately 1 year apart; (ii) confirmatory checks correlating the years in which a pesticide was reportedly used with dates of registered use of that particular pesticide; and (iii) comparison of the exposure algorithm with external exposure data. Agreement between reporting of ever/never use of specific pesticides and application practices was high, and generally ranged from 70% to > 90%. Agreement was lower (typically 50–60%) for duration or frequency of use of specific pesticides (Blair et al., 2000). The confirmatory checks on reported usage of specific pesticides established that the majority of respondents provided plausible responses for both decade of first use and total duration of use (Hoppin et al. 2002). The exposure-intensity algorithm was evaluated using measurements of 2,4-D urinary biomarkers in the AHS cohort and in two other studies (Coble et al., 2005; Acquavella et al., 2006; Thomas et al., 2010b). When combined, these studies showed that the AHS algorithm had the capacity to separate the upper tertiles of exposure intensity from the lower.

[The Working Group noted that the AHS had collected very detailed information on pesticide use and practices, and validation studies showed these data to be appropriate for estimating historical exposure to pesticides. In addition, information on exposure was collected before disease diagnosis, eliminating the potential for case-recall bias. It is however important to note that the validity studies were based on information reported at the time the exposure surveys were completed, and would not necessarily reflect the recall of information which had been reported to be good in regard to some but not all aspects, in particular for data regarding frequency and duration of use. The exposure assessment of 2,4-D in the AHS was based on the baseline questionnaire (1993–1997) that relied on recall for recent and previous pesticide-use information. One of the studies also used data from the follow-up questionnaire (1999–2003) that used recent recall to update pesticide-use information. Although use of 2,4-D may be more readily recalled than use of other less widely recognized pesticides, the validity of recalled information is nonetheless unknown. Furthermore, application procedures and other factors in the 1950s–1980s may not necessarily be similar to the parameters addressed in the AHS exposure algorithm. Moreover, it is likely that a certain proportion of exposure of farmers is attributable to non-application circumstances, such as re-entry, and contaminated work and home environments. In this regard, the publication by Bakke et al. (2009) based on corn farmers in Iowa in the AHS is illustrative. In that study, biological samples taken on non-application days revealed elevated levels of 2,4-D among farmers compared with non-farmer controls during the whole year. These levels, although an order of magnitude lower than those recorded when the farmers were applying 2,4-D, still indicated a notable contribution of non-application days to cumulative exposure over the year. This consideration is relevant when accounting for the fact that active application by most farmers amounted to only a few days during the growing season.]

The case–control studies used questionnaire-based approaches to obtain information on participant use of pesticides in agricultural settings, including specific pesticides used, days per year of use, and in one case, year of first use. Analyses were based on ever/never use of specific pesticides, with further analyses using categories of days per year of use for specific pesticides.

[The case–control studies had several strengths, including collection of information on specific pesticides including 2,4-D, using days per year of use to stratify users into categories with higher and lower exposure to 2,4-D, and collection of information from agricultural users likely to be able to accurately identify specific pesticides such as 2,4-D. Exposure assessment in the case–control studies also had several potential limitations. Exposure data were collected after case diagnosis, leading to potential case-recall bias, and general recall bias for both cases and controls is possible. The studies did not collect total years for use of 2,4-D, thus the lifetime days of use metric could not be used to develop a better exposure categorization for distinguishing participants with higher and lower lifetime exposures. Pesticide-use practice information was not collected or used to account for likely differences in exposure intensity. No validation assessments using exposure measurements were performed, nor was questionnaire-response reproducibility examined.]

The farmworker study used available information on the amount of each pesticide used on specific crops at every location in the state of California, USA. The information on pesticide location and amount was used in combination at the county level, with farmworker-union payroll-data records that provided work location information on a monthly level to stratify exposures among the farmworkers over their entire work history (Mills et al., 2005).

[The strengths of this research included the availability of high-quality and very specific records of pesticides and their amount of use for all locations and times across several decades. Use of payroll records that included information of work location removed the possibility of recall bias for workers trying to remember where and when they had worked over the many years of their career. The exposure assessment did, however, have considerable limitations. It was not possible to determine a direct match between participant work location and date with pesticide application and date. It was not possible to ascertain that participants worked in fields where 2,4-D had actually been applied, since the pesticide-use data and work-location data were linked only at a county level. There was no information on re-entry intervals between application and work dates. 2,4-D would not have been applied directly to the crops (it was most likely used as a pre-emergent herbicide) and probably not at any time that crops were present, so the farmworkers were unlikely to have had any exposure during crop picking or exposure to foliar residues. There was no evidence as to whether 2,4-D residues were present and accessible in the soil. There was no information on specific work tasks for specific locations, so the amount of soil contact could not be distinguished within and between individuals over time. There were no measurement studies to demonstrate that the exposure categorization was able to distinguish different exposure intensities between individuals. There was no information to judge whether and to what extent exposure to 2,4-D may have occurred away from the work locations.]

(b) Studies of the general population

One case–control study assessed the association between residential use of 2,4-D and non-Hodgkin lymphoma (NHL) in the general population (Hartge et al., 2005). This study used two different approaches for characterizing and classifying exposure to 2,4-D. One approach was based on a questionnaire that collected participant-recalled information on pesticide use in and around each home occupied for more than 2 years since 1970. The categories of use included lawn treatments by the participant, professional applicator, or a third person. The second approach was based on analysis of 2,4-D in house dust in the homes of cases and controls.

[The primary strength of this study was the use of two different approaches for exposure classification, firstly a computer-assisted personal-interview approach, and secondly a measurement-based approach. There were also important limitations in the exposure assessment. There was potential for recall bias among cases and controls, given the difficulty in recalling and accurately reporting lawn-herbicide uses for multiple homes over long periods of time. It was not possible to ascertain that 2,4-D was the active ingredient in the herbicides used for lawn treatments. 2,4-D in house dust may not be a good surrogate for differences in residential occupant exposures at an individual level. In a different study in the same country, there was no significant correlation between 2,4-D concentrations in house dust and in adult urine (Morgan et al., 2008).]

1.5 Regulation

The WHO Guidelines for Drinking-water Quality assign a value of 0.03 mg/L for 2,4-D (WHO, 2011). The guideline value applies to 2,4-D, since salts and esters of 2,4-D are rapidly hydrolysed to the free acid in the water (WHO, 2003, 2011).

This value was determined using an acceptable daily intake (ADI) of 0.01 mg/kg bw for the sum of 2,4-D and its salts and esters, expressed as 2,4-D, on the basis of a no-observed adverse effect level (NOAEL) of 1 mg/kg bw per day in a 1-year study of toxicity in dogs (for a variety of effects, including histopathological lesions in kidneys and liver) and a 2-year study of toxicity

and carcinogenicity in rats (for renal lesions) (JMPR, 1996), and assuming a body weight of 60 kg, drinking-water consumption of 2 L/day and a 10% allocation to drinking-water (WHO, 2003, 2011).

Information summarizing regulatory controls and related statutory measures addressing the use of 2,4-D in many regions, particularly countries of Asia, Africa, and South America, was not available to the Working Group.

2,4-D has been registered in the USA since 1948. It was the subject of a registration standard dated 16 February 1988, and a registration standard guidance document dated 1 September 1988. Through the EPA, 2,4-D is registered for use on a variety of food/feed sites, including field, fruit, and vegetable crops, and for use on turf, lawns, rights-of-way, aquatic sites, and forestry applications. 2,4-D is registered for use in terrestrial and aquatic environments (EPA, 2005).

In 2013, the Pest Management Regulatory Agency in Canada updated the re-evaluation notice for 2,4-D, stating that the 2,4-D registrants had provided the required data, which were deemed acceptable (Pest Management Regulatory Agency Canada, 2013).

The European Commission has approved products containing 2,4-D that fulfil safety requirements laid down in Article 5(1)(a) and (b) of Directive 91/414/EEC on the basis of 2,4-D being applied to winter and spring cereals, pasture, green manuring crops, grass-seeds, fallow land, borders of arable land and pastures, and 2,4-D ethylhexyl ether being applied to winter cereals (European Commission, 1991). Extension of use patterns beyond those specified requires evaluation at the member-state level. The European Commission has specified that the theoretical maximum daily intake (TMDI) for an adult of body weight 60 kg is 1.4% of the ADI of 0.05 mg/kg bw per day, based on the European Diet established by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO European Diet) (European Commission, 2001).

Concerning water contamination with 2,4-D, the EPA has set an enforceable regulation, called a "maximum contaminant level", at 0.07 mg/L (70 µg/L or ppb) (EPA, 2005).

MRLs for 2,4-D were first set in the European Union in 2002, and re-evaluated by EFSA in 2011. MRLs are specified at 1 mg/kg or less by the European Commission for most of 378 products containing 2,4-D (EFSA, 2011).

In relation to occupational exposure limits, the International Labour Organization (ILO) in collaboration with United States National Institute for Occupational Safety and Health (NIOSH) specified a threshold limit value for 2,4-D of 10 mg/m^3 as a time-weighted average (ILO, 1999). The Occupational Safety and Health Administration in the USA has adopted the following permissible exposure limits for 2,4-D: general industry, 10 mg/m^3; and construction industry, 10 mg/m^3, time-weighted average (OSHA, 2015).

Adequate margins of exposure together with details concerning appropriate personal protective equipment for workers are available through the EPA (EPA, 2005).

In various jurisdictions, statutory authorities exercise decision-making in relation to the marketing and use of various categories of consumer products. A comprehensive listing of such authorities and their respective responsibilities is beyond the scope of this *Monograph*, but examples of such authorities are given. The EPA has registered a herbicide formulation, the active ingredients of which are 2,4-D and glyphosate, to manage "resistant weeds". The EPA has also put in place restrictions to avoid pesticide drift, including a 30-foot in-field "no spray" buffer zone around the application area, no pesticide application when the wind speed is > 15 mph, and only ground applications are permitted (EPA, 2014).

2. Cancer in humans

2.1 Cohort studies

See Table 2.1

This section reviews studies that have specifically assessed exposure to 2,4-D. Studies were excluded from this review if 2,4-D was one of several chemicals or pesticides evaluated, but no specific risk estimate for 2,4-D only was provided (e.g. Kogevinas et al., 1997; Saracci et al., 1991; Boers et al., 2010; Hooiveld et al., 1998; Bueno de Mesquita et al., 1993; Coggon et al., 2015; Lynge, 1985; Lynge, 1998; Becher et al. 1996; 't Mannetje et al., 2005). [The Working Group considered that studies addressing the possible toxic effects of agent orange and related formulations could not be used to indicate the impact of 2,4-D specifically, and such studies are not addressed in the present *Monograph*.] The publications that were informative for assessing the carcinogenicity of 2,4-D are described below.

2.1.1 The International Register of Workers Exposed to Phenoxy Herbicides and Their Contaminants

The International Register of Workers Exposed to Phenoxy Herbicides and Their Contaminants was established in 1980 by IARC in association with the United States National Institute of Environmental Health Sciences to study cancer outcomes associated with these exposures (Saracci et al., 1991; Kogevinas et al., 1997). The register included 21 863 male and female workers in 36 cohorts, in 12 countries, in Europe, Australia, New Zealand, Canada, and the USA. Exposures were classified using individual job records, company-exposure questionnaires developed specifically for this study, and, in some cohorts, measurements of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and other dioxin and furan congeners in serum and adipose tissue and in the workplace.

Using the IARC International Register, Kogevinas et al. (1995, 1997) conducted nested case–control studes of the incidence of NHL (32 cases) and soft tissue sarcoma (11 cases). Cancer cases were identified by linkage of cohort rosters to death certificates and to cancer registries in each of the countries with cancer-registration systems. Cases were each matched to five controls by age, sex, and country of residence at the time of employment. A panel of three industrial hygienists reviewed company-exposure questionnaires and company records to assess exposure to 21 chemicals or mixtures. Ever exposure to 2,4-D/DP/DB (2,4-D and its precursor compounds, 2,4-dichlorophenoxypropionic acid and 2,4-dichlorophenoxybutyric acid), lagged by 5 years, was associated with an increased risk of soft tissue sarcoma (odds ratio, OR, 5.72; 95% CI, 1.14–28.65) and 1.4 (95% CI, 0.10–15.80) when adjusted for 2,4,5-T and MCPA. In analyses comparing different levels of cumulative exposure to non-exposure, the greatest increase in risk of soft tissue sarcoma in relation to 2,4-D was found for the category of highest exposure (OR, 13.71; 95% CI, 0.90–309.00), with a significant trend across exposure categories ($P = 0.01$). For NHL, the odds ratio was 1.11 (95% CI, 0.46–2.65). After adjustment for 2,4,5-T and MCPA, the odds ratio for NHL was 1.05 (95% CI, 0.26–4.28). No trend was observed for risk of NHL and level of 2,4-D exposure.

2.1.2 United Farm Workers of America

Mills et al. (2005) conducted a case–control study of lympho-haematopoietic cancers (leukaemia, 51 cases; NHL, 60 cases; multiple myeloma, 20 cases) in Hispanic farmworkers, which was nested within a cohort of members of the United Farm Workers of America labour union in California, USA. Incident cases diagnosed between 1988 and 2001 were identified from linkage of union records to the California cancer registry, and for each case, five controls were selected from

Table 2.1 Cohort studies of cancer and exposure to 2,4-D

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Kogevinas et al. (1995) Europe, Australia, New Zealand, Canada, and USA 1939–1992 Nested case–control study	Cases: 11 cases of STS (all men) and 32 cases of NHL (one woman) in a cohort identified from the International Register of Workers Exposed to Phenoxy Herbicides and Their Contaminants) (established jointly with IARC), or national cancer registration records Controls: 55 for STS, 158 for NHL; incidence-density sampling from the cohort; 5 controls per case were matched for age, sex, country of residence at the time of employment Exposure assessment method: company records; exposure information from each plant was retrieved using a questionnaire completed by factory personnel in the presence of an industrial hygienist; mortality records varied between the countries and included records from the company, union, insurance and, physicians, workers and families, and municipal and national vital records	NHL	2,4-D/DP/DB			None	No cases of STS or NHL in the Italian, Canadian, or Austrian cohorts. Risk of STS and of NHL increased with time since first exposure. In workers exposed to phenoxy herbicides with minimal or no contamination from TCDD, mortality was similar to that expected based on national rates Strengths: the study was large and included production workers and herbicide sprayers; the exposure assessment was able to classify workers by ordinal level of exposure to chemicals including 2,4-D; the study examined cancer incidence Limitations: information concerning lifestyle characteristics associated with cancer risk, such as smoking, was not available; there was no quantitative information on exposure
			Ever exposed	12	1.11 (0.46–2.65)		
			Low	4	0.73 (0.22–2.43)		
			Medium	6	2.14 (0.73–6.23)		
			High	2	0.69 (0.11–4.55)		
		NHL	2,4-D/DP/DB			2,4,5-T, MCPA	
			Ever exposed	12	1.05 (0.26–4.28)		
		STS	2.4-D/DP/DB			None	
			Ever exposed	9	5.72 (1.14–28.65)		
			Low	4	4.55 (0.61–53.41)		
			Medium	2	6.13 (0.33–129.70)		
			High	3	13.71 (0.90–309.00)		
			Trend-test *P* value: 0.01				
		STS	2.4-D/DP/DB			2,4,5-T, MCPA	
			Ever exposed	12	1.40 (0.10–15.80)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mills et al. (2005) California USA 1988–1997 Nested case–control study	Cases: 131 cases (response rate, NR) (leukaemia, 51; NHL, 60; multiple myeloma, 20) were identified by linking the cohort to the California cancer registry for 1988–2001 Controls: 655 (response rate, NR); 5 controls for each case were drawn from the cohort, not diagnosed with any cancer, and matched on sex, Hispanic ethnicity, and ± 1 yr of birth Exposure assessment method: crop and pesticide exposures were estimated by linking county/month and crop-specific job-history information from union records with California Department of Pesticide Regulation pesticide-use reports during the 20 yr before cancer diagnosis; "high exposure" can be interpreted as having worked in an area with high use	Leukaemia, total	High (vs low)	NR	1.03 (0.41–2.61)	Age, duration of union affiliation, date of first union affiliation, sex (when appropriate)	United Farm Workers of America Strengths: the study was conducted among farm workers (as opposed to pesticide applicators); included women; used objective exposure-assessment methods not prone to recall bias Limitations: small number of cases; number of cases and controls exposed was not reported; exposure assessment was based on regional pesticide-use data and did not take into account personal use of or exposure to pesticides
			High (men)	NR	0.55 (0.15–2.06)		
			High (women)	NR	3.73 (0.77–18.00)		
		NHL, total	High (vs low)	NR	3.80 (1.85–7.81)		
			High (men)	NR	3.79 (1.58–9.11)		
			High (women)	NR	5.23 (1.30–20.90)		
		Leukaemia, lymphocytic	High (vs low)	NR	1.47 (0.33–6.59)		
		Leukaemia, granulocytic	High (vs low)	NR	1.28 (0.30–5.42)		
		NHL, nodal	High (vs low)	NR	2.29 (0.90–5.82)		
		NHL, extranodal	High (vs low)	NR	9.73 (2.68–35.30)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Koutros et al. (2013) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 31 December 2007	54 412 male pesticide applicators; among the 57 310 applicators, 2898 were excluded (1563 women, 1071 prevalent cases of other types of cancer, 264 with no follow-up information) Exposure assessment method: questionnaire; see other details elsewhere; incident prostate cancers were identified from enrolment (1993–1997) until 31 December 2007 (n = 1962)	Prostate: (no family history)	Unexposed to 2,4-D (ref.)	290	1.00	Age, state, smoking, fruit servings, leisure time physical activity in winter, race	Agricultural Health Study Strengths: large cohort study in an agricultural population, thus high exposure prevalence; good exposure assessment
			IWED Q1	262	0.93 (0.78–1.11)		
			IWED Q2	256	0.88 (0.74–1.05)		
			IWED Q3	287	1.03 (0.86–1.22)		
			IWED Q4	260	0.87 (0.73–1.03)		
			Trend-test P value: 0.25				
		Prostate (with family history)	Unexposed to 2,4-D (ref.)	43	1.00	Age, state, smoking, fruit servings, leisure time physical activity in winter, race	
			IWED Q1	60	1.21 (0.8–1.82)		
			IWED Q2	68	1.29 (0.85–1.95)		
			IWED Q3	51	0.86 (0.56–1.31)		
			IWED Q4	73	1.17 (0.78–1.75)		
			Trend-test P value: 0.9				
Flower et al. (2004) Iowa and North Carolina, USA 1993–1997 for enrolment; 1975–1998 for follow-up	17 357 children (aged < 19 yr) of licensed pesticide applicators in Iowa Exposure assessment method: questionnaire; state cancer registries	Childhood cancer	Maternal use of 2,4-D (ever)	7	0.72 (0.32–1.60)	Child's age at enrolment	Agricultural Health Study There was a small number of cases in North Carolina, so these were excluded from all subsequent analyses Strengths: large cohort; assessment of specific chemicals, including 2,4-D Limitations: based on self-reported exposure; potential exposure to multiple pesticides; limited power to study a rare disease such as childhood cancer
			Paternal use of 2,4-D (prenatal)	26	1.29 (0.71–2.35)		

Table 2.1 (continued)

Reference, location, enrolment/ follow-up period, study design	Population size, description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Burns et al. (2011) Michigan, USA Enrolment, 1945–1994; follow-up until 2007	1316 men employed by Dow Chemical Company who had ever worked full time for at least 3 days between 1945–1994 in any of four buildings involved in 2,4-D manufacturing, located in Midland, MI, and were alive on 1 January 1985 (corresponding to the initiation of the Michigan state-wide cancer registry) Exposure assessment method: analyses by duration and exposure level based on JEM (from monitoring data) linked to work histories to assign a relative exposure index of 0.005, 0.05, 0.5, or 5 units to each job in in 2,4-D operations; cancer registry (SEER) cancer incidence rates for Michigan white men	All cancers combined	Cohort 3	211	0.96 (0.84–1.10)	Age	Cancer incidence was described only for Michigan. Cohort 3 was limited to persons considered to be Michigan residents for the entire period (the most restrictive but most valid analysis). Cohort 2: continued to accrue person years at risk for persons diagnosed with cancer, beyond the diagnosis date, and whomay have moved out of state. Other respiratory cancers included 4 cases of mesothelioma. Strengths: near complete follow-up; examined cancer incidence; exposures to 2,4-D varied and were assessed for each employee based on the exposure potential of a job during a period of time Limitations: small population; there was exposure to other phenoxyherbicides; lifestyle factors, such as smoking, were not adjusted for
		Other respiratory cancers	Cohort 3	5	4.76 (1.53–11.11)	Age	
		Other cancers of the urinary tract	Cohort 3	3	4.48 (0.90–13.08)	Age	
		NHL	Cohort 3	14	1.71 (0.93–2.87)	Age	
			Cohort 2, ≥ 5 yr duration of employment	4	3.08 (0.84–7.88)		
			Cohort 2, ≥ 5 exposure-years	3	2.16 (0.45–6.31)		

2,4-D/DP/DB, 2,4-dichlorophenoxyacetic acid/2,4-dichlorophenoxypropionic acid/2,4-dichlorophenoxybutyric acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; approx.., approximately; CI, confidence intervals; IWED, intensity-weighted cumulative exposure days; JEM, job-exposure matrix; MCPA, 2-methyl-4-chlorophenoxyacetic acid; NHL, non-Hodgkin lymphoma; NR, not reported; ref., reference; SEER, Surveillance, Epidemiology and End Results; SMR, standardized mortality ratio; STS, soft tissue sarcoma; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; Q, quintile; vs, versus

cancer-free union members, matched to the case by sex, Hispanic ethnicity, and year of birth. Exposure to 15 pesticides was assessed by linkage of detailed monthly employment union records to the California Department of Pesticide Regulation Pesticide Use Reports, for the two to three decades before cancer diagnosis. Specifically, each subject's employment by month, county, and crop was matched to pesticide application (pounds applied) in the county during that month, and the summed exposures were categorized by median or tertile. Logistic regression was conducted to estimate risk associated with pesticide use, with adjustment for age, sex, date of first contribution to the union, and duration of union membership. The study found that a history of high exposure to 2,4-D was associated with a 3.8-fold (95% CI, 1.85–7.81) increased risk of NHL when compared with a history of low exposure; this association appeared in women (OR, 5.23; 95% CI, 1.30–20.9), and men (OR, 3.79; 95% CI, 1.58–9.11), and was similar after adjustment for the 14 other high-use pesticides examined (OR, 3.58; 95% CI, 1.02–12.56). Leukaemia was not associated with 2,4-D (OR, 1.03; 95% CI, 0.41–2.61). [The semi-ecological exposure assessment in this study limited interpretation concerning individual exposure. On the other hand, the objective manner in which exposures were assigned reduced the possibility of recall bias.]

2.1.3 The Agricultural Health Study

In the USA, the risk of cancer of the prostate (total or aggressive) was examined in the Agricultural Health Study (AHS) for 1993–2007 (Koutros et al., 2013). During this period, 1962 incident cases of cancer of the prostate, including 919 cases of aggressive cancer, were observed among 54 412 pesticide applicators. Rate ratios and 95% confidence limits (CI) were calculated using Poisson regression to evaluate association with lifetime use of 48 pesticides using enrolment-questionnaire data (1993–1997) and follow-up questionnaire data (1999–2003), and incidence of all (total) cancers of the prostate, or aggressive cancers of the prostate (defined by a Gleason score of ≥ 7 or ≥ 8). About three quarters (74.9%) of the cases of cancer of the prostate, and the rest of the cohort (75.7%), had used 2,4-D. No excess incidence of cancer of the prostate was observed in applicators who had used 2,4-D (see Table 2.1). [This was a large study with high-quality exposure assessment.]

Flower et al. (2004) reported the results of analyses of pesticide application by parents and risk of childhood cancer in the AHS. The study included 17 357 children of pesticide applicators in Iowa. The number of cases in North Carolina was insufficient for the analyses, from which they were thus excluded. Parents provided data via questionnaires (1993–1997), and follow-up for cancer (retrospectively and prospectively) was done through the state cancer registries. Fifty incident cases of childhood cancer were identified in 1975–1998 in children aged 0–19 years; roughly half of the fathers had used 2,4-D prenatally (26 exposed cases). For all the children of the pesticide applicators, the incidence of all childhood cancers combined was increased compared with that of the general population, as were the incidence of all lymphomas combined, and of Hodgkin lymphoma. The odds ratio for mothers' use of 2,4-D and risk of childhood cancer was 0.72 (95% CI, 0.32–1.60, 7 exposed cases), and for fathers' use was 1.29 (95% CI, 0.71–2.35; 26 exposed cases). [The Working Group noted that this analysis had limited power to study rare diseases like childhood cancer.]

The risk of cutaneous melanoma was examined in the AHS for 1993–2005 (Dennis et al., 2010). Among 271 incident cases of cutaneous melanoma, no association was seen with exposure to 2,4-D. [The risk estimates for 2,4-D and melanoma were not presented, although the authors stated that "None of the 22 pesticides detailed on the enrollment questionnaire was

associated with melanoma" and 2,4-D was one of these pesticides according to the Appendix table.]

2.1.4 The Dow Chemical Company cohort

The Dow Chemical Company cohort in the USA included men involved in the manufacture, formulation, or packaging of 2,4-D and its amines or esters from 1945 until 1982 (Bond et al., 1988). Production of 2,4-D took place in four separate buildings of the plant, each building housing different activities with different levels of exposure. At least one of these buildings, referred to as the "2,4-D plant," also housed formulation and packaging of other herbicides (2,4,5-T, MCPA, and Silvex) at various times during its history. Industrial hygienists developed a job-exposure matrix for the estimation of levels of exposure to 2,4-D among employees based on department, job title, calendar year, available monitoring data and professional judgment, and estimated cumulative exposure based on a time-weighted average of all the jobs held by an individual during the follow-up period. Cumulative exposure to 2,4-D was categorized as: very low (< 0.05 mg/m^3), low (0.05–0.49 mg/m^3), moderate (0.5–4.9 mg/m^3), or high (≥ 5 mg/m^3) (Burns et al., 2011). In an analysis of cancer incidence with follow-up until 2007, there was no increase in the incidence of all cancers combined among employees who were residents in the state for the entire period (n = 1108; "cohort 3") (Burns et al., 2011). For NHL, the standardized incidence ratio (SIR) was 1.71 (95% CI, 0.93–2.87). The highest increases in risk of NHL were observed with duration ≥ 5 years (SIR, 3.08; 95% CI, 0.84–7.88) and in analyses with censoring only at the time employees moved out of the state (n = 1256) with intensity × duration ≥ 5 exposure years (SIR, 2.16; 95% CI, 0.45–6.31). There was no clear pattern in risk of NHL with decade of starting employment. These findings were similar to analyses of mortality with earlier follow-up periods to 1982 (Bond et al., 1988), 1986 (Bloemen et al., 1993), and 1994 (Burns et al., 2001). [The Working Group noted that while analyses were presented for three methods of counting person-time, the method that censored workers at the time of loss to follow-up or diagnosis (state residents for the entire period), provided the most valid estimate ("cohort 3").]

2.2. Case–control studies

2.2.1 Lympho-haematopoietic cancers

See Table 2.2

Many of the case–control studies on lymphoma and leukaemia reported on exposure to phenoxy herbicides as a broad category or report on pesticide mixtures, rather than exposure to 2,4-D, specifically (e.g. Pearce et al., 1986a, b, 1987; Wiklund et al., 1987; Pearce, 1989; Persson et al., 1989; Eriksson & Karlsson, 1992; Fontana et al., 1998; Fritschi et al., 2005; Eriksson et al., 2008; Orsi et al., 2009; Navaranjan et al., 2013). These studies were considered uninformative with regards to carcinogenicity of 2,4-D and are therefore not described further.

In the first of the case–control studies conducted by the National Cancer Institute in the midwest USA in the 1970s and 1980s, Hoar et al. (1986) studied 170 NHL cases diagnosed from 1976 through 1982 and 948 population-based controls in the state of Kansas. Participants were asked in a telephone interview about their years working or living on farmland with wheat, corn, sorghum, or pasture, and specific pesticides handled in these settings. Compared with subjects who had never farmed, ever exposure to phenoxy herbicides was associated with a 2.2-fold increased risk of NHL (OR, 2.2; 95% CI, 1.2–4.1; 24 exposed cases) and exposure to only 2,4-D (eliminating 3 cases who also used 2,4,5-T) was also associated with increased risk (OR, 2.6, 1.4–5.0). There were significant trends in increasing risk of NHL with increasing duration

Table 2.2 Case–control studies of lympho-haematopoietic cancer and exposure to 2,4-D

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Midwest USA							
Hoar et al. (1986) Kansas, USA 1976–1982	Cases: 170 (response rate, 96%); population-based cancer registry (University of Kansas Cancer Data Service) Controls: 948 (response rate, 94%); random digit dialling (age < 65 years), Medicare records (age ≥ 65 years), death certificates (deceased) Exposure assessment method: questionnaire	NHL	Phenoxyherbicides (synonymous with 2,4-D use)	24	2.2 (1.2–4.1)	Age and vital status (by matched analysis)	Studies in midwest USA This study also enrolled cases of STS and Hodgkin disease, but did not report results associated with 2,4-D exposure for these cancer sites Strengths: excellent response proportion; risk estimated by duration and frequency Limitations: duration and frequency variables were based on reported use of any herbicide (instead of being specific to 2,4-D)
			2,4-D use			Age and vital status (by matched analysis)	
			2,4-D only use (excluding 2,4,5-T)	21	2.6 (1.4–5.0)		
			2,4-D duration (yr):			Age and vital status (by matched analysis)	
			1–5	3	1.3 (0.3–5.1)		
			6–15	7	2.5 (0.9–6.8)		
			16–25	8	3.9 (1.4–10.9)		
			≥ 26	6	2.3 (0.7–6.8)		
			Trend-test *P* value: 0.002				
			2,4-D frequency (days/yr):			Age and vital status (by matched analysis)	
			1–2	6	2.7 (0.9–8.1)		
			3–5	4	1.6 (0.4–5.7)		
			6–10	4	1.9 (0.5–6.7)		
			11–20	4	3.0 (0.7–11.8)		
			≥ 21	5	7.6 (1.8–32.3)		
			Trend-test *P* value: 0.0001				
			2,4-D, first year of use			Age and vital status (by matched analysis)	
			1966 or later	5	1.9 (0.6–6.0)		
			1956–1965	9	2.9 (1.1–7.2)		
			1946–1955	8	2.1 (0.8–5.6)		
			Before 1946	2	6.2 (0.6–65.3)		
			Trend-test *P* value: 0.002				

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Zahm et al. (1990) Nebraska, USA 1983–1986	Cases: 201 (response rate, 91%); cases identified by the Nebraska Lymphoma Study Group and area hospitals; white male population Controls: 725 (response rate, 87%); random-digit dialling (age < 65 yr), Medicare records (age ≥ 65 yr), death certificates (deceased) Exposure assessment method: questionnaire; telephone interviews with subjects or next-of-kin	NHL	Mixed or applied 2,4-D	43	1.5 (0.9–2.5)	Age	Studies in midwest USA Strengths: good response proportion; information on duration and frequency, specific to 2,4-D; no evidence of recall bias since ORs for 2,4-D use were similar in subjects who recalled the exposure without prompting and in those who were probed for 2,4-D use Limitations: possibly biased exposure information from proxies
			Frequency mixing or applying 2,4-D (days/yr):			Age	
			1–5	16	1.2 (0.6–2.4)		
			6–20	12	1.6 (0.7–3.6)		
			≥ 21	3	3.3 (0.5–22.1)		
			Trend-test *P* value: 0.051				
			Duration 2,4-D used on farm (yr):			Age	
			1–5	3	0.9 (0.2–3.6)		
			6–15	11	2.8 (1.1–7.1)		
			16–20	3	0.6 (0.1–2.1)		
			≥ 21	13	1.3 (0.6–2.7)		
			Trend-test *P* value: 0.274				
			First year of use:			Age	
			Before 1945	8	1.4 (0.5–3.5)		
			1946–1955	13	1.1 (0.5–2.3)		
			1956–1965	5	2.1 (0.6–7.7)		
			1965–1986	4	1.3 (0.3–4.9)		
			Trend-test *P* value: 0.17				
			By histological subtype for personal use:			Age	
			B-cell/ever 2,4-D	NR	1.5 (0.9–2.6)		
			T-cell/ever 2,4-D	NR	2.0 (0.5–7.3)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Brown et al. (1990) Iowa and Minnesota, USA 1981–1984	Cases: 578 (response rate, 86%); state cancer registry (IA) and surveillance network of hospitals and pathology laboratories (MN) Controls: 1245 (response rate, 77–79%); random-digit dialling (age < 65 yr), Medicare records (age ≥ 65 yr), death certificates (deceased) Exposure assessment method: interviewer-administered questionnaire elicited information about use of specific pesticides	Leukaemia	Ever mixed/ handled/applied 2,4-D	98	1.2 (0.9–1.6)	Vital status, age, state, smoking, family history of lympho-haematopoietic cancer, high-risk occupation, high-risk exposures	Studies in midwest USA Reference group was non-farmers Strengths: agricultural region with high frequency of farming and pesticide use Limitations: no quantitative exposure information; white men only
		Leukaemia (AML)	Ever handled 2,4-D	28	1.5 (0.9–2.5)		
		Leukaemia (CML)	Ever handled 2,4-D	13	1.9 (0.9–3.9)		
		NHL (CLL)	Ever handled 2,4-D	45	1.3 (0.8–2.0)		
Cantor et al. (1992) Iowa and Minnesota, USA 1980–1983	Cases: 622 (response rate, 89%); Iowa State Health Registry and surveillance of Minnesota hospital and pathology laboratory records Controls: 1245 (response rate, 77–79%); random-digit dialling (age < 65 yr), Medicare records (age ≥ 65 yr), death certificates (deceased) Exposure assessment method: questionnaire collected data on use history of specific pesticides	NHL	Handled 2,4-D	115	1.2 (0.9–1.6)	Vital status, age, state, smoking, family history of lympho-haematopoietic cancer, high-risk occupation, high-risk exposures	Studies in midwest USA Reference group was non-farmers; no difference in results by state Strengths: agricultural region with high frequency of farming occupation and pesticide use Limitations: non-quantitative exposure assessment; white men only
			Handled before 1965	86	1.3 (0.9–1.8)		
			Handled without PPE	89	1.2 (0.9–1.7)		
			Iowa – ever handled 2,4-D	51	1.2 (0.8–1.9)		
			Minnesota – ever handled 2,4-D	35	1.4 (0.9–2.3)		

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
De Roos et al. (2003) Iowa, Minnesota, Nebraska, Kansas, USA 1980s (pooled analysis)	Cases: 870 (response rate, NR); white men with NHL Controls: 2569 (response rate, NR); frequency matched on age, race, state of residence using 2 : 1 ratio in Iowa and Minnesota, 4 : 1 in Kansas and Nebraska; random-digit dialling for living cases aged < 65 yr and from the Health Care Financing Administration for those aged ≥ 65 yr; controls for deceased cases from deaths records in each state, matched for age and year of death Exposure assessment method: telephone interviews with subjects or next-of-kin in Kansas and Nebraska, and in-person in Iowa and Minnesota	NHL	Use of 2,4-D (hierarchical regression)	123	0.9 (0.6–1.2)	Age, location, other pesticides	Subjects missing data for any of 47 pesticides were excluded (25% of subjects)

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Other studies in North America							
Woods et al. (1987) Western Washington, USA 1981–1984	Cases: 576 (response rate, NR); cases identified from population-based tumour registry that covered 13 counties of western Washington State from 1974 Controls: 694 (response rate, NR) chosen by random-digit dialling (aged 20–64 yr) or at random from Health Care Financing Administration records covering social security recipients in the study area (aged 65–79 yr) Exposure assessment method: questionnaire	NHL	Ever occupationally exposed to 2,4-D Farmers who "regularly worked with 2,4-D"	NR NR	0.73 (0.40–1.30) 0.68 (0.3–1.4)	Age	Strengths: large study population Limitations: number exposed to 2,4-D, NR

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McDuffie et al. (2001) Six provinces, Canada 1991–1994	Cases: 517 (response rate, 67.1%) men from cancer registries and hospitals; newly diagnosed men (aged ≥ 19 yr) Controls: 1506 (response rate, 48%); random sample from health insurance and voting records; men (aged ≥ 19 yr) frequency-matched on province and age Exposure assessment method: mailed questionnaire followed by telephone interview for subjects reporting ≥ 10 h/ yr of pesticide exposure	NHL	Mixed/sprayed 2,4-D Frequency (days/ yr): > 0 and ≤ 2 > 2 and ≤ 5 > 5 and ≤ 7 > 7	111 111 55 36 9 11	1.32 (1.01–1.73) – 1.17 (0.83–1.64) 1.39 (0.91–2.13) 1.38 (0.60–3.15) 1.22 (0.60–2.49)	Age, province, medical history variables, family history of cancer	Men only Strengths: large sample; information on individual pesticides Limitations: low response rate
Hartge et al. (2005) Iowa, Washington (Seattle metropolitan area), Michigan (Detroit metropolitan area), California (Los Angeles county), USA 1998–2000	Cases: 679 (response rate, 59%); population cancer registries (SEER) Controls: 510 (response rate, 44); random-digit dialling (age < 65 yr); Medicare records (≥ age 65 yr), stratified by age, sex, race, centre Exposure assessment method: environmental monitoring; measurement in household dust samples and interview	NHL	2,4-D (ng/g): Below detection limit < 500 500–999 1000–9999 > 10 000	 147 257 86 165 24	 1.00 1.10 (0.78–1.55) 0.91 (0.58–1.45) 0.66 (0.45–0.98) 0.82 (0.41–1.66)	Study site, age, sex, race, education	2,4-D half-life in dust is probably shorter than latency period. Frequency and intensity of residential exposures are low compared with those exposed occupationally Strengths: exposure based on measurement (rather than recall) Limitations: low response rate

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Metayer et al. (2013) Northern and central California, USA 1995–2008	Cases: 296 childhood leukaemia, 269 ALL (response rate, 91%); enrolled from paediatric clinical centres Controls: 333 (response rate, 82%); enrolled from birth certificates Exposure assessment method: environmental monitoring; measurement in household dust sample	ALL	2,4-D log-transformed concentration (ng/g)	252	0.96 (0.85–1.08)	Sex, age, Hispanic ethnicity, maternal race, income, season of dust sampling, year of dust sampling, neighbourhood type (urban, rural, etc.)	Strengths: exposure measurement (rather than self-report) Limitations: 2,4-D half-life in dust may be shorter than latency period

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Mills et al. (2005) California, USA 1987–2001 Nested case–control study	Cases: 131 (response rate, 100%); labour union members, Hispanic population; cases identified from linkage to state-wide population-based cancer registry Controls: 655 (response rate, 100%); 5 controls per case were selected from the union, who had not been diagnosed with any cancer at time of case diagnosis, and matched on sex, Hispanic ethnicity, year of birth Exposure assessment method: detailed month-by-month work histories from union records linked to pesticide-use records from California Department of Pesticide Regulation & Pesticide Use Reports to match pesticide applications given the month/year/ location/crop	NHL	High (vs low)			Age, sex, duration of union affiliation, date of first union affiliation	United Farm Workers of America Strengths: large database with objective exposure assignment (e.g. not based on recall) Limitations: semi-ecological exposure assessment limited interpretation about individual exposure
			High (vs low)	60	3.80 (1.85–7.81)		
			Men	45	3.79 (1.58–9.11)		
			Women	15	5.23 (1.30–20.9)		
		NHL, nodal	High (vs low)	38	2.29 (0.90–5.82)	Same	
		NHL, extranodal	High (vs low)	22	9.73 (2.68–35.30)	Same	
		Leukaemia	High (vs low)	51	1.03 (0.41–2.61)	Same	
			Men	35	0.55 (0.15–2.06)		
			Women	16	3.73 (0.77–18.00)		
		Lymphocytic leukaemia	High (vs low)	23	1.47 (0.33–6.59)	Same	
		Granulocytic leukaemia	High (vs low)	20	1.28 (0.30–5.42)	Same	

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Sweden							
Hardell et al. (1994) Umea, Sweden 1974–1978	Cases: 105 (response rate, NR); Department of Oncology, Umea Controls: 335 (response rate, NR); national population registry (living) or national registry for causes of death (deceased) Exposure assessment method: questionnaire	NHL	Occupational or leisure-time use of 2,4-D only	3	13.0 (1.2–360.0)	NR	Strengths: separate risk estimate for 2,4-D Limitations: small number exposed to 2,4-D, specifically
Nordström et al. (1998) Sweden 1987–1990	Cases: 121 (response rate, NR); male patients with HCL reported to the Swedish Cancer Registry 1987–1992 Controls: 484 (response rate, NR); age- and county-matched controls from the national population registry Exposure assessment method: mailed, self-administered questionnaire (by subject or next-of-kin) plus supplemental telephone call for clarification of unclear information	NHL (HCL)	2,4-D	2	1.6 (0.3–8.3)	Age	A minimum exposure of 1 working day(8 h) and an induction period of ≥ 1 yr were used in the coding of exposures Strengths: comprehensive population registration Limitations: small number of ever-exposed cases and controls for the analysis; the reference group was not specified

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Europe							
Miligi et al. (2003) Italy 1990–1993	Cases: 1575 (response rate, 80–83%) Controls: 1232 (response rate, 74%) Exposure assessment method: questionnaire	NHL (ICD 200 & 200) and CLL (ICD 204.1)	Ever occupationally exposed to 2,4-D, men Ever occupationally exposed to 2,4-D, women	6 7	0.7 (0.3–1.9) 1.5 (0.4–5.7)	Age, area	
Miligi et al. (2006a) Italy 1990–1993 Case–control	Cases: 1145 NHL and CLL, 205 MM, 430 leukaemia, 258 HD (80%); all incident cases of malignancies of the haematolymphopoietic system, aged 20–74 yrs, residents of the study area Controls: 1232 (response rate, 74%); age- and sex-matched from the general population Exposure assessment method: questionnaires reviewed by agronomists to assign pesticide-exposure histories	NHL (ICD 200 & 200) and CLL (ICD 204.1)	Ever occupationally exposed to 2,4-D Probability of use > low and no protective equipment	17 9	0.9 (0.5–1.8) 4.4 (1.1–29.1)	Sex, age, area	Association with ever use of 2,4-D did not differ between men and women, as reported in an earlier publication (Miligi et al., 2003) Strengths: expert assessment of exposure

Table 2.2 (continued)

Reference, location, enrolment/ follow-up period	Population size (response rate), description, exposure assessment method	Organ site	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cocco et al. (2013a) Europe (Czech Republic, France, Germany, Italy, Ireland, Spain) 1998–2003	Cases: 2348 (response rate, 88%); referring centres Controls: 2462 (response rate, 52–81%); population controls (Germany, Italy); hospital controls (Czech Republic, France, Ireland, Spain) Exposure assessment method: subjects reporting work in agriculture received job-specific questionnaire eliciting further details on tasks and exposures	B-cell lymphoma CLL	2,4-D	2	0.6 (0.1–3.5)	Age, sex, education, centre Age, sex, education, centre	Strengths: detailed exposure questionnaire combined with expert assessment Limitations: low response proportion for population controls

ALL, acute lymphoblastic/lymphocytic leukaemia; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myeloid leukaemia; 2,4-D, 2,4-dichlorophenoxyacetic acid; DEET, N,N-Diethyl-meta-toluamide; HCL, hairy cell leukaemia; NHL, non-Hodgkin lymphoma; NR, not reported; OR, odds ratio; PPE, personal protective equipment; SEER, Surveillance, Epidemiology, and End Results; STS, soft tissue sarcoma; vs, versus; yr, year

(years) (trend-test *P* value, 0.002) and frequency (days/year) (trend-test *P* value, 0.0001) of exposure, with the highest risks observed in the third of four exposure categories with 16–25 years exposure (OR, 3.9; 95% CI, 1.4–10.9) or ≥ 21 days/year exposure (OR, 7.6; 95% CI, 1.8–32.3). [The Working Group noted that the information on duration and frequency were based on reported patterns of use of any herbicide, rather than being specific to 2,4-D. This study also enrolled cases of soft tissue sarcoma and Hodgkin, disease, but did not report results associated with 2,4-D exposure for these cancer sites.]

The study conducted by the United States National Cancer Institute (NCI) in Nebraska included 201 cases of NHL and 725 controls, identified between 1983 and 1986 (Zahm et al., 1990). The study was limited to white men. There were elevated, but non-significant increases in risk of NHL observed in association with having having ever mixed or applied 2,4-D (OR, 1.5; 95% CI, 0.9–2.5) and with ≥ 21 days per year use on the farm (OR, 3.3; 95% CI, 0.5–22.1), and a trend in increasing risk by frequency of use (*P* = 0.051). There were no apparent patterns by duration or year of first use. Risk estimates were elevated for T-cell and B-cell NHL, but the trend by days per year was significant only for B-cell NHL (*P* = 0.045). Possible confounding of the results for 2,4-D by use of other pesticides was evaluated. Exclusion of users of 2,4,5-T did not change the risk estimates for handling 2,4-D. The risk associated with having ever mixed or applied 2,4-D was attenuated with exclusion of all organophosphate users (OR, 1.1; CI, not reported), but was increased with adjustment for fungicides (OR, 1.8; 95% CI, 1.1–3.0). For farmers who mixed or applied 2,4-D on > 20 days per year, simultaneous adjustment for organophosphates and fungicides resulted in a risk estimate of 3.1 (CI not reported). Risk estimates for use of 2,4-D were similar in subjects who recalled the exposure without prompting and in subjects whose use of 2,4-D was only reported in response to a specific probe (OR, 1.5 in both groups [CI not reported]), suggesting little recall bias; however, the associations were higher when limited to proxy respondents. [The Working Group noted possible biased exposure information from proxies. This study also enrolled cases of Hodgkin disease, multiple myeloma, and chronic lymphocytic leukaemia, but did not report results associated with 2,4-D exposure for these other cancer sites.]

In Iowa and Minnesota, USA, cases of NHL (*n* = 622) (Cantor et al., 1992) and leukaemia (*n* = 578) (Brown et al., 1990) diagnosed in 1980–1984 were frequency-matched to 1245 population controls. Only white men were included in the analyses. There were no strong associations of 2,4-D use in relation to either NHL or leukaemia in this study, in analyses of ever use, frequency of use, year of first use, use without personal protective equipment, and use by state, or when considering multiple potential confounders including vital status, age, state, smoking, family history of lympho-haematopoietic cancer, high-risk occupation, and high-risk exposures. For subjects who had ever used 2,4-D, the odds ratios were: NHL, 1.2 (95% CI, 0.9–1.6) (Cantor et al., 1992); chronic lymphocytic leukaemia [a subtype of NHL], 1.3 (95% CI, 0.8–2.0) (Brown et al., 1990); and all leukaemia combined, 1.2, (95% CI, 0.9–1.6) (Brown et al., 1990).

A pooled analysis by De Roos et al. (2003) of men from the three United States NCI case–control studies conducted in the midwest USA was limited to men with complete data for 47 pesticides (*n* = 870 cases, and 2569 controls from Hoar et al. (1986); Zahm et al. (1990); Cantor et al. (1992)). About 75% of the original study population was included, but examination of risk factors for NHL, including family history and farming history, indicated no difference in the association of those factors with case status between the original population and the included subset. No association was found between ever use of 2,4-D and risk of NHL (OR, 0.9; 95% CI, 0.6–1.2), with adjustment for other individual

pesticides using hierarchical regression. [This estimate was limited to a smaller study population than in the individual studies, due to exclusion of participants with missing data for any of the multiple pesticides examined; nevertheless, the sample size was large. The study suggested that any confounding of the association between NHL and exposure to 2,4-D by exposure to other pesticides is away from the null. The hierarchical regression method "shrinks" (adjusts) unstable estimates towards a prior distribution; however, the shrunken estimate for 2,4-D (OR, 0.9) did not differ substantially from the pooled estimate before shrinkage (OR, 0.8).]

Woods et al. conducted a case–control study of NHL in western Washington State, USA, in the early 1980s, in which a detailed occupational history was obtained, including exposure to phenoxy herbicides (Woods et al., 1987; Woods & Polissar, 1989). In a comparison of 576 cases and 694 controls, there was no observed difference with respect to ever having occupational exposure to 2,4-D (OR, 0.73; 95% CI, 0.4–1.3) or for farmers who reported having "regularly worked with" 2,4-D (OR, 0.68; 95% CI, 0.3–1.4). There were no details provided on the number of cases and controls exposed to 2,4-D. [This study had a strong exposure assessment, including expert review of occupational histories and verification of exposure with a third party in instances where exposure was uncertain. Response proportions were not provided.]

A large study of NHL in men was conducted in six provinces of Canada, between 1991 and 1994 (McDuffie et al., 2001). A total of 517 cases were identified from provincial cancer registries and hospitals, and 1506 controls were identified from provincial health insurance records, computerized telephone listings, and voters' lists. Information about pesticide exposure was collected by means of a mailed questionnaire, followed by a telephone interview for subjects reporting 10 hours per year or more of pesticide exposure. In analyses of individual phenoxyherbicides, the odds ratio for 2,4-D was 1.32, (95% CI, 1.01–1.73). There was no dose–response pattern in analyses of 2,4-D exposure by frequency of use (days/year). Similar effect estimates were presented in a later paper by (Pahwa et al., 2012), in which no interaction was found between exposure to 2,4-D and asthma or allergy in the relationship with NHL. Hohenadel et al. (2011) found no interaction between exposure to 2,4-D and malathion in this study population, and reported no association between exposure to 2,4-D and risk of NHL in the absence of malathion exposure (OR, 0.94; 95% CI, 0.67–1.33) (Hohenadel et al., 2011) [The Working Group noted that the response proportion was 48% for population controls, making selection bias a concern].

Two studies estimated the risk of lympho-haematopoietic cancer associated with exposure to 2,4-D based on measurement of 2,4-D in samples of household dust. Hartge et al. (2005) conducted a study of NHL in Iowa, and the metropolitan region of Seattle, Washington State, Detroit, Michigan, and Los Angeles County, California, USA. Population controls were identified by random-digit telephone dialling and through Medicare records. Dust samples were collected from participating households from the subjects' vacuum-cleaner bag, and 2,4-D concentration (in ng/g) was measured by gas chromatography/mass spectrometry. Comcentrations of 2,4-D and dicamba were higher in the carpets of subjects reporting residential use of herbicides. There was no association between 2,4-D concentration and risk of NHL, and no pattern in the dose–response relationship with increasing concentration. The risk estimate for the highest category of 2,4-D exposure at > 10 µg/g compared with concentrations below the detection limit was 0.82 (95% CI, 0.41–1.66). [The interpretation of this study was limited by the fact that the half-life of 2,4-D in house dust is unknown.]

Metayer et al. (2013) conducted a study of childhood acute lymphocytic leukaemia

in northern and central California, USA, in which samples of household dust were collected according to a standardized protocol during a household visit. There was no association between the concentration of 2,4-D in household dust (measured by gas chromatography/mass spectrometry) and risk of childhood acute lymphocytic leukaemia (OR, 0.96; 95% CI, 0.85–1.08). [The half-life of 2,4-D in house dust is unknown.]

Two case–control studies conducted in Sweden used national registration systems for identification of cases and population controls. Hardell et al. (1994) conducted a study on NHL in Umea, Sweden, in which they identified 105 cases and 335 controls from 1974 until 1978. Controls were identified from the national population registry (living) and national registry for causes of death (deceased), and were matched to cases on age, sex, place of residence, vital status and year of death (for the deceased). Exposure information was obtained by questionnaire (completed by proxy for deceased subjects), and exposure was defined as ever use of the pesticide in occupation or during leisure time. There was a significantly increased risk of NHL with use of 2,4-D among those without exposure to any other phenoxy herbicide, based on only 3 exposed cases and 1 exposed control (OR, 13; 95% CI, 1.2–360). There was no estimate presented for exposure to 2,4-D with statistical adjustment for other phenoxy herbicides. [The extreme imprecision of the risk estimate for 2,4-D from this study limited interpretation about the possible magnitude of the association.]

Nordström et al. (1998) identified 121 male patients with hairy cell leukaemia who reported to the Swedish Cancer Registry between 1987–1982 and 484 age- and country-matched population-based controls identified from national registries. Information on pesticide use was collected by mailed questionnaire, with clarification of information by follow-up telephone calls, if needed. Exposure was defined as a minimum of one working day of exposure (8 hours) and a latency period of ≥ 1 year. The association with ever having been exposed to 2,4-D, specifically, was reported for hairy cell leukaemia only, based on two exposed cases and five exposed controls (OR, 1.6; 95% CI, 0.3–8.3).

In a large case–control study on NHL (including chronic lymphocytic leukaemia; $n = 1145$) in Italy in which exposure was assessed through consultation by industrial hygienists and agronomists, there was no association with ever use of 2,4-D (OR, 0.9, 95% CI, 0.5–1.8; 17 exposed cases) (Miligi et al., 2006a). Greater than low probability of 2,4-D use was not associated with risk of NHL in men (OR, 0.7; 0.3–1.9; 6 exposed cases) or women (OR, 1.5; 0.4–5.7; 7 exposed cases) (Miligi et al., 2003); however, an increased risk was observed with greater than low probability of 2,4-D use in combination with lack of protective equipment (OR, 4.4; 95% CI, 1.1–29.1; 9 exposed cases) (Miligi et al., 2006a).

The Epilymph study is a large case–control study of lymphoma (including NHL, Hodgkin lymphoma, chronic lymphocytic leukaemia, and multiple myeloma) conducted in six European countries. Cocco et al. (2013a) evaluated pesticide exposures in the Epilymph study based on expert assessment of detailed work histories coupled with a crop-exposure matrix. Exposure to 2,4-D was not associated with risk of B-cell lymphoma in this study (OR, 0.6; 95% CI, 0.1–3.5), based on two exposed cases and four exposed controls. [Findings for 2,4-D were mentioned in the text of the paper with a reference to Table 4. Therefore, the Working Group interpreted the table entry labelled "2,4-dichlorophenol" as the association between 2,4-dichlorophenoxyacetic acid and B-cell lymphoma.]

2.2.2 Other cancer sites

Most available case–control studies on soft tissue sarcoma evaluating exposure to phenoxy herbicides provided effect estimates for wide exposure categories and did not provide

estimates specifically for exposure to 2,4-D (Hardell & Sandström, 1979; Eriksson et al., 1981; Smith et al., 1984; Vineis et al., 1987; Smith & Christophers, 1992). Two studies including soft tissue sarcoma did not provide odds ratios specific for exposure to 2,4-D, but indicated that risks were not associated with exposure to 2,4-D (Hoar et al., 1986; Woods et al., 1987). One case–control study on gastric cancer and one on nasal and nasopharyngeal carcinoma evaluating exposure to phenoxy herbicides provided effect estimates for wide exposure categories and did not provide estimates specifically for exposure to 2,4-D (Hardell et al., 1982; Ekström et al., 1999). These studies were considered to be uninformative about the carcinogenicity of 2,4-D and are not reviewed further here.

A case–control study on glioma was conducted among non-metropolitan residents of Iowa, Michigan, Minnesota and Wisconsin, USA (Yiin et al., 2012). The study included 798 histologically confirmed cases of primary intracranial glioma (45% with proxy respondents) and 1175 population-based controls, aged 18–80 years. Subjects were interviewed face-to-face. Information on exposure from questionnaire responses was evaluated by an experienced industrial hygienist. An inverse association was observed for non-farm occupational use of 2,4-D (OR, 0.56; 95% CI, 0.28–1.10); a similar result was observed when excluding proxy respondents (6 cases) (OR, 0.49; 95% CI, 0.20–1.22). Reported house and garden use of 2,4-D was also inversely associated with risk (OR, 0.64; 95% CI, 0.47–0.88; OR after excluding proxy respondents, 0.76; 95% CI, 0.51–1.11) [The number of exposed subjects was very small. Effect estimates were not presented for farm use of 2,4-D].

Mills et al. (2005) conducted a registry-based case–control study of cancer of the breast in Hispanic members of a farm labour union in California, USA. The study included 128 incident cases of cancer of the breast (1988–2001) diagnosed among past or present members of a large agricultural labour union and identified from the California cancer registry. The controls were 640 cancer-free members of the same trade union matched for ethnicity and the case's attained age at the time of diagnosis. Exposure was determined from detailed employment records that were linked to pesticide information obtained from the Pesticide Databank, a database of historical pesticide-use records collected by the California Department of Food and Agriculture. Exposure to 2,4-D was associated with increased risk of cancer of the breast (OR, 2.14; 95% CI, 1.06–4.32; 21 cases with "high" 2,4-D exposure) among cases diagnosed in the second part of the study period (1995–2001), but not in the first part (1988–1994), after adjusting for age, date of first union affiliation, duration of union affiliation, fertility, and socioeconomic level. [The overall odds ratio for 2,4-D as calculated by the Working Group was 1.40 (95% CI, 0.91–2.17]. Exposure assessment was semi-ecological in this study. There was no adjustment for several potential confounders.]

2.3 Meta-analyses

Schinasi & Leon (2014) conducted a meta-analysis of NHL and exposure to pesticides in agricultural settings. Case–control and cohort studies were included if they were published in English language, used interviews, questionnaires or exposure matrices to assess occupational exposure to agricultural pesticides, and reported quantitative associations of NHL overall or by subtype with specific active ingredients or chemical groups. Five papers on case–control studies contributed to the meta relative-risk estimates for the relationship between occupational exposure to 2,4-D and NHL overall (Zahm et al., 1990; Cantor et al., 1992; Mills et al., 2005; Miligi et al., 2006b; Pahwa et al., 2012). The meta relative-risk for exposure to 2,4-D and NHL was 1.40 (95% CI, 1.03–1.91, I^2 = 61.5%). Sensitivity analyses examining the influence of sex, period

of diagnosis, and geographical region did not substantially change the meta relative risk (RR) for 2,4-D.

A meta-analysis of 2,4-D and cancer by Goodman et al. (2015) included a larger number of peer-reviewed observational epidemiological studies of NHL reported before 9 October 2014 that reported quantitative measures of association specifically for 2,4-D, and also estimated cancer of the stomach or prostate in relation to exposure to 2,4-D. In the main analysis, there were nine studies on NHL (Woods & Polissar, 1989; Hardell et al., 1994; Kogevinas et al., 1995; De Roos et al., 2003; Hartge et al., 2005; Mills et al., 2005; Miligi et al., 2006b; Burns et al., 2011; Hohenadel et al., 2011), three studies on cancer of the stomach (Lee et al., 2004; Mills & Yang, 2007; Burns et al., 2011), and two studies on cancer of the prostate (Band et al., 2011; Burns et al., 2011). Risk estimates and confidence intervals extracted from the original studies were log-transformed before analysis. Exposure to 2,4-D was not associated with NHL (RR, 0.97; 95% CI, 0.77–1.22; I^2 = 28.8%, $P_{heterogeneity}$ = 0.19). For cancer of the stomach, the relative risk was 1.14 (95% CI, 0.62–2.10; I^2 = 54.9%, $P_{heterogeneity}$ = 0.11), and for cancer of the prostate it was 1.32 (95% CI, 0.37–4.69; I^2 87.0%, $P_{heterogeneity}$ = 0.01). The tests of heterogeneity of effect by exposure source did not reveal heterogeneity by study design, type of exposure (agricultural or other), geographical location, or sex. Sensitivity analyses indicated that the results were robust to most factors considered. However, after substitution of: (i) a pooled analysis that adjusted for multiple pesticides (De Roos et al., 2003) by the three individual studies (Hoar et al., 1986; Zahm et al., 1990; Cantor et al., 1992) that were not adjusted; and (ii) a study considering 2,4-D in the absence of malathion (Hohenadel et al., 2011) by a study that considered ever exposure to 2,4-D (Pahwa et al., 2012); and (iii) the unadjusted rather than the adjusted estimate from another study (Mills et al., 2005), the meta relative risk for NHL increased to 1.34 (95% CI, 1.04–1.72), and heterogeneity also increased (I^2 = 56.3%, P = 0.011).

The Working Group carried out an additional meta-analysis of 2,4-D and non-Hodgkin lymphoid neoplasms (including NHL, multiple myeloma, hairy cell leukaemia, and chronic lymphocytic leukaemia) (see Table 2.3 for the key characteristics of the studies included in the Working Group meta-analysis and a comparison with the previously published meta-analyses). Thirteen reports were included in the main (primary) analysis and 15 reports were included in a secondary analysis. When the risk estimates for the primary analysis were displayed on a funnel plot, the plot was nearly symmetric [this was interpreted to show no significant evidence of publication bias] (Fig. 2.1). Where available, risk estimates were selected for the primary analysis that adjusted for use of pesticides other than 2,4-D: De Roos et al. (2003) adjusted for more than 40 pesticides, Kogevinas et al. (1995) adjusted for 2,4,5-T and MCPA, Mills et al. (2005) adjusted for 14 pesticides. In addition, a risk estimate for 2,4-D in the absence of exposure to malathion was selected from Hohenadel et al. (2011), the risk estimate for 2,4-D in the absence of exposure to DEET was selected from Pahwa et al. (2006), and a risk estimate in the absence of any other phenoxy herbicide was selected from Hardell et al. (1994). Estimates adjusted for other pesticide use were not available for a further seven studies included in the meta-analysis: Woods & Polissar (1989), Miligi et al. (2006b), Cocco et al. (2013b) (B-cell lymphoma), Nordström et al. (1998) (hairy cell leukaemia), Brown et al. (1993) (multiple myeloma), Brown et al. (1990) (chronic lymphocytic leukaemia), and Burns et al. (2011). Hartge et al. (2005) was not included in the Working Group meta-analysis, in contrast to Goodman et al. (2015), due to the use of exposure measurement via dust, which was qualitatively different from the other studies (Table 2.3). In this analysis, 2,4-D was not associated with risk of NHL (RR, 1.04; 95% CI; 0.88–1.22; I^2 = 6%,

Table 2.3 Selected characteristics of studies included in the Working Group meta-analysis (primary and secondary analysis), and comparison with previously published meta-analyses

Study reference	Relative risk (95% CI)	Included in Working Group analysis[a]		Included in previously published meta-analysis		Exposure–response reported	Study design	Study outcome	Comments
		Primary analysis	Second analysis	Goodman et al. (2015)	Schinasi & Leon (2014)				
Burns et al. (2011)	1.71 (0.93–2.87)					√	Cohort	NHL	Dow cohort of 2,4-D production workers; analysed with three methods of counting person-time. We chose cohort 3 for the primary analysis because it is the most valid since it was censored at lost-to-follow-up or date of diagnosis
	1.36 (0.74–2.29)			√		√	Cohort	NHL	Cohort 2
	0.79 (0.09–2.87)					√	Cohort	MM	Cohort 3
	1.50 (0.85–2.43)	√	√			√	Cohort	NHL + MM	Cohort 3 combined SMR for NHL and MM
Cocco et al. (2013a)	0.60 (0.10–3.50)	√	√				Case–control	NHL	B-cell lymphoma
Nordström et al. (1998)	1.60 (0.30–8.30)	√	√				Case–control	HCL	HCL
Mills et al. (2005)	3.80 (1.85–7.81)		√		√		Cohort	NHL	This estimate was rounded to 3.80 (1.85–7.81) for analysis in Schinasi & Leon (2014)
	3.58 (1.02–12.56)	√		√			Cohort	NHL	Adjusted for use of 14 other pesticides The upper bound of the 95% CI was reported as 12.58 in Goodman et al. (2015)
Kogevinas et al. (1995)	1.11 (0.46–2.65)		√	√		√	Cohort	NHL	
	1.05 (0.26–4.28)	√				√	Cohort	NHL	Adjusted for use of the pesticides 2,4,5-T and MCPA
Pahwa et al. (2012)	1.27 (0.98–1.65)		√		√		Case–control	NHL	This estimate was rounded to 1.30 (1.00–1.70) for analysis in Schinasi & Leon (2014)

Table 2.3 (continued)

Study reference	Relative risk (95% CI)	Included in Working Group analysis[a]		Included in previously published meta-analysis		Exposure–response reported	Study design	Study outcome	Comments
		Primary analysis	Second analysis	Goodman et al. (2015)	Schinasi & Leon (2014)				
Miligi et al. (2006a)	0.90 (0.50–1.80)	√	√	√	√		Case–control	NHL + CLL	
	4.40 (1.10–29.10)						Case–control	NHL + CLL	Probability of use > low (rated by hygienist) and lack of protective equipment. This estimate reflects the highest exposure
Hartge et al. (2005)	0.89 (0.49–1.59)			√		√	Case–control	NHL	Adjusted for use of other pesticides Excluded from the Working Group meta-analysis because 2,4-D was measured in dust, which is different from the exposure assessment for the other studies
Hardell et al. (1994)	13.00 (1.20–360)	√	√	√			Case–control	NHL	Adjusted for use of other pesticides Estimate for "2,4-D only"without exposure to other phenoxyherbicides
Cantor et al. (1992)	1.20 (0.90–1.60)		√		√		Case–control	NHL	Ever handled; 115 exposed cases
	1.20 (0.90–1.70)				√		Case–control	NHL	Highest exposed: handled without protective equipment; 89 exposed cases
Zahm et al. (1990)	1.50 (0.90–2.50)		√		√	√	Case–control	NHL	Adjusted for use of other pesticides Presents a more thorough analysis than Weisenburger (1990), but with complete overlap in patients
	1.50 (0.80–2.60)					√	Case–control	NHL	2,4-D use without 2,4,5-T
Hoar et al. (1986)	2.60 (1.40–5.00)					√	Case–control	NHL	Adjusted for use of other pesticides 2,4-D use without 2,4,5-T
	2.30 (1.30–4.30)		√			√	Case–control	NHL	Overall 2,4-D exposure

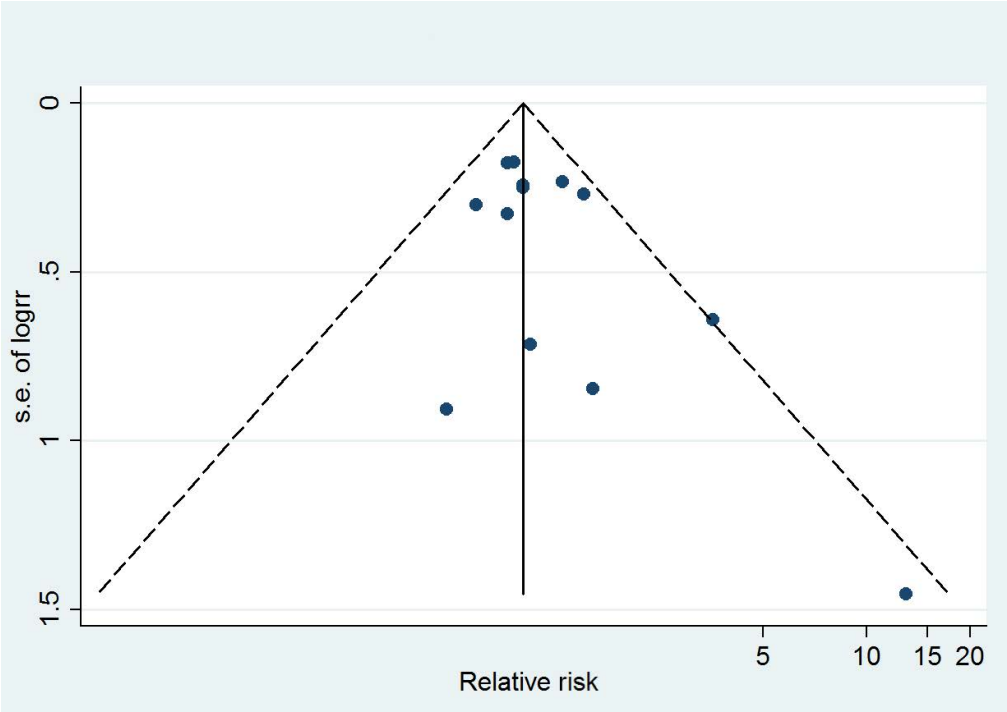
Table 2.3 (continued)

Study reference	Relative risk (95% CI)	Included in Working Group analysis[a]		Included in previously published meta-analysis		Exposure–response reported	Study design	Study outcome	Comments
		Primary analysis	Second analysis	Goodman et al. (2015)	Schinasi & Leon (2014)				
Hohenadel et al. (2011)	0.94 (0.67–1.33)	√		√			Case–control	NHL	Adjusted for use of other pesticides. Subset of 2,4-D without malathion exposure (49 exposed cases), but may have been exposed to other pesticides
De Roos et al. (2003)	0.90 (0.60–1.20)	√					Case–control	NHL	Adjusted for use of other pesticides. Hierarchical regression; pooled analysis that replaces Cantor et al. (1992), Zahm et al. (1990), and Hoar et al. (1986)
	0.80 (0.60–1.10)			√			Case–control	NHL	Adjusted for use of other pesticides. Logistic regression
Woods & Polissar (1989)	0.73 (0.40–1.30)	√	√	√			Case–control	NHL	The same estimate was reported in Woods et al. (1987)
Brown et al. (1993)	1.00 (0.60–1.60)	√	√				Case–control	MM	
McDuffie et al. (2001)	1.32 (1.01–1.73)					√	Case–control	NHL	
Brown et al. (1990)	1.30 (0.80–2.00)	√	√				Case–control	CLL	
Pahwa et al. (2006)	1.25 (0.93–1.68)		√				Case–control	MM	
	1.00 (0.62–1.61)	√					Case–control	MM	Adjusted for use of other pesticides.

[a] The "primary analysis" prioritized inclusion of estimates adjusted for other pesticides, when available, whereas the "second analysis" included estimates unadjusted for concomitant use of pesticides from the same studies.

2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; CI, confidence interval; CLL, chronic lymphocytic leukaemia; HCL, hairy cell leukaemia; MCPA, 2-methyl-4-chlorophenoxyacetic acid; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; SMR, standardized mortality ratio

Fig. 2.1 Funnel plot (with pseudo 95% confidence limits) of cohort and case–control studies assessing exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) and non-Hodgkin lymphoid neoplasms: primary analysis

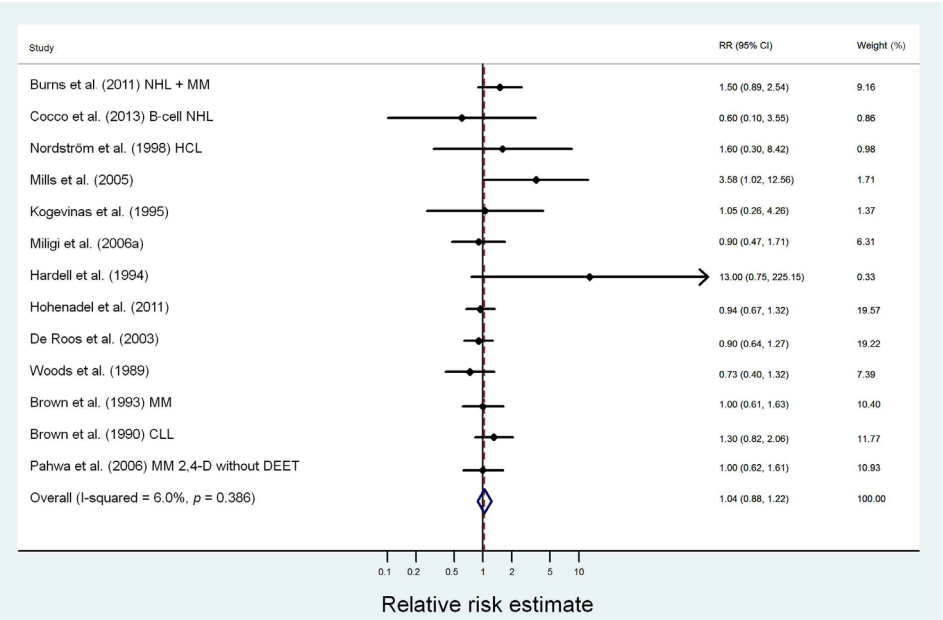


The figure plots the risk estimates for the 13 reports included in the primary analysis
s.e, standard error
Prepared by the Working Group

Table 2.4 Summary of results of the Working Group meta-analysis (primary and secondary analysis) of epidemiological studies on 2,4-dichlorophenoxyacetic acid (2,4-D) and non-Hodgkin lymphoid neoplasms

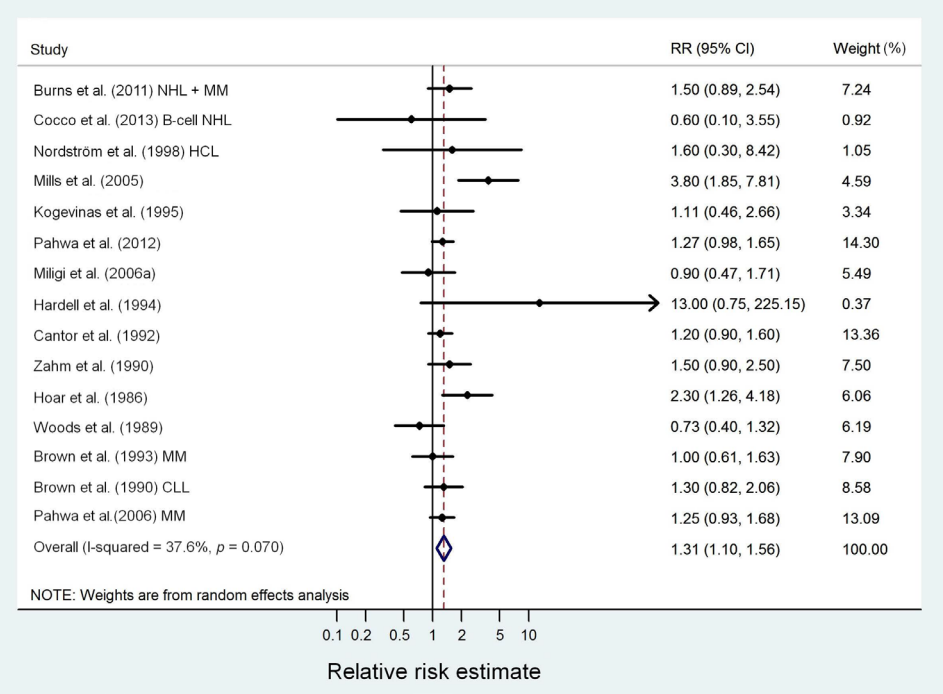
Analysis	No. of studies included	I^2	Meta-relative risk (95% CI)	Comments
Overall				
Most adjusted (primary analysis)	13	6.00%	1.04 (0.88–1.22)	De Roos et al. (2003) replaces the individual case–control studies in the midwest USA
Least adjusted (secondary analysis)	15	37.60%	1.31 (1.10–1.56)	Least adjusted estimates when possible, compare to primary analysis
By outcome				
Non-Hodgkin lymphoma	9	36.30%	1.06 (0.80–1.40)	Primary analysis restricted to non-Hodgkin lymphoma only, subtypes excluded
Multiple myeloma	3	0.00%	0.99 (0.71–1.39)	Restricted to multiple myeloma
Chronic lymphocytic leukaemia	2	0.00%	1.15 (0.79–1.67)	Restricted to chronic lymphocytic leukaemia

Fig. 2.2 Forest plot of cohort and case–control studies assessing exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) and non-Hodgkin lymphoid neoplasms: primary analysis)



Prepared by the Working Group

Fig. 2.3 Forest plot of cohort and case–control studies assessing exposure to 2,4-dichlorophenoxyacetic acid (2,4-D) and non-Hodgkin lymphoid neoplasms: secondary analysis)



Prepared by the Working Group

$P_{heterogeneity}$ = 0.386) (Fig. 2.2; Table 2.4). A sensitivity analysis showed positive associations and a large heterogeneity when risk estimates that were adjusted for other pesticides (meta relative risk, 1.31; 95% CI, 1.10–1.56; I^2 = 37.6%) (Fig. 2.3; Table 2.4). For both the above, an analysis of the effect of omitting each study in turn did not substantially affect the overall meta relative risk.

3. Cancer in Experimental Animals

2,4-D and its butyl, isopropyl, and isooctyl esters were previously reviewed and evaluated for carcinogenicity by the *IARC Monographs* Working Groups for Volume 15 and Supplement 7 (IARC, 1977, 1987) on the basis of studies of oral administration in rats and mice, and subcutaneous administration in mice. All the studies considered at the time of these evaluations were found to have limitations, inadequate reporting, or inadequate (small) numbers of animals. In addition, only one dose group was used in most of the studies. The previous Working Group (IARC, 1987) concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of 2,4-D. For the present *Monograph*, the Working Group evaluated all relevant studies of carcinogenicity in experimental animals, including those published since the evaluations made in 1977 and 1987.

3.1 Mouse

See Table 3.1

3.1.1 Oral administration

Groups of 18 male and 18 female (C57BL/6 × C3H/Anf)F_1 mice and groups of 18 male and female (C57BL/6 × AKR)F_1 mice were given commercial 2,4-D (purity, 90%) at a dose of 46.4 mg/kg body weight (bw) per day in 0.5% gelatin (in distilled water) by gavage at age 7–28 days, followed by a diet containing 149 mg/kg bw ad libitum for 18 months. Additional groups of 18 male and 18 female (C57BL/6 × AKR)F_1 mice were given 2,4-D at a dose of 100 mg/kg bw per day by gavage, and subsequently fed a diet containing 2,4-D at 323 mg/kg bw for 18 months. Groups of 18 males and 18 females served as vehicle controls in all experiments. No significant increase in tumour incidences occurred in any of the treatment groups. Similar results were obtained in groups of 18 male and 18 female (C57BL/6 × C3H/Anf)F_1 and (C57BL/6 × AKR)F_1 mice given the 2,4-D isopropyl ester (purity, 99%), butyl ester (purity, 99%), or isooctyl ester (purity, 97%) at a dose of 46.4 mg/kg bw per day in 0.5% gelatin by stomach tube, at age 7–28 days, followed by diets containing the esters at a dose of 111, 149, or 130 mg/kg bw, respectively, for 18 months (NTIS, 1968; Innes et al., 1969). [The Working Group noted the inadequate number of treated and control animals, and the use of a single dose level.]

Groups of 50 male and 50 female B6C3F_1 CRL BR mice were given diets containing 2,4-D (purity, 96.4%) at a concentration of 0, 5, 62.5, or 125 mg/kg bw (males), and 0, 5, 150 or 300 mg/kg bw (females) for 104 weeks. There were no treatment-related deaths or clinical signs of toxicity in either sex. Body weight, body-weight gain, and food consumption were generally similar among the groups of males throughout the study. Body-weight gains of females at 300 mg/kg bw were non-significantly decreased during the first 18 months of the study. There were no treatment-related increases in the incidence of any tumour type in males or females (Charles et al., 1996a).

In a study submitted to the EPA (1997), groups of 50 male and 50 female B6C3F_1 mice were given diets containing 2,4-D (purity, 97.5%) at a dose of 0, 1, 15, or 45 mg/kg bw for 104 weeks. There were no treatment-related effects on survival or body weight. There were no treatment-related increases in tumour incidences in any treated group of males or females.

Table 3.1 Studies[a] of carcinogenicity with 2,4-D and its esters in mice

Strain (sex), age at start Duration Reference	Dosing regimen No. of animals/group at start	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
2,4-D				
(C57BL/6 × C3H/Anf)F_1 (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D (purity, 90%) at a dose of 46.4 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D at 149 mg/kg bw per day, up to 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 16/18, 15/18 Survival, F: 16/18, 16/18
(C57BL/6 × AKR)F_1 (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D (purity, 90%) at a dose of 46.4 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D at 149 mg/kg bw per day, up to age 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 18/18, 16/18 Survival, F: 15/18, 16/18
(C57BL/6 × AKR)F_1 (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D (purity, 90%) at a dose of 100 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D at 323 mg/kg bw per day, up to age 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 18/18, 11/18 Survival, F: 15/18, 13/18
B6C3F_1 CRL BR (F, M), age 6–7 wk 104 wk Charles et al. (1996a)	Diets containing 2,4-D (purity, 96.4%) at a concentration of 0, 5, 62.5, 125 mg/kg bw per day (M) or 0, 5, 150, 300 mg/kg bw per day (F) for 104 wk 50 M and 50 F/group	*Liver* Hepatocellular adenoma: M: 12/50, 9/50, 13/50, 16/50 F: 5/50, 11/50, 8/50, 10/50 Hepatocellular carcinoma: M: 6/50, 3/50, 7/50, 4/50 F: 1/50, 2/50, 0/50, 1/50	 NS NS NS	Strengths: covered most of the lifespan Body-weight gain in females decreased during the first 18 mo of the study, but was not affected in males All mice survived to the end of the study

Table 3.1 (continued)

Strain (sex), age at start Duration Reference	Dosing regimen No. of animals/group at start	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
B6C3F$_1$ (F, M), age NR 104 wk EPA (1997)	Diets containing 2,4-D (purity, 97.5%) at a concentration of 0, 1, 15, or 45 mg/kg bw per day, for 104 wk 50 M and 50 F/group	No increase in the incidence of any tumour type (M or F) Pulmonary adenoma, multiplicity: 14.6 ± 0.8, 15.0 ± 0.8, 18.7 ± 0.9, 14.9 ± 0.8	– NS	Strengths: covered most of the lifespan Survival, NR
(C57BL/6 × C3H/Anf)F1 (F, M), age 28 days 18 mo NTIS (1968)	Single s.c. injection of 2,4-D (purity, 90%) at a dose of 0, or 215 mg/kg bw in DMSO 24 M and 24 F/group (controls) 18 M and 18 F/group (215 mg/kg bw)	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 23/24, 16/18 Survival, F: 23/24, 17/18
(C57BL/6 × AKR)F1 (F, M), age 28 days 18 mo NTIS (1968)	Single s.c. injection of 2,4-D (purity, 90%) at a dose of 0, or 215 mg/kg bw in DMSO 24 M and 24 F/group (controls) 18 M and 18 F/group (215 mg/kg bw)	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 22/24, 18/18 Survival, F: 24/24, 18/18
2,4-D esters				
(C57BL/6 × C3H/Anf)F$_1$ (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D isopropyl ester (purity, 99%) at a dose of 46.6 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D isopropyl ester at 111 mg/kg bw per day, up to 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 16/18, 18/18 Survival, F: 16/18, 18/18
(C57BL/6 × AKR)F$_1$, (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D isopropyl ester (purity, 99%) at a dose of 46.6 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D isopropyl ester at 111 mg/kg bw per day, up to 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 18/18, 17/18 Survival, F: 15/18, 14/18

Table 3.1 (continued)

Strain (sex), age at start Duration Reference	Dosing regimen No. of animals/group at start	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
(C57BL/6 × C3H/Anf)F_1, (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D butyl ester (purity, 99%) at a dose of 46.4 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D butyl ester at 149 mg/kg bw per diet, up to 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 16/18, 17/18 Survival, F: 16/18, 17/18
(C57BL/6 × AKR)F_1, (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D butyl ester (purity, 99%) at a dose of 46.4 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D butyl ester at 149 mg/kg bw per day, up to 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 18/18, 18/18 Survival, F: 15/18, 16/18
(C57BL/6 × C3H/Anf)F_1 (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D isooctyl ester (purity, 97%) at a dose of 46.4 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D butyl ester at 130 mg/kg bw per day, up to 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 16/18, 16/18 Survival, F: 16/18, 16/18
(C57BL/6 × AKR)F_1 (F, M), age 7 days 18 mo NTIS (1968); Innes et al. (1969)	2,4-D isooctyl ester (purity, 97%) at a dose of 46.4 mg/kg bw per day in 0.5% gelatin/distilled water by gavage at age 7–28 days, then diet containing 2,4-D butyl ester at 130 mg/kg bw per day, up to 18 mo; controls were given vehicle only by gavage, followed by untreated diet 18 M and 18 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 18/18, 17/18 Survival, F: 15/18, 16/18

Table 3.1 (continued)

Strain (sex), age at start Duration Reference	Dosing regimen No. of animals/group at start	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
(C57BL/6 × C3H/Anf)F_1 (F, M), age 28 days 18 mo NTIS (1968)	Single s.c. injection of 2,4-D isopropyl ester (purity, 99%) at a dose of 0, or 100 mg/kg bw in corn oil 24 M and 24 F/group (controls) 18 M and 18 F/group (100 mg/kg bw)	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 17/24, 16/18 Survival, F: 21/24, 18/18
(C57BL/6 × AKR)F_1 (F, M), age 28 days 18 mo NTIS (1968)	Single s.c. injection of 2,4-D isopropyl ester (purity, 99%) at a dose of 0, or 100 mg/kg bw in corn oil 24 M and 24 F/group (controls) 18 M and 18 F/group (100 mg/kg bw)	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 16/24, 16/18 Survival, F: 19/24, 16/18
(C57BL/6 × C3H/Anf)F_1 (F, M), age 28 days 18 mo NTIS (1968)	Single s.c. injection of 2,4-D butyl ester, (purity, 99%) at a dose of 0, or 21.5 mg/kg bw in corn oil 24 M and 24 F/group (controls) 18 M and 18 F/group (21.5 mg/kg bw)	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 17/24, 16/18 Survival, F: 21/24, 18/18
(C57BL/6 × AKR)F_1 (F, M), age 28 days 18 mo NTIS (1968)	Single s.c. injection of 2,4-D butyl ester, (purity, 99%) at a dose of 0, or 21.5 mg/kg bw in corn oil 24 M and 24 F/group (controls) 18 M and 18 F/group (21.5 mg/kg bw)	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 16/24, 18/18 Survival, F: 19/24, 16/18
(C57BL/6 × C3H/Anf)F_1 (F, M), age 28 days 18 mo NTIS (1968)	Single s.c. injection of 2,4-D isooctyl ester (purity, 97%) at a dose of 0, or 21.5 mg/kg bw in corn oil 24 M and 24 F/group (controls) 18 M and 18 F/group (21.5 mg/kg bw)	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 17/24, 16/18 Survival, F: 21/24, 16/18

Table 3.1 (continued)

Strain (sex), age at start Duration Reference	Dosing regimen No. of animals/group at start	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
$(C57BL/6 \times AKR)F_1$ (F, M), age 28 days 18 mo NTIS (1968)	Single s.c. injection of 2,4-D isooctyl ester (purity, 97%) at a dose of 0, or 21.5 mg/kg bw in corn oil	*Haematopoietic system* Reticulum cell sarcoma: M: 0/16, 0/18 F: 0/19, 5/17	NS [$P < 0.02$, Fisher exact test]	Strengths: covered most of the lifespan Limitations: inadequate numbers of animals; only one dose group; study poorly reported Survival, M: 16/24, 18/18 Survival, F: 19/24, 17/18
CD-1 (M), age 3 wk (weanling) 15 wk Blakley et al. (1992) Co-carcinogenicity study	Drinking-water containing a commercial amine formulation of 2,4-D (purity, NR) at a dose of 0, 10, 25, or 50 mg/kg bw per day, for 15 wk. After 3 wk of treatment, mice were given a single i.p. injection of urethane at 1.5 g/kg bw in saline, and were killed after the 15-wk exposure period 25/group	*Lung* Pulmonary adenoma, multiplicity: 6.7 ± 0.7, 12.0 ± 1.7*, 11.3 ± 2.1, 9.5 ± 1.6	*$P = 0.0207$	Limitations: short duration, inadequate numbers of animals, only included males
CD-1 (F), age 6–7 wk 19 wk Lee et al. (2000) Co-carcinogenicity study	Pregnant mice given drinking-water containing a commercial amine formulation of 2,4-D (purity, NR) at a dose of 0, 15, 65, or 650 mg/kg bw on days 6–16 of gestation. At age 7 wk, female offspring were given a single i.p. injection of urethane at 1.5 g/kg bw in saline, and killed 19 wk after birth 25/group	*Lung* Pulmonary adenoma, multiplicity: 14.6 ± 0.8, 15.0 ± 0.8, 18.7 ± 0.9, 14.9 ± 0.8	NS	Limitations: short duration, inadequate numbers of animals, only included females In-utero exposure to 2,4-D did not affect the number of urethane-induced adenomas

[a] All studies are full studies of carcinogenicity, unless otherwise stated

–, no significance test performed; 2,4-D, 2,4-dichlorophenoxyacetic acid; bw, body weight; DMSO, dimethyl sulfoxide; F, female; i.p., intraperitoneal; M, male; mo, month; NA, not applicable; NR, not reported; NS, not significant; s.c., subcutaneous; wk, week

3.1.2 Subcutaneous injection

Groups of 18 male and 18 female (C57BL/6 × C3H/Anf)F_1 mice and groups of 18 male and female (C57BL/6 × AKR)F_1 mice were given single subcutaneous injections of 2,4-D (purity, 90%) at a dose of 215 mg/kg bw in dimethyl sulfoxide (DMSO) at age 28 days, and observed for 18 months. At termination of the study, 16–18 mice in the four treated groups were still alive. Groups of 24 male and 24 female mice served as vehicle controls in all experiments. There were no treatment-related increases in tumour incidences in any of the treatment groups.

Tumour incidences were not increased in groups of 18 male and 18 female (C57BL/6 × C3H/Anf)F_1 mice, and 18 male and 18 female (C57BL/6 × AKR)F_1 mice given single subcutaneous injections of 2,4-D butyl esters (purity, 90%) at a dose of 21.5 mg/kg bw, or 2,4-D isopropyl esters (purity, 90%) at a dose of 100 mg/kg bw, in corn oil. However, in (C57BL/6 × AKR)F_1 mice similarly injected with the isooctyl ester of 2,4-D (purity, 97%) at a dose of 21.5 mg/kg bw in corn oil, 5 out of 17 female (C57BL/6 × AKR)F_1 mice developed reticulum cell sarcoma [$P < 0.02$, versus 0 out of 19 controls]; there was no increase in tumour incidence in males treated with the isooctyl ester, or in (C57BL/6 × C3H/Anf)F_1 male and female mice treated with the isooctyl ester (NTIS, 1968). [The Working Group noted the inadequate number of treated and control animals, the use of a single injection and of a single dose. The Working Group also noted that reticulum cell sarcoma may be classified by current terminology as histiocytic sarcoma or as a type of mixed cell malignant lymphoma.]

3.1.3 Coadministration with a known carcinogen

Groups of 25 male CD-1 mice were given drinking-water containing a commercial amine formulation of 2,4-D [containing 2,4-D at 140 g/L; the content of other chemicals was not reported] at a concentration of 0, 10, 25, or 50 mg/kg bw per day for 15 weeks. After 3 weeks, the mice were given a single intraperitoneal injection of urethane at a dose of 1.5 g/kg bw in saline. The effect of 2,4-D on urethane-induced formation of pulmonary adenoma was evaluated 84 days after urethane injection. Treatment with 2,4-D significantly increased the multiplicity of pulmonary adenoma (6.7 ± 0.7, 12.0 ± 1.7*, 11.3 ± 2.1, 9.5 ± 1.6; *$P = 0.0207$) (Blakley et al., 1992).

Pregnant CD-1 mice were given drinking-water containing a commercial amine formulation of 2,4-D [the chemical content was not reported] at a concentration of 0, 15, 65, or 650 mg/kg bw on days 6–16 of gestation. At age 7 weeks, female offspring were given a single intraperitoneal injection of urethane at a dose of 1.5 g/kg bw in saline. The effect of 2,4-D on urethane-induced formation of pulmonary adenoma was evaluated in female offspring 19 weeks after birth. Treatment with 2,4-D did not significantly increase the multiplicity of pulmonary adenoma (14.6 ± 0.8, 15.0 ± 0.8, 18.7 ± 0.9, 14.9 ± 0.8, respectively) (Lee et al., 2000).

3.2 Rat

See Table 3.2

Oral administration

Groups of 25 male and 25 female Osborne-Mendel rats (age, 3 weeks) were given diets containing 2,4-D (purity, 96.7%) at a concentration of 0, 5, 25, 125, 625, or 1250 ppm for 2 years. All rats were killed and necropsied after 2 years,

Table 3.2 Studies[a] of carcinogenicity with 2,4-D in rats

Strain (sex), age at start Duration Reference	Dosing regimen No. animals/group at start	Results For each target organ: incidence (%) and/or multiplicity of tumours	Significance	Comments
Osborne-Mendel (F, M), age 3 wk 2 yr Hansen et al. (1971)	Diets containing 2,4-D (purity, 96.7%) at a concentration of 0, 5, 25, 125, 625, or 1250 ppm for 2 years 25 M and 25 F/group	No increase in the incidence of any tumour type (M or F)	–	Strengths: covered most of the lifespan Limitations: detailed histopathological examination of tissues does not appear to have been consistent across all dose groups: all tissues from 6 males and 6 females from the 1250-ppm and control groups examined, and certain tissues in other groups; inadequate numbers of animals No significant differences in survival, mean body weights, or relative organ weights between controls (M and F) and dosed rats
F344 (F, M), age NR 104 wk EPA (1997)	Diets containing 2,4-D (purity, 97.5%) at a dose of 0, 1, 5, 15, or 45 mg/kg bw per day, for 104 wk 60 F and 60 M/group	*Brain* Astrocytoma: M: 1/60, 0/60, 0/60, 2/58, 6/60 F: 0/60, 1/60, 2/60, 1/60, 1/60	 $P = 0.0026$, trend NS	Strengths: covered most of the lifespan Decrease in body-weight gains in females at the highest dose Survival, NR
F344 (F, M), age 5 wk 104 wk Charles et al. (1996a); EPA (1997)	Diets containing 2,4-D (purity, 96.4%) et a dose of 0, 5, 75, or 150 mg/kg bw per day, for 104 wk 50, 50, 50, 50 28, 25, 33, 36	*Brain* Astrocytoma: M: 0/50, 0/50, 0/50, 1/50 F: 1/50, 0/50, 0/50, 1/50	 NS NS	Strengths: covered most of the lifespan Body-weight gains and average food consumption were decreased throughout the study at the highest dose level (M and F) Survival, M: 28/50, 25/50, 33/50, 36/50 Survival, F: 35/50, 39/50, 40/50, 35/50

[a] All studies are full studies of carcinogenicity, unless otherwise stated

2,4-D, 2,4-dichlorophenoxyacetic acid; bw, body weight; DMSO, dimethyl sulfoxide; F, female; i.p., intraperitoneal; M, male; mo, month; NA, not applicable; NR, not reported; NS, not significant; s.c., subcutaneous; wk, week

except for one rat at the highest dose that died during the experiment. Microscopic examinations were conducted on heart, lung, liver, spleen, kidney, stomach, intestines, pancreas, pituitary, thyroid, adrenal, bone (including marrow), ovary and uterus (or testis and prostate), tumours, and other gross lesions from six males and six females from the group at the highest dose and the control group. Only the liver, kidney, spleen, ovary (or testis), tumours and other gross lesions from rats at other doses were subjected to microscopic examination. Treatment with 2,4-D did not affect survival, body weight, or organ weights at any dose. There were no treatment-related increases in tumour incidences in any treated group of males or females (Hansen et al., 1971). [The Working Group noted that microscopic evaluations were not performed on a standard comprehensive list of tissues and organs across all dose groups].

In a study submitted to the United States EPA (EPA, 1997), groups of 60 male and 60 female F344 rats were given diets containing 2,4-D (purity, 97.5%) at a dose of 0 (control), 1, 5, 15, or 45 mg/kg bw per day for 2 years. Body-weight gains were significantly decreased in females at the highest dose, relative to controls. The incidences of astrocytoma in the brain in the control group, and the groups at 1, 5, 15, and 45 mg/kg bw were: 1/60, 0/60, 0/60, 2/58, and 6/60 in males, respectively; and 0/60, 1/60, 2/60, 1/60, and 1/60 in females, respectively. In males, the incidence of astrocytoma showed a statistically significant positive trend ($P = 0.0026$), but the incidence in the group at the highest dose was not statistically significant.

In a study of combined long-term toxicity and carcinogenicity, groups of 50 male and 50 female F344 rats were given diets containing 2,4-D (purity, 96.4%) at a dose of 0, 5, 75, or 150 mg/kg bw for up to 104 weeks. There were no treatment-related deaths. Body-weight gains and average food consumption were decreased throughout the study in males and females at the highest dose. There was no treatment-related increase in the incidence of any tumour, including astrocytoma of the brain. The incidence of astrocytoma was: 0/50, 0/50, 0/50, 1/50 in males, respectively; and 1/50, 0/50, 0/50, 1/50 in females, respectively (Charles et al. 1996a; EPA, 1997).

[The Working Group noted that the study by Charles et al. (1996a) was designed to address the finding of a significant positive trend in the incidence of astrocytoma of the brain (with no pairwise significance) found in the study submitted to the EPA (1997). In the study by Charles et al. (1996a), the rats were given higher doses, and there was no significant increase in the incidence of astrocytoma of the brain (Charles et al., 1996a; EPA, 1997).]

3.3 Dog

A hospital-based case–control study of pet dogs examined the risk of developing canine malignant lymphoma associated with the use of 2,4-D in and about the dog owner's home. Dogs with histopathologically confirmed malignant lymphoma, newly diagnosed over a 4-year period, were identified using computerized medical-record abstracts from three veterinary medical teaching hospitals in the states of Colorado, Indiana, and Minnesota, USA. Two comparison control groups, matched by age group, but not by sex, were chosen from dogs seen at the same teaching hospital in the same year as the identified case, with one-to-one matching for each control group. The first control group (tumour control) was selected from all other tumour cases diagnosed at the teaching hospital, excluding transitional cell carcinoma of the lower urinary tract because of a potential etiology related to chemical exposures. The second control group (non-tumour control) was selected from dogs seen for any other diagnostic reason, excluding those with conditions possibly related to chemical exposures (e.g. nonspecific

dermatitis, neuropathies). Owners of dogs in the case and control groups were sent a standardized questionnaire requesting information about the demographic characteristics of all people living in their home, basic information about the dog's life history, household use of chemicals (in and about the home), including those directly applied to the pet, and personal use of chemicals of whatever kind on the lawn and garden and/or the employment of commercial companies applying such chemicals. In addition, owners were asked about opportunities for exposure of their pets to lawn and garden chemicals, including frequency of access to the yard. The questionnaire did not provide a list of chemicals that the owner could consult in responding to the various questions regarding home, lawn, and gardening chemicals. Information from the self-administered questionnaire and/or a telephone interview of owners of 491 cases, 466 non-tumour controls, and 479 tumour controls indicated that owners of dogs that developed malignant lymphoma had applied 2,4-D herbicides to their lawn and/or employed commercial lawn-care companies to treat their yard significantly more frequently than control owners (OR, 1.3; 95% CI, 1.04–1.67). The risk of canine malignant lymphoma rose to a twofold (OR, 2.0; 95% CI, 0.92–4.15) non-significant excess with four or more lawn applications of 2,4-D per year by the owner. The findings in this study were consistent with those of studies of occupational exposure in humans, which have reported modest associations between agricultural exposure to 2,4-D and increased risk of NHL, the histology and epidemiology of which are similar to those of canine malignant lymphoma (Hayes et al., 1991). [The Working Group noted that details on the assessment procedures were described very briefly, and that information on the type of chemicals applied to the lawns of the respondents by commercial companies was not provided.]

Hayes et al. (1995), responding to a review of their earlier article (Hayes et al., 1991) by Carlo et al. (1992), presented additional data and a subsequent analysis showing that risk estimates did not vary by type of control group (tumour control or non-tumour control), by method of response (self-administered or telephone interview) or by geographical area of recruitment of the subjects.

A re-analysis of the original data was reported by Kaneene & Miller (1999). This re-analysis included a reclassification of exposure to 2,4-D, and a considerable number of the animals characterized as exposed in the original analysis by Hayes et al. (1991) were considered to be non-exposed in the re-analysis. The odds ratio for owner and/or commercial application was slightly lower and non-significant in the re-analysis (OR, 1.2; 95% CI, 0.87–1.65) compared to that in the original article (OR, 1.3; 95% CI, 1.04–1.67) by Hayes et al. (1991). In the re-analysis, there was also no clear dose–response relationship found for level of lawn treatment by the owner, although a non-significant elevated odds ratio was still observed in the highest quartile of exposure (four or more applications per year by the owner) versus non-exposed (OR, 2.4; 95% CI, 0.87–6.72) [no *P* value for trend was reported]. [The Working Group noted that although Kaneene & Miller (1999) extensively revised the exposure assessment, the re-analysis still lacked information on type of commercial application, as did the original analysis by Hayes et al. (1991). In addition, the classification as non-exposed of many animals that had been previously classified as exposed to applications by commercial companies in the original article may have led to exposure misclassification of the non-exposed group.]

[Overall, results from the original article, the subsequent analysis and the re-analysis were difficult to interpret due to potential exposure misclassification.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetics

4.1.1 Humans

(a) Absorption, distribution, and excretion

2,4-D and its salts and esters are readily absorbed after exposure. Dermal absorption has been demonstrated experimentally in humans, with about 5% of the dermally applied dose recovered in the urine in two studies (Feldmann & Maibach, 1974; Harris & Solomon, 1992). However, another study reported that absorption ranged from 7% to 14% depending on the vehicle (water or acetone), and on whether a mosquito repellent (DEET; *N*,*N*-diethyl-*meta*-toluamide) was also applied (Moody et al., 1992). A review of studies of dermal absorption reported a weighted average (± standard deviation) absorption rate of 5.7% (± 3.4%) (Ross et al., 2005). In skin from human cadavers, the relative percentage of dermal absorption of 2,4-D from contaminated soil increased as soil loadings of 2,4-D decreased (Duff & Kissel, 1996). Absorption after inhalation has not been directly measured experimentally in humans.

Experimental studies in humans demonstrated essentially complete absorption in the gastrointestinal tract after oral exposure (Kohli et al., 1974; Sauerhoff et al., 1977). 2,4-D has been detected in plasma after oral exposure, with terminal plasma half-lives of 7–16 hours, and urinary elimination half-lives of 10–30 hours (Sauerhoff et al., 1977). A somewhat slower elimination half-life of around 33 hours was reported in another study (Kohli et al., 1974). However, both results suggested that 2,4-D would not accumulate with repeated exposure. Additionally, Sauerhoff et al. (1977) reported a distribution volume of around 300 mL/kg, suggesting some distribution to tissues. Like other organic acids of low relative molecular mass, 2,4-D is reversibly bound to plasma proteins, which explains the relatively low distribution volume. For instance, several studies have suggested that 2,4-D binds to human serum albumin (Rosso et al., 1998, Purcell et al., 2001). Rosso et al. (1998) reported that 2,4-D has a poor ability to penetrate membranes.

Available data suggested that excretion of 2,4-D is largely, if not completely, via the urine, regardless of the route of exposure (Feldmann & Maibach, 1974; Kohli et al., 1974; Sauerhoff et al., 1977). The rate of excretion exceeds that which would be expected by passive glomerular filtration, consistent with a role for active transport in the kidney. Based on uptake measured in human kidney slices, organic anion transporter 1 (OAT1) appeared to be largely responsible for the ability of the kidney to excrete 2,4-D (Nozaki et al., 2007). While mainly expressed in the kidney, OAT1 is also expressed at lower levels in the human brain (Burckhardt & Wolff, 2000), and thus may play a role in the blood–brain distribution of 2,4-D (Gao & Meier, 2001).

(b) Metabolism

The salts and esters of 2,4-D are hydrolysed in vivo to 2,4-D, which undergoes a minor amount of metabolism in humans. In one study, 75% of an administered oral dose was excreted unchanged in the urine after 96 hours (Kohli et al., 1974). In another study of oral dosing, 13% of the administered dose was excreted as a 2,4-D hydrolysable conjugates, with about 82% excreted as unchanged 2,4-D (Sauerhoff et al., 1977). The identities of metabolites were not determined in these studies. One study using human CYP3A4 expressed in yeast reported metabolism of 2,4-D to 2,4-dichlorophenol (2,4-DCP) (Mehmood et al., 1996), but no data confirming metabolism of 2,4-D to 2,4-DCP in exposed humans were available.

4.1.2 Experimental systems

The toxicokinetics of 2,4-D has been studied in multiple species, but only studies in mammals are discussed here.

(a) Absorption, distribution, and excretion

2,4-D is readily absorbed by all experimental animal species tested. Absorption from the lung has been measured in rats in one study, although 2,4-D was administered through a tracheal cannula to anaesthetized animals (Burton et al., 1974). The rate of absorption was found to be rapid, with a half-time of 1.4 minutes and no evidence of saturation up to a concentration of 10 mM (Burton et al., 1974). Rapid and nearly complete absorption via the oral route has been reported in multiple species, including mice, rats, hamsters, dogs, pigs, and calves (Erne 1966; Pelletier et al., 1989; Griffin et al., 1997; van Ravenzwaay et al., 2003). Dermal absorption has also been measured in multiple species, including mice, rats, rabbits, and monkeys, with absorption rates between 6% and 36% (Grissom et al., 1985; Pelletier et al., 1989; Moody et al., 1990; Knopp & Schiller 1992). Multiple experimental studies have also reported that 2,4-D absorption is enhanced by ethanol consumption or application of topical sunscreens, moisturizers, or insect repellents (Pelletier et al., 1990; Brand et al., 2003, 2007a, b, c; Pont et al., 2003).

After absorption, 2,4-D readily distributes to tissues via systemic circulation. In all species tested, it is detected in the plasma after oral or dermal exposure. In a study in rats given radiobelled 2,4-D by oral or dermal administration, peak concentrations of radiolabel in the blood and kidney were reached within 30 minutes of administration by either route (Pelletier et al., 1989). In another study in rats given radiolabelled 2,4-D by oral administration, peak concentrations in tissues were reached 6–20 hours after exposure, with the highest levels in the lung, heart, liver, spleen, and kidney, and the lowest levels in adipose tissue and brain (Deregowski et al., 1990). In a study of toxicokinetics in male and female mice and rats given 2,4-D as an oral dose at 5 mg/kg bw, the highest peak concentrations were found in the kidney (Griffin et al., 1997). At a higher oral dose of 200 mg/kg bw, blood concentrations were nearly equal to or higher than kidney concentrations in mice and hamsters (Griffin et al., 1997). In studies in rats, rabbits, and goats, administration of 2,4-D during pregnancy or lactation lead to distribution of 2,4-D to the fetus or to milk (Kim et al., 1996; Sandberg et al., 1996; Stürtz et al., 2000; Barnekow et al., 2001; Stürtz et al., 2006; Saghir et al., 2013).

Like other organic acids of low relative molecular mass, 2,4-D is reversibly bound to plasma proteins (Arnold & Beasley, 1989). In studies in rats, dogs, and goats given 2,4-D orally, the binding fraction was reported to be more than 85% (Örberg, 1980; Griffin et al., 1997; van Ravenzwaay et al., 2003). Plasma binding explains the relatively low apparent volume of distribution (van Ravenzwaay et al., 2003). Binding specifically to bovine serum albumins has been measured in vitro (Mason, 1975; Fang & Lindstrom 1980).

In rats, a disproportionate increase in plasma concentrations of 2,4-D, consistent with saturation of elimination, occurs as doses increase (Saghir et al., 2006). The lowest dietary dose at which this disproportionality has been observed was between 25 and 79 mg/kg bw per day, depending on sex and life-stage (pups, adults, pregnant, lactating) (Saghir et al., 2013).

Several studies in rats and rabbits have examined the distribution of 2,4-D to the brain at higher exposures (Kim et al., 1988; Tyynelä et al., 1990; Kim et al., 1994). OAT1 is expressed in the rat and mouse brain (Burckhardt & Wolff, 2000), and may play a role in the distribution of 2,4-D to the brain (Gao & Meier, 2001). Brain concentrations of 2,4-D at exposures above 100 mg/kg bw appear higher than would be

expected based on passive diffusion, given the plasma protein binding of 2,4-D (Tyynelä et al., 1990). Additionally, no increase in permeability of the blood–brain barrier was found (Kim et al., 1988). Mechanistic studies suggest that alterations in OAT1 or other transporters at higher exposures of 2,4-D may be responsible for this accumulation (Pritchard, 1980; Kim et al., 1983).

Excretion of 2,4-D is largely via the urine. After oral administration at 5–50 mg/kg bw, the percentage of 2,4-D recovered in the urine was 81–97% in rats, 71–81% in hamsters, and 54–94% in mice (Griffin et al., 1997; van Ravenzwaay et al., 2003). As in humans, it appears that organic anion transporters, such as OAT1, are responsible for active transport into the kidney, which facilitates excretion (Imaoka et al., 2004). In dogs only 20–38% was recovered in urine after 72 hours, with an additional 10–24% in the faeces (van Ravenzwaay et al., 2003). Moreover, the half-life in dogs appears to be much longer than in other species, even accounting for allometric differences due to differences in body size, leading to much higher body burdens of 2,4-D for a given exposure (van Ravenzwaay et al., 2003; Timchalk, 2004).

(b) Metabolism

The salts and esters of 2,4-D are hydrolysed in vivo to 2,4-D. In most experimental systems, 2,4-D undergoes only limited metabolism to conjugates before excretion. In one study of oral administration, no metabolites were detected in rats, glycine and taurine conjugates of 2,4-D were detected in hamsters and mice, and glucuronide conjugates of 2,4-D were detected in hamsters only (Griffin et al., 1997). In mice, these conjugates accounted for less than 20% of urinary excretion at 5 mg/kg bw, but almost 50% at 200 mg/kg bw. In hamsters, which were only exposed to 2,4-D at 200 mg/kg bw, metabolites accounted for less than 13% of urinary excretion. No metabolites were detected in a study of goats given 2,4-D (Örberg, 1980). In a study comparing rats and dogs, no metabolite in rat urine exceeded 2% of the administered dose. Numerous metabolites were detected in dogs (van Ravenzwaay et al., 2003); the most abundant 2,4-D conjugates were those with glycine (about 34% of the administered dose at 120 hours) and glucuronide (7%), and were more abundant than the parent compound, 2,4-D (1%) (van Ravenzwaay et al., 2003). [The Working Group noted that these data suggested that dogs have a lower capacity to excrete 2,4-D than other species studied.] After administration of 2,4-D, 2,4-DCP has been reported in the rat kidney (Aydin et al., 2005), goat milk and fat, and chicken eggs and liver (Barnekow et al., 2001).

4.1.3 Modulation of metabolic enzymes

No data on modulation of metabolic enzymes in humans were available to the Working Group. At the median lethal dose (LD_{50}, 375 mg/kg), a single gavage dose of 2,4-D induced cytochrome P450 (CYP1A1, CYP1A2, and CYP1B1) mRNAs in the mammary gland, liver, and kidney of female Sprague-Dawley rats (Badawi et al., 2000).

In mouse liver, dietary exposure to 2,4-D at a concentration of 0.125% (w/w) induced total cytochrome oxidase activity and the activities of cytosolic and microsomal epoxide hydrolases (Lundgren et al., 1987). A less pronounced increase in total cytosolic glutathione transferase activity was observed. Total protein levels of CYP450 and cytosolic epoxide hydrolase were induced [probably due to induction of CYP4A mediated by peroxisome proliferator-activated receptor (PPAR).]

4.2 Mechanisms of carcinogenesis

4.2.1 Genotoxicity and related effects

2,4-D has been studied in a variety of assays for genotoxic and related potential. Table 4.1, Table 4.2, Table 4.3, and Table 4.4 summarize the studies carried out in exposed humans, in

Table 4.1 Genetic and related effects of 2,4-D in exposed humans

Tissue	Cell type (if specified)	End-point	Test	Description of exposed and controls	Response, significance	Comments	Reference
Blood	Lymphocytes isolated	Chromosomal damage	Micronucleus formation	12 applicators spraying only 2,4-D and 9 non-exposed controls	–		Figgs et al. (2000)
Blood	Lymphocytes	Chromosomal damage	Micronucleus formation	12 male applicators exposed solely to 2,4-D during a 3-month period	–		Holland et al. (2002)
Blood	Lymphocytes	Chromosomal damage	Chromosomal aberrations, V(D)J region rearrangements	24 forest/roadside pesticide applicators exposed to 2,4-D; 15 non-exposed controls	+ (chromosome translocations, inversions, deletions, $P = 0.003$; breaks, $P = 0.017$; gaps, $P = 0.006$)	Chromosome aberrations associated with the amount of herbicide applied; no association for workers who applied 2,4-D only, or with urinary 2,4-D concentrations V(D)J region rearrangement frequencies positively correlated (r = 0.54) with urinary 2,4-D concentrations ($P = 0.003$)	Garry et al. (2001)
Blood	Lymphocytes	DNA damage	Comet assay	10 subjects exposed to a pesticide mixture (atrazine, alachlor, cyanazine, 2,4-D, malathion) for 22.2 yr (range, 4–30 yr); 10 controls, matched for sex, age, and smoking status	(+)	DNA damage remained elevated, but was significantly decreased 6 mo after exposure cessation [Causative effect of 2,4-D alone could not be demonstrated]	Garaj-Vrhovac & Zeljezic (2000)
Blood	Lymphocytes	DNA and chromosomal damage	Comet assay (DNA damage), chromosomal aberrations, micronucleus formation, sister-chromatid exchanges	20 subjects exposed to a pesticide mixture (atrazine, alachlor, cyanazine, 2,4-D, malathion) for an average of 22.2 yr; 20 controls, matched for sex, age, and smoking status	(+)	Damage remained elevated, but was significantly decreased 8 mo after exposure cessation [Causative effect of 2,4-D alone could be demonstrated]	Zeljezic & Garaj-Vrhovac (2001)

Table 4.1 (continued)

Tissue	Cell type (if specified)	End-point	Test	Description of exposed and controls	Response, significance	Comments	Reference
Blood	Lymphocytes	DNA and chromosomal damage	Comet assay, (DNA damage), chromosomal aberrations, micronucleus formation, sister-chromatid exchanges	10 subjects exposed to a pesticide mixture (atrazine, alachlor, cyanazine, 2,4-D, malathion); 20 controls, matched for sex, age, and smoking status	(+)	[Causative effect of 2,4-D alone could not be demonstrated]	Garaj-Vrhovac & Zeljezic (2002)
Blood	Lymphocytes	Chromosomal damage	Chromosomal aberrations	42 male workers (Idaho, USA) occupationally exposed to 2,4-D, DDT, malathion, ethyl parathion, endosulfan, atrazine, dicamba, among other pesticides; 16 age-matched healthy controls	(+)	[Causative effect of 2,4-D alone could not be demonstrated]	Yoder et al. (1973)
Blood	Lymphocytes	Chromosomal damage	Chromosomal aberrations	19 spraying foliage in forestry workers exposed to 2,4-D and MCPA for 6–28 days	(–)	[Causative effect of 2,4-D alone could not be demonstrated]	Mustonen et al. (1986)
Blood	Lymphocytes	Chromosomal damage	Chromosomal aberrations	19 (2 female, 17 male) herbicide production workers exposed to 2,4-D and 2,4,5-trichlorophenol for 10–30 yr	(+) ($P < 0.05$)	[Causative effect of 2,4-D alone could not be demonstrated]	Kaioumova & Khabutdinova (1998)
Blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchanges	35 males spraying foliage in forestry with 2,4-D and MCPA, and 15 controls not working with herbicides	(–)	[Causative effect of 2,4-D alone could not be demonstrated]	Linnainmaa (1983)

[], comments not provided or present in the original reference
+, positive
–, negative
+/–, equivocal (variable response in several experiments within an adequate study)
(+) or (–), positive or negative result in a study of limited quality
2,4-D, 2,4-dichlorophenoxyacetic acid; DDT, dichlorodiphenyltrichloroethane; MCPA, 2-methyl-4-chlorophenoxyacetic acid; mo, month; yr, year

human cells in vitro, in other mammals in vivo and in vitro, and in non-mammalian systems, respectively.

(a) Humans

(i) Exposed humans

See Table 4.1

No induction of micronucleus formation in circulating blood lymphocytes was reported in applicators exposed only to 2,4-D (Figgs et al., 2000; Holland et al., 2002). In a study of chromosomal aberrations in lymphocytes of forest/roadside herbicide applicators, an association was reported with the amount of herbicide applied, but no association was observed among workers who applied 2,4-D only, or with urinary 2,4-D concentrations (Garry et al., 2001). An association was reported between 2,4-D urine levels and V(D)J rearrangements (Garry et al., 2001). [The Working Group noted that the V(D)J rearrangements may not reflect a genotoxic effect.]

A causative effect of 2,4-D alone could not be demonstrated in several studies of pesticide mixtures. Induction of DNA breaks as measured by the comet assay was seen in lymphocytes from male production workers exposed to a pesticide mixture including 2,4-D (Garaj-Vrhovac & Zeljezic, 2000, 2001, 2002). Chromosomal aberrations were induced in lymphocytes of male agricultural workers (Yoder et al., 1973; Garaj-Vrhovac & Zeljezic, 2001, 2002), but not in male forestry workers (Mustonen et al., 1986). Induction of micronucleus formation in lymphocytes was seen in males exposed to a pesticide mixture including 2,4-D (Garaj-Vrhovac & Zeljezic, 2001, 2002) or in an herbicide plant producing 2,4-D and 2,4,5-trichlorophenol (Kaioumova & Khabutdinova, 1998). For sister-chromatid exchange, positive results were reported in males exposed to a pesticide mixture including 2,4-D (Garaj-Vrhovac & Zeljezic, 2001; Zeljezic & Garaj-Vrhovac, 2002). However, a separate study showed no effect on sister-chromatid exchange in male forestry workers exposed to 2,4-D and MCPA (Linnainmaa, 1983).

(ii) Human cells in vitro

See Table 4.2

No induction of DNA strand breaks by 2,4-D was detected by ^{32}P labelling in exposed leukocytes (Sreekumaran Nair et al., 2002). Comet assay results were positive in lymphocytes isolated from smokers, but not non-smokers, exposed to 2,4-D (Sandal & Yilmaz, 2011). Apurinic/apyrimidinic sites in human fibroblasts were induced by a commercial formulation containing 2,4-D dimethylamine salt, but not by 2,4-D or the 2,4-D trimethylamine salt (Clausen et al., 1990).

Regarding induction of chromosomal aberration by 2,4-D, both positive (Pilinskaia, 1974; Korte & Jalal, 1982) and negative (Mustonen et al., 1986) results have been reported in human lymphocytes. Positive results were reported in human lymphocytes exposed to 2,4-D-based formulations (Mustonen et al., 1986; Zeljezic & Garaj-Vrhovac, 2004).

Regarding micronucleus formation, inconclusive results have also been observed in whole blood or lymphocyte cultures treated with 2,4-D or a 2,4-D-based formulation (Holland et al., 2002). Micronuclei were induced after exposure of lymphocytes to a 2,4-D-based formulation in the presence or absence of metabolic activation (Zeljezic & Garaj-Vrhovac, 2004).

Positive results were seen in an assay for sister-chromatid exchange in human lymphocytes treated with 2,4-D (Korte & Jalal, 1982). Soloneski et al. (2007) reported induction of sister-chromatid exchange in lymphocyte cultures by 2,4-D or by a formulation containing 2,4-D dimethylamine salt, but only when erythrocytes were present in the cultures (Soloneski et al., 2007). [The Working Group noted that the findings of Soloneski et al. (2007) suggest that erythrocytes may play a role in 2,4-D metabolic activation or lipid peroxidation induction.]

Table 4.2 Genetic and related effects of 2,4-D in human cells in vitro

Tissue, cell line	End-point	Test	Results	Concentration (LED or HID)	Comments	Reference
2,4-D						
Leukocytes	DNA damage	^{32}P post labelling	–	80 µg/mL		Sreekumaran Nair et al. (2002)
Lymphocytes, non-smokers	DNA damage	Comet assay	–	10 µM		Sandal & Yilmaz (2011)
Lymphocytes, smokers	DNA damage	Comet assay	+	10 µM		Sandal & Yilmaz (2011)
Fibroblasts	DNA damage	AP sites	–	100 mM		Clausen et al. (1990)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	50 µg/mL		Korte & Jalal (1982)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	2 µg/mL		Pilinskaia (1974)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	0.35 mM		Mustonen et al. (1986)
Whole blood cultures	Chromosomal damage	Micronucleus formation	(+)	0.001–1.0 mM	Only 2 donors; modest dose-dependent (P = 0.012) induction in 1 out of 2 donors	Holland et al. (2002)
Lymphocytes	Chromosomal damage	Micronucleus formation	(+)	0.3 mM	Only 2 donors; slight induction at cytotoxic concentration in both donors	Holland et al. (2002)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	10 µg/mL		Korte & Jalal (1982)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	10 µg/mL	Only when erythrocytes were present	Soloneski et al. (2007)
2,4-D-based formulation						
Fibroblasts	DNA damage	AP sites	+	10 mM	2,4-D-based formulation or 2,4-D DMA-HCl salt; 2,4-D TMA-HCl salt was without effect at 100 mM	Clausen et al. (1990)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	0.4 µg/mL	With or without metabolic activation	Zeljezic & Garaj-Vrhovac (2004)
Lymphocytes	Chromosomal damage	Chromosomal aberrations	+	0.5 mM		Mustonen et al. (1986)
Lymphocytes	Chromosomal damage	Micronucleus formation	+	0.4 µg/mL	With or without metabolic activation	Zeljezic & Garaj-Vrhovac (2004)

Table 4.2 (continued)

Tissue, cell line	End-point	Test	Results	Concentration (LED or HID)	Comments	Reference
Whole blood cultures or lymphocytes	Chromosomal damage	Micronucleus formation	(–)	0.3 mM	Two 2,4-D-based formulations; only 2 donors; slight induction (from 2/1000 to 6.7/1000) in 1 out of 2 donors that was within the range of baseline variability (3-12/1000)	Holland et al. (2002)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	10 µg/mL	Formulation containing 2,4-D DMA; positive results only when erythrocytes were present	Soloneski et al. (2007)

+, positive
–, negative
+/–, equivocal (variable response in several experiments within an adequate study)
(+) or (–), positive/negative results in a study of limited quality
2,4-D, 2,4-dichlorophenoxyacetic acid; AP sites, apurinic/apyrimidinic sites; DMA, dimethylamine; HID, highest ineffective dose; LED, lowest effective dose; TMA, trimethylamine

Table 4.3 Genetic and related effects of 2,4-D, and its metabolites, salts and esters, in non-human mammals in vivo

Species, strain	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
2,4-D								
Mouse, Swiss	Germ cells	Mutation	Dominant lethal	–	125 mg/kg bw 75 mg/kg bw	i.p. × 1 p.o. × 5	2,4-D	Epstein et al. (1972)
Mouse, $B6C3F_1$	Thymocytes	Mutation	T-cell receptor (*V(D)J*)	–	100 mg/kg bw	p.o. × 1 × 4 days	2,4-D	Knapp et al. (2003)
Mouse, Swiss	Bone-marrow cells, spermatocytes	Chromosomal damage	Chromosomal aberrations	+	3.3 mg/kg bw	p.o. × 3 or 5 days	2,4-D	Amer & Aly (2001)
Mouse, CD-1	Bone-marrow cells	Chromosomal damage	Micronucleus formation	–	400 mg/kg bw	p.o. × 1	2,4-D	Charles et al. (1999b)
Mouse	Bone-marrow cells	Chromosomal damage	Chromosomal aberrations	+	100 mg/kg bw	p.o. × 1	2,4-D	Pilinskaia (1974)
Mouse, C57BL	Bone-marrow cells	Chromosomal damage	Chromosomal aberrations	+	3.5 mg/kg bw	i.p. × 1	2,4-D	Venkov et al. (2000)
Mouse, Swiss	Bone-marrow cells	Chromosomal damage	Chromosomal aberrations	–	3.38 mg/kg bw	i.p. every 3 days for 55 days	2,4-D; offspring of maternal treated mice	Yilmaz & Yuksel (2005)
Mouse, CD-1	Bone-marrow cells	Chromosomal damage	Micronucleus formation	–	1/8 dermal LD_{50}	Topically × 1, 24 h before analysis	2,4-D	Schop et al. (1990)
Mouse, CD-1	Hair follicle	Chromosomal damage	Nuclear aberration assay	+	1/8 dermal LD_{50}	Topically × 1, 24 h before analysis	2,4-D	Schop et al. (1990)
Mouse, CBA	Bone-marrow cells	Chromosomal damage	Micronucleus formation	–	100 mg/kg bw	i.p. × 1	2,4-D Negative results at 24 hours and 7 days after treatment	Jenssen & Renberg (1976)
Mouse, ICR	Bone-marrow cells	Chromosomal damage	Micronucleus formation	–	400 mg/kg bw	p.o. × 1	2,4-D Negative results at 1, 2, and 3 days after treatment	EPA (1990b)

Table 4.3 (continued)

Species, strain	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, NIH	Bone-marrow and spermatogonia cells	Chromosomal damage	Sister-chromatid exchange	+	100 mg/kg bw	p.o. × 1	2,4-D	Madrigal-Bujaidar et al. (2001)
Rat, Han Wistar	Hepatocytes	DNA damage	UDS assay	–	1000 mg/kg bw	p.o. × 1	2,4-D	Charles et al. (1999a)
Rat, Wistar	Hepatocytes, kidney cells	DNA damage	Alkaline elution	+	7 mg/kg bw	i.p. × 1	2,4-D	Kornuta et al. (1996)
Rat, Wistar	Spleen, lung, bone-marrow cells	DNA damage	Alkaline elution	+	70 mg/kg bw	i.p. × 1	2,4-D	Kornuta et al. (1996)
Rat, Wistar	Bone-marrow cells	Chromosomal damage	Chromosomal aberrations	+	35 mg/kg bw	i.p. × 1 × 2 days	2,4-D	Adhikari & Grover (1988)
2,4-D metabolites, salts and esters								
Mouse, Swiss	Bone-marrow cells, spermatocytes	Chromosomal damage	Chromosomal aberrations	+	180 mg/kg bw	i.p. × 1 × 3 or 5 days	2,4-DCP	Amer & Aly (2001)
Mouse, CD-1	Bone-marrow cells	Chromosomal damage	Micronucleus formation	–	443 mg/kg bw (	p.o. × 1	2,4-D DEA	Charles et al. (1999b)
					397 mg/kg bw		2,4-D DMA	
					376 mg/kg bw		2,4-D IPA	
					542 mg/kg bw		2,4-D TIPA	
					375 mg/kg bw		2,4-D BEE	
					500 mg/kg bw		2,4-D EHE	
					400 mg/kg bw		2,4,-D IPE	
Mouse, ICR	Bone-marrow cells	Chromosomal damage	Micronucleus formation	–	600 mg/kg bw	p.o. × 1	2,4-D DMA Negative results at 1, 2, and 3 days after treatment	EPA (1990b)

Table 4.3 (continued)

Species, strain	Tissue	End-point	Test	Results	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Mouse, ICR	Bone-marrow cells	Chromosomal damage	Micronucleus formation	–	500 mg/kg bw	p.o. × 1	2,4-D IOE Negative results at 1, 2, and 3 days after treatment	EPA (1990a)
Mouse, C57BL/6	Bone-marrow cells	Chromosomal damage	Sister-chromatid exchange	–	20–40 mg/kg bw	i.p. × 1	Mixtures of 2,4-D, 2,4,5-T, and TCDD [causative effect of 2,4-D alone could not be demonstrated]	Lamb et al. (1981)
2,4-D-based formulations								
Rat, Wistar	Circulating lymphocytes	Chromosomal damage	Sister-chromatid exchange	–	100 mg/kg bw	p.o. × 1 × 14 day		Linnainmaa (1984)
Hamster, Chinese	Circulating lymphocytes	Chromosomal damage	Sister-chromatid exchange	–	100 mg/kg bw	p.o. × 1 × 9 days		Linnainmaa (1984)
Dog	Malignant lymphoma	Mutation	c-N-*ras* amplification	–	NR	Lawns treated with herbicides containing 2,4-D		Edwards et al. (1993)

+, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality
2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; BEE, butoxyethyl ester; DCP, dichlorophenol; DEA, diethanolamine; DMA, dimethylamine; EHE, ethylhexyl ester; HID, highest ineffective dose; IOE, isooctyl ester; i.p, intraperitoneal; IPA, isopropylamine ; IPE, isopropyl ester; LED, lowest effective dose; NR, not reported; p.o., oral administration; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TIPA, tri-isopropanolamine; UDS, unscheduled DNA synthesis

(b) Experimental systems

(i) Non-human mammals in vivo

See Table 4.3

In mice, dominant-lethal tests with 2,4-D gave negative results (Epstein et al., 1972). Positive results for chromosomal aberration were reported in bone marrow cells and spermatocytes after oral or intraperitoneal treatment with 2,4-D (Pilinskaia, 1974; Venkov et al., 2000; Amer & Aly, 2001) or after exposure to the metabolite 2,4-DCP (Amer & Aly, 2001). Negative results were seen in the offspring of mice treated with 2,4-D by intraperitoneal administration (Yilmaz & Yuksel, 2005). Micronuclei were not induced in bone marrow cells of mice exposed to 2,4-D topically (Schop et al., 1990), by intraperitoneal administration (Jenssen & Renberg, 1976), or orally (EPA, 1990b; Charles et al., 1999b). 2,4-D salts and esters also gave negative results after oral administration (EPA, 1990a, b; Charles et al., 1999b). Sister-chromatid exchange was induced in bone marrow and spermatogonial cells by 2,4-D administered orally (Madrigal-Bujaidar et al., 2001). Positive results were reported in an assay for nuclear aberration in hair follicles after topical exposure to 2,4-D (Schop et al., 1990).

In rats, inconsistent results were seen for induction of DNA strand breaks. There was no DNA damage induction in hepatocytes evaluated by an assay for unscheduled DNA synthesis after oral exposure to 2,4-D (Charles et al., 1999a). Induction of DNA damage by alkaline elution assay was seen in cells of the liver, kidney, spleen, lung, and bone marrow after intraperitoneal exposure to 2,4-D (Kornuta et al., 1996). Chromosomal aberrations were induced in bone-marrow cells after intraperitoneal exposure to 2,4-D (Adhikari & Grover, 1988). No increase in sister-chromatid exchange in circulating lymphocytes was seen after intragastric exposure to a 2,4-D formulation in rats or Chinese hamsters (Linnainmaa, 1984).

In dogs, no association was reported between exposure to 2,4-D and amplification or mutation of c-N-*ras* in lymphoma specimens (Edwards et al., 1993).

(ii) Non-human mammalian cells in vitro

See Table 4.4

In rat cells, no DNA damage was seen in hepatocytes evaluated by assay for unscheduled DNA synthesis after exposure to 2,4-D acid, or to its salts and esters (EPA, 1990a; Charles et al., 1999a).

In Chinese hamster V79 cells, 2,4-D was mutagenic in the hypoxanthine-guanine phosphoribosyl transferase (*HGPRT*) assay (Pavlica et al., 1991). In Chinese hamster ovary (CHO) cells, no mutagenic effect was reported in the *HGPRT* assay after exposure to 2,4-D salts and esters in the presence or absence of metabolic activation (Gollapudi et al., 1999).

Results were variable for DNA strand-break induction by the comet assay. Positive results were observed in Syrian hamster embryo cells exposed to 2,4-D (Maire et al., 2007), and in CHO cells exposed to 2,4-D or 2,4-D DMA (González et al., 2005). Negative results were reported at higher concentrations of 2,4-D in CHO cells (Sorensen et al., 2005).

Sister-chromatid exchange was induced in CHO cells exposed to 2,4-D in the presence (Linnainmaa, 1984), or absence (González et al., 2005) of metabolic activation, or exposed to 2,4-D DMA in the absence of metabolic activation (González et al., 2005). A 2,4-D formulation slightly increased the frequency of sister-chromatid exchange with, but not without, metabolic activation (Linnainmaa, 1984). Negative results were reported in an assay for chromosomal aberration in lymphocytes exposed to 2,4-D salts and esters with and without metabolic activation (Gollapudi et al., 1999).

(iii) Non-mammalian systems

See Table 4.5

Table 4.4 Genetic and related effects of 2,4-D in non-human mammalian cells in vitro

Species	Tissue, cell line	End-point	Test	Results		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
2,4-D, esters and salts								
Rat	Hepatocytes	DNA damage	UDS assay	–	NT	2,4-D (96.9 µg/mL) 2,4-D IOE (25 µg/mL) 2,4-D DMA (100 µg/mL)		EPA (1990a)
Rat	Hepatocytes	DNA damage	UDS assay	– – – – – – – –	NT	2,4-D (96.9 µg/mL) 2,4-D DEA (369 µg/mL) 2,4-D DMA (66.2 µg/mL) 2,4-D IPA (250.5 µg/mL) 2,4-D TIPA (354.5 µg/mL) 2,4-D BEE (478 µg/mL) 2,4-D EHE (24.5 µg/mL) 2,4-D IPE (194.2 µg/mL)		Charles et al. (1999a)
Chinese hamster	V79 cells	Mutation	*HGPRT* mutation assay	+	NT	10 µg/mL	2,4-D	Pavlica et al. (1991)
Syrian golden hamster	SHE cells	DNA damage	Comet assay	+	NT	11.5 µM	2,4-D	Maire et al. (2007)
Chinese hamster	CHO cells	DNA damage	Comet assay	+	NT	2 µg/mL	2,4-D or 2,4-D DMA	González et al. (2005)
Chinese hamster	CHO cells	DNA damage	Comet assay	–	NT	1600 µM	2,4-D, alone or after reaction with redox-modified clay	Sorensen et al. (2005)
Chinese hamster	CHO cells	Chromosomal damage	Sister-chromatid exchange	–	+	10^{-4} M	2,4-D, slight increase over control values	Linnainmaa (1984)
Chinese hamster	CHO cells	Chromosomal damage	Sister-chromatid exchanges	+	NT	2 µg/mL	2,4-D or 2,4-D DMA	González et al. (2005)
Rat	Lymphocytes	Chromosomal damage	Chromosomal aberrations	–	–	2,4-D BEE (1400 µg/mL) 2,4-D IPA (3068 µg/mL) 2,4-D TIPA (5000 µg/mL)	For 2,4-D IPA, HIC of 1500 µg/mL with metabolic activation	Gollapudi et al. (1999)

Table 4.4 (continued)

Species	Tissue, cell line	End-point	Test	Results		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Chinese hamster	CHO cells	Mutation	*HGPRT* assay	–	–	2,4-D BEE (1400µg/mL) 2,4-D IPA (3000 µg/mL) 2,4-D TIPA (5000 µg/mL)	For 2,4-D BEE, HIC of 700 µg/mL without metabolic activation	Gollapudi et al. (1999)
2,4-D-based formulation								
Chinese hamster	CHO cells	Chromosomal damage	Sister-chromatid exchanges	+	–	10^{-5} M	Slight increase over control values	Linnainmaa (1984)

+, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality
2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; 2,4-D, 2,4-dichlorophenoxyacetic acid; BEE, butoxyethyl ester; CHO, Chinese hamster ovary; DCP, dichlorophenol; DEA, diethanolamine; DMA, dimethylamine; EHE, ethylhexyl ester; HIC, highest ineffective concentration; IOE, isooctyl ester; IPA, isopropylamine; IPE, isopropyl ester; LEC, lowest effective concentration, NT, not tested; SHE, Syrian hamster embryo; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TIPA, tri-isopropanolamine; UDS, unscheduled DNA synthesis

In chickens, positive results in sister-chromatid exchanges in embryo cells were reported after exposure to 2,4-D or a 2,4-D formulation (Arias, 2003, 2007).

In fish, DNA strand-breaks, chromosomal aberrations, micronucleus formation, and other abnormalities indicative of genotoxicity were seen after exposure to 2,4-D in *Clarias batrachus* (Ateeq et al., 2002b, 2005), *Channa punctatus* (Farah et al., 2003, 2006), and *Oncorhynchus mykiss* (Martínez-Tabche et al., 2004). DNA strand breaks were induced by 2,4-D or a formulation containing 2,4-D DMA in a carp cell line in vitro (Bokán et al., 2013).

In freshwater snails, exposure to a formulation containing 2,4-D DMA had mutagenic effects in the dominant-lethal assay (Estevam et al., 2006). No induction of micronucleus formation was seen in haemocytes of Mediterranean mussels exposed to 2,4-D (Raftopoulou et al., 2006).

In *Drosophila melanogaster*, mutagenicity was observed with 2,4-D in some assays for sex-linked recessive lethal (Tripathy et al., 1993; Kale et al., 1995) and somatic mutation and recombination (SMART) (Graf & Würgler, 1996), but not others (Vogel & Chandler, 1974; Zimmering et al., 1985). Mutagenicity was observed by the wing-spot test after exposure to 2,4-D (Tripathy et al., 1993; Kaya et al., 1999) or to 2-(2,4-dichlorophenoxy) propionic acid (Surjan, 1989). No mutagenic effect was seen for the white-ivory eye-spot test after 2,4-D exposure (Graf & Würgler, 1996). No chromosomal aberrations were induced in germline cells exposed to a 2,4-D-based formulation (Woodruff et al., 1983).

In plants, positive results were reported in assays for point mutation and homologous recombination in *Arabidopsis thaliana* after exposure to 2,4-D (Filkowski et al., 2003). In bean seedlings (*Phaseolus vulgaris*), DNA damage was induced by comet assay and random amplified polymorphic DNA (RAPD) assay after exposure to 2,4-D (Cenkci et al., 2010). 2,4-D induced chromosomal aberrations in *Allium cepa* (Kumari & Vaidyanath, 1989; Ateeq et al., 2002a), and in *A. ascalonicum* (Pavlica et al., 1991), and induced sister-chromatid exchange in *A. sativum* (Doleźel et al., 1987). In *Vicia faba*, chromosomal aberration was induced by 2,4-D by when plants were sprayed, but not when seeds were soaked (Amer & Ali, 1974).

A 2,4-D-based formulation (containing butyl ester) was tested in 12 plant species and induced chromosome aberration in three species, giving positive results when applied to roots (*Chrysanthemum leucanthemum*), germinated seeds (*Secale cereale),* or bulbs (*Allium cepa*) (Mohandas & Grant, 1972). Increased frequencies (although slight) of sister-chromatid exchange were seen in *Triticum aestivum* (Murata, 1989).

In *Saccharomyces cerevisiae*, mutagenic effects were reported with 2,4-D in assays for reverse mutation and mitotic gene conversion (Venkov et al., 2000). A separate study reported no mutagenicity in the assay for mitotic gene conversion after exposure to 2,4-D (Fahrig, 1974). 2,4-D-based formulations gave positive results in mitotic gene-conversion assays (Siebert & Lemperle, 1974; Zetterberg et al., 1977), but not in the host-mediated assay (Zetterberg et al., 1977).

In *Salmonella typhimurium*, 2,4-D and its salts and esters did not demonstrate mutagenicity in TA98, TA100, TA1535, TA1537, or TA1538 in the assay for reverse mutation in the presence or absence of metabolic activation (Moriya et al., 1983; Mortelmans et al., 1984; EPA, 1990b; Charles et al., 1999a). A 2,4-D-based formulation gave negative results in TA1535 and TA1538 strains, and in strains TA1530 and TA1531 in the host-mediated assay (Zetterberg et al., 1977). In *Escherichia coli*, no mutagenic effect of 2,4-D was seen in WP2 *hcr* in the reverse mutation assay with or without metabolic activation (Moriya et al., 1983).

In *Bacillus subtilis*, no mutagenic effect was seen with 2,4-D in the Rec mutation assay (Shirasu et al., 1976), and results were inconclusive

Table 4.5 Genetic and related effects of 2,4-D in non-mammalian systems

Phylogenetic class	Species, strain, tissue	End-point	Test	Results		Agent, concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Chicken	White Leghorn 288-Shaver strain Chick embryos	Chromosomal damage	Sister-chromatid exchange	+	NT	0–4 mg/embryo	Injection × 1 into the egg air cell Dose-related increase with 2,4-D (borderline significance) or a 2,4-D-based formulation	Arias (2003)
Chicken	White Leghorn 288-Shaver strain Chick embryos	Chromosomal damage	Sister-chromatid exchange	+	NT	4 mg/embryo	Injection × 1 into the egg air cell Induction with 2,4-D (after 10 days) or a 2,4-D formulation (after 4 days)	Arias (2007)
Fish	Catfish (*Clarias batrachus*), circulating erythrocytes	DNA damage	Comet assay	+	NA	25 ppm	2,4-D	Ateeq et al. (2005)
Fish	Catfish (*Clarias batrachus*), circulating erythrocytes	Chromosomal damage	Micronucleus formation	+	NA	25 ppm	2,4-D	Ateeq et al. (2002b)
Fish	Air-breathing *Channa punctatus*, kidney cells	Chromosomal damage	Chromosomal aberrations	+	NA	75 ppm	2,4-D	Farah et al. (2006)
Fish	Air-breathing *Channa punctatus*, circulating erythrocytes	Chromosomal damage	Micronucleus formation	+	NA	25 ppm	2,4-D	Farah et al. (2003)
	Air-breathing *Channa punctatus*, circulating erythrocytes	Chromosomal damage	Micronucleus formation	+	NA	75 ppm	2,4-D	Farah et al. (2006)
Fish	Rainbow trout (*Oncorhynchus mykiss*), gill cells	DNA damage	Comet assay	+	NA	5 mg/l	2,4-D Water not changed during experiment; 1–8 days	Martínez-Tabche et al. (2004)
Snails	*Biomphalaria glabrata*, germ cells	Mutation	Dominant lethal assay	+	NA	75 ppm	2,4-D DMA formulation)	Estevam et al. (2006)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results		Agent, concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Mussels	Mediterranean mussel (*Mytilus galloprovincialis*), haemocytes	Chromosomal damage	Micronucleus induction	–	NA	0.03 mg/l	2,4-D	Raftopoulou et al. (2006)
Insects	*Drosophila melanogaster*, germ-line cells	Mutation	Wing-spot test, sex-linked recessive lethal	+	NT	5 mM	2,4-D In feeding media	Tripathy et al. (1993)
	Drosophila melanogaster, germ-line cells	Mutation	Sex-linked recessive lethal	+	NT	10 000 ppm	2,4-D In feeding media	Kale et al. (1995)
	Drosophila melanogaster, germ-line cells	Mutation	Sex-linked recessive lethal	–	NT	9 mM	2,4-D In feeding media	Vogel & Chandler (1974)
	Drosophila melanogaster, germ-line cells	Mutation	Sex-linked recessive lethal	–	NT	10 000 ppm	2,4-D In feeding media	Zimmering et al. (1985)
	Drosophila melanogaster, somatic cells	Mutation	Wing-spot test	+	NT	2.5 mM	2,4-D In feeding media × 2 days	Graf & Würgler (1996)
	Drosophila melanogaster, somatic cells	Mutation	White-ivory eye spot test	–	NT	2.5 mM	2,4-D In feeding media × 3 days	Graf & Würgler (1996)
	Drosophila melanogaster, somatic cells	Mutation	Wing-spot test	+	NT	10 mM	2,4-D In feeding media	Kaya et al. (1999)
	Drosophila melanogaster, somatic cells	Mutation	Wing-spot test	+	NT	0.031 w/w	2-(2,4-D) propionic acid In feeding media	Surjan (1989)
	Drosophila melanogaster with ring-X and doubly marked Y chromosome, germ-line cells	Chromosomal damage	Chromosomal aberrations	–		1000 ppm	2,4-D formulation (not otherwise specified) In feeding media	Woodruff et al. (1983)
Plant systems	*Arabidopsis thaliana* line166 and 166A	Mutation	Point mutation	+	NA	3 µg/l	2,4-D. A→G, but not T→G, reversions	Filkowski et al. (2003)
	Arabidopsis thaliana line 651	Mutation	Homologous recombination assay	+	NA	3 µg/l	2,4-D	Filkowski et al. (2003)
	Common bean, *Phaseolus vulgaris*	DNA damage	Comet assay	+	NA	0.1 ppm	2,4-D	Cenkci et al. (2010)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results		Agent, concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Plant systems (cont.)	Onion, *Allium cepa*	Chromosomal damage	Chromosomal aberrations	+	NA	1 ppm	2,4-D	Ateeq et al. (2002a)
	Shallot, *Allium ascalonicum*	Chromosomal damage	Chromosomal aberrations	+	NA	45 µM	2,4-D	Pavlica et al. (1991)
	Garlic, *Allium sativum*	Chromosomal damage	Sister-chromatid exchanges	+	NA	5 µM	2,4-D	Doleźel et al. (1987)
Lower eukaryote (yeast, mould, fungi)	*Saccharomyces cerevisiae* D7 ts1	Mutation	Reverse mutation, gene conversion	+	NA	8 mM	2,4-D	Venkov et al. (2000)
	Saccharomyces cerevisiae	Mutation	Mitotic gene conversion	–	NT	Dose, NR	2,4-D	Fahrig (1974)
	Saccharomyces cerevisiae D4	Mutation	Mitotic gene conversion	+	NA	1000 ppm/16 h	2,4-D formulation	Siebert & Lemperle (1974)
	Saccharomyces cerevisiae D4, D5	Mutation	Mitotic gene conversion	+	NA	0.6 mg/mL/3 h (D4)0.3mg/mL/3 h (D5)	2,4-D formulation, in buffer at pH 4.50	Zetterberg et al. (1977)
	Saccharomyces cerevisiae D4	Mutation	Host-mediated assay	–	NA	6 mg/o.p.	Formulated product of 2,4-D as sodium salt	Zetterberg et al. (1977)
Prokaryote (bacteria)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538; *Escherichia coli* WP2 *hcr*	Mutation	Reverse mutation	–	–	5000 µg/plate	2,4-D	Moriya et al. (1983)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537	Mutation	Reverse mutation	–	–	10 mg/plate	2,4-D	Mortelmans et al. (1984)
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	Mutation	Reverse mutation	–	–	10 000 µg/plate	Tested were 2,4-D, 2,4-D DMA and 2,4-D IOE	EPA (1990b)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results		Agent, concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Prokaryote (bacteria) (cont.)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Mutation	Reverse mutation	–	–	2,4-D acid (9610 µg/plate)		Charles et al. (1999a)
				–	–	2,4-D DEA (10 332 µg/plate)		
				–	–	2,4-D DMA (6620 µg/plate)		
				–	–	2,4-D TIPA (7090 µg/plate)		
				–	–	2,4-D BEE (4780 µg/plate)		
				–	–	2,4-D EHE (9800 µg/plate)		
				–	–	2,4-D IPE (4855 µg/plate)		
				–	–	2,4-D IPA (1670 µg/plate)		
	Salmonella typhimurium TA1535, TA1538	Mutation	Reverse mutation	–	–	0.08 mg/mL	2,4-D formulation	Zetterberg et al. (1977)
	Salmonella typhimurium TA1530, TA1531	Mutation	Host-mediated assay	–	NA	6 mg, p.o.	2,4-D formulation	Zetterberg et al. (1977)
	Bacillus subtilis M 45 Rec$^-$, H17 Rec$^+$	Mutation	Rec assay	–	NT	Dose not provided	2,4-D	Shirasu et al. (1976)
	Bacillus subtilis M 45 Rec$^-$, H17 Rec$^+$	Mutation	Rec assay	+/–	NA	2,4-D (10 mg/mL) 2,4-D formulation (7.2 mg/mL)		Grabińska-Sota et al. (2000, 2002)
Acellular systems	Isolated DNA from bacteriophage PM2	DNA damage	DNA single-strand breaks (AP sites)	–	NT	100 mM	2,4-D	Clausen et al. (1990)
	Isolated DNA from bacteriophage PM2	DNA damage	DNA single-strand breaks (AP sites)	+	NT	10 mM	2,4-D DMA formulation	Clausen et al. (1990)

Table 4.5 (continued)

Phylogenetic class	Species, strain, tissue	End-point	Test	Results		Agent, concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Acellular systems (cont.)	Isolated DNA from bacteriophage PM2	DNA damage	DNA single-strand breaks (AP sites)	+	NT	25 mM	2,4-D; only positive when DNA was pre-incubated with $CuCl_2$	Jacobi et al. (1992)

+, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality
2,4-D, 2,4-dichlorophenoxyacetic acid; 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; BEE, butoxyethyl ester; DCP, dichlorophenol; DEA, diethanolamine; DMA, dimethylamine; EHE, ethylhexyl ester; HIC, highest ineffective concentration; IOE, isooctyl ester; IPA, isopropylamine; IPE, isopropyl ester; LEC, lowest effective concentration; NA, not applicable; NR, not reported; NT, not tested; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TIPA, tri-isopropanolamine

at high concentrations of 2,4-D (Grabińska-Sota et al., 2000), and with a 2,4-D-based formulation (Grabińska-Sota et al., 2000, 2002).

2,4-D did not induce apurinic/apyrimidinic sites in bacteriophage PM2 DNA, while positive results were reported with a formulation containing 2,4-D DMA (Clausen et al., 1990). In a separate study, 2,4-D or the 2,4-D DMA formulation only gave positive results after pre-incubation with copper chloride ($CuCl_2$) (Jacobi et al., 1992).

2,4-D interacts with DNA as a groove binder rather than an intercalating agent, based on ultraviolet, fluorescence, and viscosity measurements, and alternating current voltammetry assays (Ahmadi & Bakhshandeh, 2009).

4.2.2 Receptor-mediated effects

(a) Humans

(i) Exposed humans

An increase in hypothyroidism with reported ever-use of 2,4-D and other herbicides has been found in male pesticide applicators in the Agricultural Health Study, USA (Goldner et al., 2013). Garry et al. (2001) reported a correlation between urinary concentrations of 2,4-D and serum concentrations of luteinizing hormone, but not follicle-stimulating hormone, in male pesticide applicators who used a hand-held backpack sprayer. Total testosterone levels in winter were directly correlated with peak levels of 2,4-D in the urine in the application season.

(ii) Human cells in vitro

In human prostate cancer cells (androgen receptor-expressing 22Rv1 and PC3/AR+), 2,4-D alone did not exhibit androgenic activity, but potentiated 5-alpha-dihydroxytestosterone (DHT) androgenic activities. DHT-mediated translocation of the androgen receptor to the nucleus, and androgen-induced transactivation were also increased in the presence of 2,4-D (Kim et al., 2005). [These findings suggested that 2,4-D has the potential to alter DHT-induced transcriptional activity of the androgen receptor.] Similarly, in-vitro reporter gene assays of estrogen receptor, androgen receptor, and thyroid hormone receptor showed no agonist or antagonist activity of 2,4-D against hormone receptors, but 2,4-D enhanced the activity of testosterone through the androgen receptor (Sun et al., 2012).

2,4-D did not interact in vitro with human estrogen, androgen, or steroidogenesis pathways in Tier 1 assays in the Endocrine Disruptor Screening Program run by the United States EPA. 2,4-D gave negative results in assays for estrogen receptor-mediated transcriptional activation (HeLa-9903-ERα transactivation assay), aromatase enzymatic activity inhibition (recombinant human CYP19 aromatase inhibition assay), and interference with steroidogenesis (H295R steroidogenesis assay) (Coady et al., 2014).

In co-transfected Hepa 1 cells, 2,4-D induced transactivation by human PPAR of rat acyl-coenzyme A oxidase (acyl-CoA oxidase) and rabbit CYP4A6 (Pineau et al., 1996).

(b) Experimental systems

(i) Non-human mammals in vivo

In rats, a single dose of 2,4-D has been shown to interfere with thyroid-hormone transport (Malysheva & Zhavoronkov, 1997). 2,4-D has been reported to bind competitively to the thyroxine (T4)-binding site of transthyretin, a carrier of thyroid hormones, and 2,4-D dichlorophenoxybutyric acid reduced plasma total T4 (TT4) in rats (Van den Berg et al., 1991).

In an F1-extended one-generation study of reproductive toxicity with 2,4-D in rats, a slight decrease in follicular size was reported in 3 out of 12 dams at the highest dose, but no other consistent pattern of thyroid effects was evident (Marty et al., 2013).

2,4-D has been reported to cause proliferation of peroxisomes in mouse and rat liver (Kawashima et al., 1984; Lundgren et al., 1987).

(ii) Non-human mammalian cells in vitro

Maloney & Waxman (1999) reported that 2,4-D did not activate mouse PPARα or PPARγ using a transactivation assay in vitro. 2,4-D did not interact in vitro with rodent estrogen or androgen pathways in Tier 1 assays in the Endocrine Disruptor Screening Program run by the EPA. Specifically, 2,4-D gave negative results in assays for estrogen-receptor binding (rat uterine cytosol estrogen-receptor binding assay), and for androgen receptor-binding (rat prostate cytosol androgen-receptor binding assay) (Coady et al., 2014).

(iii) Non-mammalian systems in vivo

Estrogenic activity, as determined by the vitellogenin assay, has been reported after exposure of rainbow trout to 2,4-D (Xie et al., 2005). Plasma vitellogenin levels were 93 times higher in juvenile rainbow trout exposed to 2,4-D (1.64 mg/L) for 7 days than in control untreated fish. No effects of 2,4-D (up to 113 mg acid equivalents/L) were reported in the amphibian metamorphosis assay, and the only effect reported in the fish short-term reproduction assay was decreased fecundity at the highest concentration tested (96.5 mg acid equivalents/L) (Coady et al., 2013).

4.2.3 Oxidative stress

(a) Humans

(i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

In human erythrocytes in vitro, 2,4-D (10, 50, 100, 250, 500 ppm) induced dose-related decreases in superoxide dismutase activity, and increases in glutathione peroxidase activity. 2,4-D (500 ppm) decreased the level of reduced glutathione in erythrocytes by 18% compared with controls (Bukowska, 2003). In a follow-up study, 2,4-D increased protein carbonyl group content, but had no effect on the denaturation of haemoglobin (Bukowska et al., 2008).

(b) Experimental systems

(i) In vivo

2,4-D increased oxidative stress in ventral prostate, ovary, and mammary gland in the offspring of pregnant rats exposed to 2,4-D by oral gavage at a dose of 70 mg/kg bw per day from day 16 of gestation to 23 days after delivery. The pups were studied on postnatal days 45, 60, or 90. In ventral prostate, 2,4-D increased the concentration of hydroxyl radicals and the rate of lipid and protein oxidation at all ages studied. The activity of certain antioxidant enzymes was increased, but this was insufficient to counteract the oxidative stress. In mammary tissue, 2,4-D promoted oxidative stress, mainly during puberty and adulthood. In the ovary, 2,4-D increased lipid peroxides and altered the activity of several antioxidant enzymes (Pochettino et al., 2013).

2,4-D induced reactive oxygen species and altered antioxidant enzymes in the developing rat brain after exposure in breast milk. Maternal exposure to 2,4-D (100 mg/kg bw per day between postnatal days 9 and 25) had no effect on body weights of pups or lactating mothers. Levels of reactive oxygen species were increased in the neonatal midbrain, striatum, and prefrontal cortex. Glutathione content was significantly decreased in midbrain, and striatum, and there were alterations in levels (either increased or decreased) of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) in at least one of the brain regions studied, with the exception of the hypothalamus (Ferri et al., 2007).

2,4-D (600 ppm in drinking-water; from day 14 of pregnancy until 14 days after delivery)

induced hepatic oxidative stress and hepatotoxicity in adult and suckling rats (Troudi et al., 2012). In dams and pups, malondialdehyde levels increased, while decreases were seen in the activities of liver antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase). Similarly, 2,4-D induced oxidative stress in the liver of mothers and fetuses after exposure of pregnant rats to 2,4-D (100 mg/kg bw per day) on days 1–19 of gestation. Coadministration of vitamin E (100 mg/kg bw) partially abrogated the oxidative stress (elevated malondialdehyde levels, decreased catalase activity, decreased total antioxidant capacity) induced by 2,4-D. 2,4-D had no effect on the sex ratio, the number of implantations, or the viable and resorbed fetuses. However, lower body weight and a higher rate of morphological and skeletal defects were seen in fetuses of dams treated with 2,4-D (Mazhar et al., 2014).

In rats exposed to a 2,4-D-based formulation (15, 75, and 150 mg/kg via oral gavage for 4 weeks), a significant reduction in the activity of hepatic antioxidant enzymes (glutathione reductase and catalase) was observed (Tayeb et al., 2010). Hepatotoxicity was demonstrated by increased liver weights, histological changes, and elevated levels of serum enzyme markers. Hepatotoxicity and altered lipid metabolism were reported in a follow-up study using the same dosing regime, in which rats showed increased levels of hepatic malondialdehyde and altered levels of liver antioxidant enzyme (Tayeb et al., 2013). Olive oil was found to protect against hepatic oxidative stress induced by the 2,4-D formulation (Nakbi et al., 2010). A further study with the 2,4-D formulation at the same doses (15, 75, and 150 mg/kg bw via oral gavage) reported oxidative stress in the kidney after 28 days (Tayeb et al., 2012). The 2,4-D-based formulation significantly increased levels of malondialdehyde, and altered the activities of renal catalase and superoxide dismutase. Glutathione peroxidase was significantly decreased in rats exposed at 150 mg/kg bw. Dose-dependent increases were seen in the severity of histopathological evidence of tubular and glomerular damage, and the number of pyknotic nuclei. Decreased uric acid and increased plasma levels of urea and creatinine were also reported.

(ii) In vitro

In an in-vitro study using freshly isolated rat hepatocytes, 2,4-D rapidly depleted glutathione and protein thiols, and induced lipid peroxidation (Palmeira et al., 1995).

In *Saccharomyces cerevisiae*, a 2,4-D-based formulation (2,4-D sodium monohydrate) induced concentration-dependent increases in levels of hydroxyl radicals detected by electron paramagnetic resonance spectroscopy (Teixeira et al., 2004). The effect was consistently greater in a mutant lacking the cytosolic Cu-Zn-superoxide dismutase enzyme (*Δsod1*).

4.2.4 Immunosuppression

(a) Humans

(i) Exposed humans

Faustini et al. (1996) reported reductions in immunological parameters in blood samples from 10 farmers 1–12 days after agricultural exposure to chlorophenoxy herbicides. Compared with values before exposure, the following were significantly reduced ($P < 0.05$): circulating helper (CD4) and suppressor T-cells (CD8), CD8 dim, cytotoxic T lymphocytes (CTL), natural killer cells (NK), and CD8 cells expressing the surface antigens HLA-DR (CD8-DR), and lymphoproliferative response to mitogen stimulations. Lymphocyte mitogenic proliferative responses remained significantly decreased 50–70 days after exposure, while other values were similar to pre-exposure levels. Figgs et al. (2000) reported increased proliferation of peripheral blood lymphocytes (replicative index) after spraying in 12 applicators of 2,4-D ($P = 0.016$). Increases were independent of tobacco

and alcohol use. 2,4-D concentrations ranged from 1.0 to 1700 µg/g creatinine per L urine), and increased logarithmically with spraying time. No change was seen in complete blood counts or lymphocyte immunophenotypes.

[The Working Group noted that the findings of Faustini et al. (1996) and those of Figgs et al. (2000) appeared to be contradictory, since Faustini *et al*. observed a decrease in lymphocyte proliferation after exposure to chlorophenoxy herbicides, while Figgs et al. observed a small increase.]

(ii) Human cells in vitro

Holland *et al*. evaluated micronuclei (see Section 4.2.1) and reported a dose-dependent inhibition of the replicative index after exposure to 2,4-D in whole blood or isolated lymphocytes from two non-smoking males (aged 31 and 43 years) (Holland et al., 2002). Inhibition was also observed in a separate experiment using isolated lymphocytes from five non-smokers (three females and two males, aged 26–45 years). On the other hand, with low concentrations of a 2,4-D-based formulation (0.005 mM), the replicative index was slightly increased in both experiments (12–15%; $P = 0.052$). No change in mitotic index was seen with either the formulation or pure 2,4-D (Holland et al., 2002).

(b) Experimental systems

(i) Mouse

There were numerous studies of the immunotoxic effects of 2,4-D in mice. Blakley reported immunostimulatory effects with 2,4-D in three studies. In the first study, sheep erythrocyte-stimulated antibody production and lipopolysaccharide-stimulated B-lymphocyte mitogenesis were enhanced in female BDF1 mice (age, 6 weeks) given the n-butyl ester of 2,4-D (2,4-D content, 100 or 200 mg/kg bw). 2,4-D had no effect on T-lymphocyte mitogenesis induced by concanavalin A (Blakley, 1986).

In a second study, production of antibodies to sheep erythrocytes was suppressed at higher single dermal exposures to the n-butyl ester of 2,4-D (2,4-D content, ≤ 500 mg/kg bw) in female CD-1 mice (Blakley & Schiefer, 1986). No effect of acute exposure was seen on T- and B-lymphocyte proliferative responses to concanavalin A or lipopolysaccharide, respectively. However, short-term exposure to 2,4-D n-butyl ester (2,4-D content, up to 300 mg/kg bw, for 3 weeks) enhanced the B- and T-lymphocyte proliferative responses, while having no effect on antibody production.

In the third study (Blakley & Blakley, 1986), the immune response was altered at age 6 weeks in the female offspring of CD-1 mice given 2,4-D n-butyl ester on day 11 of gestation (2,4-D content, up to 200 mg/kg bw). At the highest exposure (200 mg/kg), a slight decrement was reported in the T-lymphocyte proliferative response, and B-lymphocyte stimulation by lipopolysaccharide was significantly reduced. However, when the decrement in background (unstimulated) mitogenic rates was taken into account, no net suppression by 2,4-D was seen. No effect on the humoral immune response (antibody production against sheep erythrocytes) was seen during gestation.

Two studies reported on the immunodepressive effects of 2,4-D in mice (Zhamsaranova et al., 1987; Sapin et al., 2003). [The Working Group noted that the doses and other experimental details were not available for review.]

Lee at al. (2001) demonstrated a suppression of lymphocyte stimulation by concanavalin A in the offspring (age, 7 weeks) of pregnant CD-1 mice exposed on days 6–16 of gestation to a commercial 2,4-D formulation (up to 1.0% in drinking-water; equivalent to 2,4-D amine derivative at 650 mg/kg per day). Body weight and kidney weights were reduced in the offspring of groups at 0.1% and 1.0%. At 1.0% in drinking-water, the formulation increased relative counts of B cells, and reduced counts of T cytotoxic or suppressor

cells. No effect was seen on the humoral immune response or peritoneal macrophage phagocytic function.

In contrast, Salazar et al. (2005) reported significant immunosuppressive effects on humoral immunity in C57BL/6 mice treated with 2,4-D. A 2,4-D formulation (dimethylamine salt of 2,4-D, 47.2%; active ingredient, 150 mg/kg bw, given by intraperitoneal administration) decreased by two to three times the number of phosphorylcholine-specific IgM and IgG antibody-secreting B cells in bone marrow, showing an effect on humoral immunity. In serum, titers of phosphorylcholine-specific immunoglobulins IgM, IgG2b, and IgG3 were decreased by three to four times in mice exposed to 2,4-D. In the spleen, however, 2,4-D produced no change in the number of antibody-producing cells in mice treated with 2,4-D [a finding of little relevance because antibodies are mainly produced by bone marrow-derived cells].

In C57Bl/6 female mice, de la Rosa et al. (2003) reported decreased bone marrow pre-B and IgM(+) B-cell populations 7 days after intraperitoneal exposure to a 2,4-D-based formulation (dimethylamine salt, 47.2%; 200 mg/kg bw per day). However, a 1 : 1 mixture of formulations of propanil and 2,4-D decreased pre-B and IgM(+) B cells at a lower dose (each formulation, 50 mg/kg bw) and an earlier time-point (2 and 7 days). De la Rosa et al. went on to demonstrate reduction in thymus-weight to body-weight ratios and thymocyte depletion at 2 days, and inhibition of thymic T-cell repopulation at 7 days, after exposure to the mixture of formulations of propanil and 2,4-D (each formulation, 150 mg/kg bw per day, by intraperitoneal administration) (de la Rosa et al., 2005). Treatment with the 2,4-D-based formulation only (150 mg/kg bw) had no effect on thymus weight. In another study with mixtures, a herbicide formulation containing 2,4-D and picloram (up to 0.42% in drinking-water for 26 days) had an immunosuppressive effect in female CD-1 mice, reducing antibody production in response to sheep erythrocytes (Blakley, 1997).

(ii) Rat

The first of several studies to report an immunosuppressive effect with 2,4-D in experimental animals was that by Kenigsberg (1975), who reported that the amine salt of 2,4-D suppressed the immune response of rats to *Salmonella* bacteria. A separate group of investigators reported that the amino salt of 2,4-D (2.0 and 20 mg/kg bw daily, intragastric administration) decreased the monocytic-precursor count in the bone marrow in 124 non-inbred white rats (Imel'baeva et al., 1999). The capacity for colony formation was increased, monocytopoiesis was activated, and monocyte migration to peripheral blood was increased. In a third study in rats (Mufazalova et al., 2001), a single dose of the 2,4-D amine salt (240 mg/kg bw) induced phasic changes in blood levels of peripheral leukocytes, and alterations in the microbicidal activity of peritoneal macrophages that persisted for 60 days. The activity of polymorphonuclear leukocytes was also affected. In contrast, Blakley et al. reported no alteration in lymphocyte or macrophage function in male Fisher 344 rats exposed to the amine salt of 2,4-D (10.0 mg/kg, by gavage in olive oil vehicle, twice per week, for 28 days) (Blakley et al., 1998). Specifically, there were no changes in lymphocyte cell-surface marker expression or blastogenesis, phagocytic function of peritoneal macrophages, or antibody production (anti-sheep erythrocytes) (Blakley et al., 1998). No changes in body weight, or organ- to body-weight ratios were seen. [The Working Group noted that effects were seen in studies at high doses, but not in a that used lower doses administered less frequently.]

Marty et al. (2013) evaluated developmental immunotoxicity in CD rats fed diets containing 2,4-D (100, 300, or 600 ppm in females, and 800 ppm in males). Adults and F1 offspring were evaluated for immune function using the sheep

erythrocyte antibody-forming cell assay, and the NK cell assay. At 600 ppm in females, reductions were seen in antibody plaque-forming cells in the spleen (54% decrease) and the number of antibody plaque-forming cells per 10^6 splenocytes (27% decrease), although neither effect attained statistical significance. [The Working Group noted that the effect on bone-marrow antibody plaque-forming cells was not evaluated; since antibodies are mainly produced by bone marrow-derived cells, this limited the value of this study.]

4.2.5 Inflammation

(a) Humans

No data from studies in exposed humans, or human cells in vitro, were available to the Working Group.

(b) Experimental systems

Fukuyama et al. (2009) evaluated allergic reactions in BALB/c mice topically sensitized (nine times in 3 weeks) and subsequently challenged with 2,4-D (dermal or intratracheal exposure). One day after challenge, immediate-type respiratory reactions were induced by 2,4-D. In bronchoalveolar lavage fluid, there was a rise in total IgE levels and an influx of eosinophils, neutrophils, and chemokines (MCP-1, eotaxin, and MIP-1beta). Serum IgE levels also increased. Additionally, surface antigen expression on B cells increased in lymph nodes, and Th2 cytokine production (IL-4, IL-5, IL-10, and IL-13) was elevated in lymph-node cells. [The Working Group noted that these results indicated that 2,4-D is a respiratory allergen capable of causing inflammatory responses in the respiratory tract of mice.]

In subsequent studies, the same group treated mice (age, 4 weeks) orally with parathion (0, 0.4, or 1.2 mg/kg bw) or methoxychlor (0, 100, or 300 mg/kg), and then 4 weeks later with 2,4-D-butyl (0%, 2.5%, 5%, or 10%) (Fukuyama et al., 2010). Parathion or methoxychlor markedly reduced the concentration of 2,4-D-butyl required to yield a positive response in the local lymph-node assay (i.e. the concentration estimated to yield a stimulation index of 3). Thus, 2,4-D may be a more potent allergen and inducer of inflammation if there is simultaneous exposure to other pesticides.

In a study in BALB/c mice, 2,4-D-specific IgE antibodies were detected after intraperitoneal administration of 2,4-D, but 2,4-D applied epicutaneously did not result in delayed-type hypersensitivity (Cushman & Street, 1982).

4.2.6 Altered cell proliferation or death

(a) Humans

(i) Exposed humans

In a small cohort (n = 12) of applicators spraying 2,4-D, a significant increase in the replicative index (a measure of cell proliferation) in peripheral blood lymphocytes in the absence of micronucleus induction was observed (Figgs et al., 2000; see Section 4.2.1).

(ii) Human cells in vitro

In-vitro exposure of isolated lymphocytes to a low dose of 2,4-D (0.005 mM) increased the replicative index, but not the mitotic index (Holland et al., 2002; see Section 4.2.4). At higher concentrations, cytotoxicity was reported in transformed human haematopoietic cells (Venkov et al., 2000) and isolated human lymphocytes (Soloneski et al., 2007); the latter study also pointed to a delay in cell-cycle progression only when erythrocytes were present. In HepG2 cells, lower concentrations of 2,4-D appeared to induce a G_1-phase arrest, while higher concentrations prolonged S- or G_2-phase; 2,4-D also significantly disrupted mitochondrial membrane potential, and increased the proportion of annexin-positive cells (Tuschl & Schwab, 2003, 2005). In isolated human lymphocytes, the dimethylammonium salt of 2,4-D initiated apoptosis in peripheral blood lymphocytes via

a direct effect on mitochondria and disruption of caspase-9 (Kaioumova et al., 2001a). A reduction in HepG2 cell proliferation (determined by incorporation of bromodeoxyuridine) and downregulation of CDC-like kinase 1 (*CLK1*) was noted by Bharadwaj et al. (2005). Several studies have indicated induction of cytotoxicity in human cells after exposure to formulations containing 2,4-D (e.g. Witte et al., 1996; Holland et al., 2002).

(b) Experimental systems

(i) In vivo

No effects on mitotic index or cell proliferation kinetics in murine bone-marrow cells were observed (Madrigal-Bujaidar et al., 2001). Exposure to a formulation containing picloram and 2,4-D caused testicular germ-cell depletion in rats (Oakes et al., 2002). As discussed above (see Section 4.2.3), 2,4-D induced hepatotoxicity and oxidative damage in male Wistar rats (Tayeb et al., 2013). The oxidative damage profile varied among tissues (Pochettino et al., 2013). It has also been reported that 2,4-D is a peroxisome proliferator in rodents (Vainio et al., 1982; Lundgren et al., 1987). Despite observations characteristic of peroxisome proliferators in the liver, 2,4-D appears to primarily induce an architectural change in the outer part of the kidney medulla, characterized by foci of tubules containing basophilic epithelial cells in rodents (primarily in rats, but also in mice) (Ozaki et al., 2001). Renal effects were also noted in fish (Ozcan Oruc et al., 2004). The dimethylammonium salt of 2,4-D caused cell depletion in the white pulp of the spleen and in the cortex of the thymus in rats (Kaioumova et al., 2001b).

(ii) In vitro

At millimolar concentrations in vitro, 2,4-D induced apoptosis associated with mitochondrial cytochrome *c* release and caspase-3 activation in cerebellar granule cells isolated from Wistar rats (age, 8 days) (De Moliner et al., 2002). In CHO cells, an inhibition of protein synthesis associated with polyamine metabolism has been observed, associated with inhibition of cell growth, DNA and protein biosynthesis, and cell accumulation at the G_1-/S-phase boundary (Rivarola et al., 1985, 1992). Ornithine decarboxylase activity was inhibited, and reductions were seen in spermine and spermidine concentrations, but not putrescine (Rivarola & Balegno, 1991).

At concentrations (low micromolar) capable of inducing cell transformation in the Syrian hamster embryo assay, no effect on levels of apoptosis was observed, including unchanged expression of Bcl2 and Bax (Maire et al., 2007). Compared with other herbicides, 2,4-D was the least potent in uncoupling oxidative phosphorylation in rat liver mitochondria (Zychlinski & Zolnierowicz, 1990).

4.2.7 Other mechanisms

Few studies were identified concerning exposure to 2,4-D and immortalization, DNA repair, or epigenetic end-points. Regarding immortalization, the Agricultural Health Study reported that the mean relative telomere length in buccal cells decreased significantly in association with increased lifetime days of use of 2,4-D ($P = 0.004$), among other pesticides (Hou et al., 2013). Epigenetic end-points were addressed in a few studies in plants, in which 2,4-D altered methylation status (Miassod & Cecchini, 1979; Leljak-Levanić et al., 2004).

4.3 Data relevant to comparisons across agents and end-points

4.3.1 General description of the database

The analysis of the in-vitro bioactivity of the agents reviewed in *IARC Monographs* Volume 113 (i.e. 2,4-D, lindane, and DDT) was informed by data from high-throughput screening assays generated by the Toxicity Testing in the

21st Century (Tox21) and Toxicity Forecaster (ToxCast™) research programmes of the government of the USA (Kavlock et al., 2012; Tice et al., 2013). At its meeting in 2014, the Advisory Group To Recommend Priorities for the *IARC Monographs* programme encouraged inclusion of analysis of high-throughput and high-content data (including from curated government databases) (Straif et al., 2014).

Lindane, DDT (*p,p*'-DDT, *o,p*'-DDT, *p,p*'-DDE, *p,p*'-DDD), and 2,4-D were among the approximately 1000 chemicals tested across the full assay battery of the Tox21 and ToxCast research programmes as of 27 April 2015. This assay battery includes 342 assays, for which data on 821 assay end-points (several assays include multiple end-point readouts) are publicly available on the website of the ToxCast research programme (EPA, 2015a). Detailed information about the chemicals tested, assays used, and associated procedures for data analysis is also publicly available (EPA, 2015b). It should be noted that the metabolic capacity of the cell-based assays is variable, and generally limited.

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

In order to explore the bioactivity profiles of the agents being evaluated in *IARC Monographs* Volume 113 with respect to their potential impact on mechanisms of carcinogenesis, the 821 available assay end-points in the ToxCast/Tox21 database were first mapped to the 10 key characteristics of known human carcinogens (Smith et al., 2016). Working Group members and *IARC Monographs* staff made independent assignments for each assay type to one or more "key characteristics." The assignment was based on the biological target being probed by each assay. The consensus assignments comprise 254 assay end-points that mapped to 6 of the 10 "key characteristics" as shown below. Within each key characteristic, the assays were further divided by the Working Group into subsets of similar end-points.

1. *Is electrophilic or can undergo metabolic activation (31 end-points)*: the assay end-points mapped to this characteristic measure CYP450 inhibition (29 end-points) and aromatase inhibition (2 end-points). All 29 assays for CYP inhibition are cell-free. These assay end-points are not direct measures of electrophilicity or metabolic activation.
2. *Is genotoxic (0 end-points):* no assay end-points were mapped to this characteristic.
3. *Alters DNA repair or causes genomic instability (0 end-points):* no assay end-points were mapped to this characteristic.
4. *Induces epigenetic alterations (11 end-points):* assay end-points mapped to this characteristic measure targets associated with DNA binding (e.g. transcription factors) (4 end-points) and transformation catalysts (e.g. histone deacetylase) (7 end-points).
5. *Induces oxidative stress (18 end-points):* the assay end-points mapped to this characteristic measure oxidative stress via cell imaging (7 end-points), markers of oxidative stress (e.g. nuclear factor erythroid 2-related factor, NRF2) (6 end-points), and metalloproteinase (5 end-points).
6. *Induces chronic inflammation (45 end-points):* the assay end-points mapped to this characteristic measure cellular adhesion (14 end-points), cytokines (e.g. IL8) (29 end-points), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) activity (2 end-points).
7. *Is immunosuppressive (0 end-points):* no assay end-points were mapped to this characteristic.
8. *Modulates receptor-mediated effects (92 end-points):* a large and diverse collection of cell-free and cell-based assay end-points measuring nuclear and other receptor

bioactivity, specifically, AhR (2 end-points), androgen receptor (11 end-points), estrogen receptor (18 end-points), farnesoid X receptor (FXR) (7 end-points), peroxisome proliferator-activated receptor (PPAR) (12 end-points), pregnane X receptor_vitamin D receptor (PXR_VDR) (7 end-points), retinoic acid receptor (RAR) (6 end-points), others (29 end-points), were mapped to this characteristic.

9. *Causes immortalization (0 endpoints):* no assay end-points were mapped to this characteristic.
10. *Alters cell proliferation, cell death, or nutrient supply (68 end-points):* assay end-points mapped to this characteristic measure cytotoxicity (41 end-points), mitochondrial toxicity (7 end-points), cell cycle (16 end-points), and cell proliferation (4 end-points).

By matching assays to key characteristics, additional insights could be obtained on the bioactivity profile for each compound specifically for the purpose of evaluating their potential to interact with or affect mechanisms involved in carcinogenesis. In addition, for each chemical, the results of the in-vitro assays that represent each "key characteristic" can be compared to the results for a larger compendium of substances with similar in-vitro data, so that a particular chemical can be aligned with other chemicals with similar toxicological effects. Nonetheless, the available assays do not cover the full spectrum of targets that may be associated with these mechanisms, and metabolic capacity in many of the assays is limited, which could account for any absence of bioactivity. Conversely, the presence of bioactivity alone does not definitively imply that the agent exhibits that key characteristic, as the assay data are considered along with other information, both in vivo and in vitro.

The Working Group then extracted information from the ToxCast database concerning whether a chemical was "active" or "inactive" for each of the selected assay end-points (Sipes et al., 2013; EPA, 2015b). In the analysis by the Working Group, each "active" was given a value of 1, and each "inactive" was given a value of 0. Thus, by assigning all active compounds a value of 1, the micromolar "potency" estimates from the concentration–response data were not explicitly modelled.

Next, to integrate the data across individual assay end-points into the cumulative score for each "key characteristic," the toxicological prioritization index (ToxPi) approach (Reif et al., 2010) and associated software (Reif et al., 2013; Filer et al., 2014) were used. In the Working Group's analyses, the ToxPi score provides a visual measure of the potential for a chemical to be associated with a "key characteristic" relative to 181 chemicals that have been previously evaluated by the *IARC Monographs* and that have been screened by ToxCast. Assay end-point data were available in ToxCast for these 181 chemicals, and not for other chemicals previously evaluated by IARC. ToxPi is a dimensionless index score that integrates multiple, different, assay results and displays them visually. Within each subset of end-points ("slice"), data are translated into ToxPi slice-wise scores for all compounds as detailed below and in the publications describing the approach and the associated software package (Reif et al., 2013). Within each individual slice for a given chemical, the distance from the origin represents the relative chemical-elicited activity of the component assays (i.e. slices extending farther from the origin were associated with "active" calls on more assays). The overall score for a chemical, visualized as a radial ToxPi profile, is the aggregation of all slice-wise scores.

The list of ToxCast/Tox21 assay end-points included in the analysis by the Working Group, description of the target and/or model system for each end-point (e.g. cell type, species, detection technology, etc.), their mapping to 6 of the 10 "key characteristics" of known human carcinogens, and the decision as to whether each

chemical was "active" or "inactive" are available as supplemental material to *Monographs* Volume 113 (IARC, 2016). The output files generated for each "key characteristic" are also provided in the supplemental material, and can be opened using ToxPi software that is freely available for download without a licence (Reif et al., 2013).

4.3.3 Specific effects across 6 of the 10 "key characteristics" based on data from high-throughput screening in vitro

The relative effects of 2,4-D were compared with those of 181 chemicals selected from the more than 800 chemicals previously evaluated by the *IARC Monographs* and also screened by the Tox21/ToxCast programmes, and with those of the other compounds evaluated in the present volume of the *IARC Monographs* (Volume 113) and with their metabolites. Of these 181 chemicals previously evaluated by the *IARC Monographs* and screened in the ToxCast/Tox21 programmes, 8 are classified in Group 1 (*carcinogenic to humans*), 18 are in Group 2A (*probably carcinogenic to humans*), 59 are in Group 2B (*possibly carcinogenic to humans)*, 95 are in Group 3 (*not classifiable as to its carcinogenicity to humans*), and 1 is in Group 4 (*probably not carcinogenic to humans).* The results are presented in a dot plot as a rank order of all compounds in the analysis arranged in the order of their relative activity. The relative positions of lindane, DDT (*p*,*p*'-DDT, *o*,*p*'-DDT, *p*,*p*'-DDE, *p*,*p*'-DDD), and 2,4-D in the ranked list are also shown on the *y*-axis. The colour scheme legend (lower left in each plot) annotates each compound according to its previous *IARC Monographs* group classification. The legend key (lower right graphic in each plot) lists components of the ToxPi chart as subcategories that comprise assay end-points in each characteristic, as well as their respective colour-coding (see Section 4.3.2 and IARC, 2016). The ToxPi profile and numeric score is shown for the highest-ranked chemical in each analysis (directly above the legend key) to represent the maximum ToxPi score and for 2,4-D (upper frame).

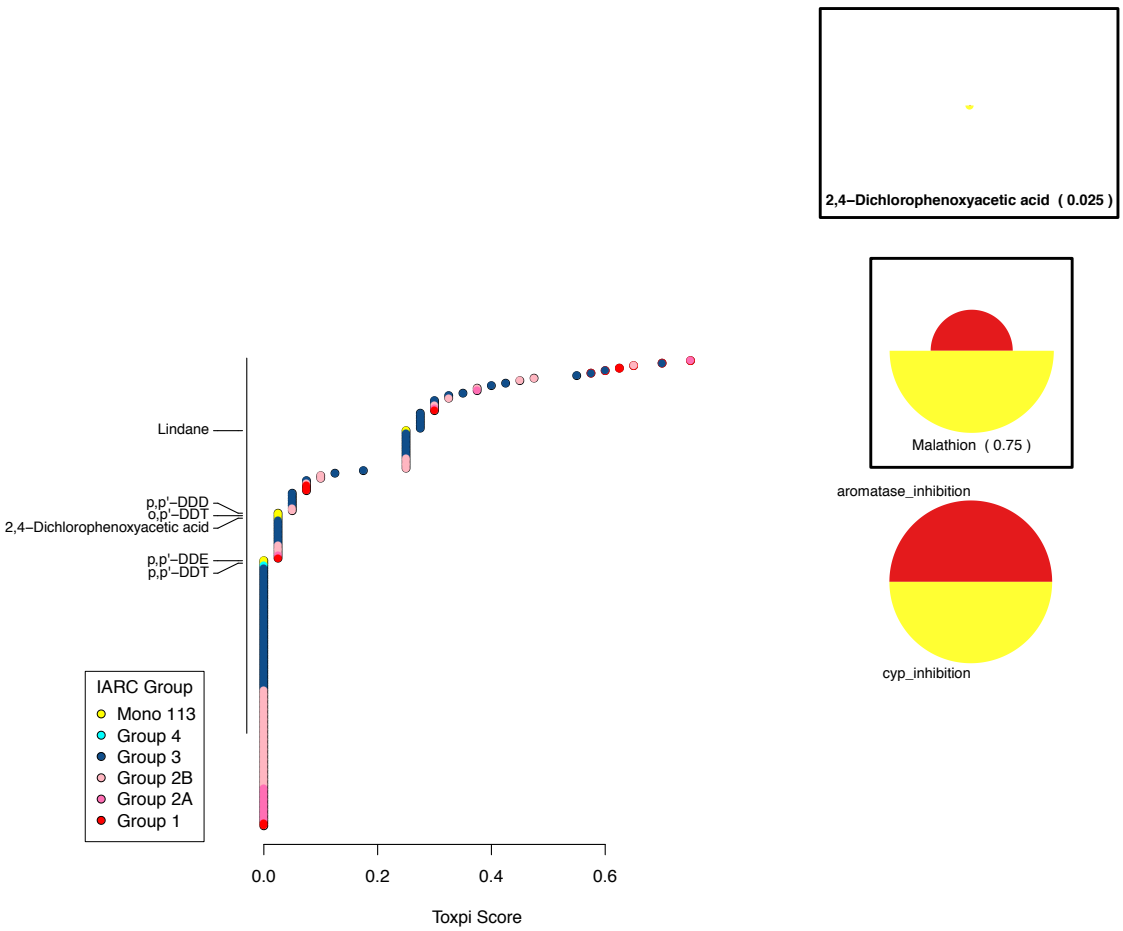
Characteristic (1) *Is electrophilic or can undergo metabolic activation*: 2,4-D was tested in all 31 assay end-points mapped to this key characteristic, and found to be active for 1 of the 29 assay end-points related to CYP inhibition. In comparison, the highest-ranked chemical, malathion (IARC Group 2A; IARC, 2017), was active for 20 out of 29 assay end-points for CYP inhibition, and for 1 out of 2 assay end-points related to aromatase inhibition (Fig. 4.1).

Characteristic (4) *Induces epigenetic alterations:* 2,4-D was tested for all 11 assay end-points mapped to this characteristic, and showed activity for 1 of the transformation-catalyst assay end-points. In comparison, the highest-ranked chemical, captan (IARC Group 3; IARC, 1983), was active for 0 out of 4 DNA binding-assay end-points, and 5 out of 7 transformation-catalyst (e.g. histone modification) assay end-points (Fig. 4.2).

Characteristic (5) *Induces oxidative stress*: 2,4-D was tested for all 18 assay end-points mapped to this characteristic, and was not active for any end-point. In comparison, the highest-ranked chemical, carbaryl (IARC Group 3; IARC, 1976), was active for 2 out of 5 metalloproteinase-assay end-points, 3 out of 7 oxidative-stress assay end-points, and 3 out of 6 oxidative-stress marker assay end-points (Fig. 4.3).

Characteristic (6) *Induces chronic inflammation*: 2,4-D was tested for all 45 assay end-points mapped to this characteristic, and was not active for any end-point. In comparison, the highest-ranked chemical, 4,4'-methylenedianiline (IARC Group 2B; IARC, 1986), was active for 2 out of 14 cellular-adhesion assay end-points, and 2 out of 29 cytokine-assay end-points (Fig. 4.4).

Fig. 4.1 ToxPi ranking for 2,4-dichlorophenoxyacetic acid (2,4-D) using ToxCast assay end-points mapped to metabolic activation

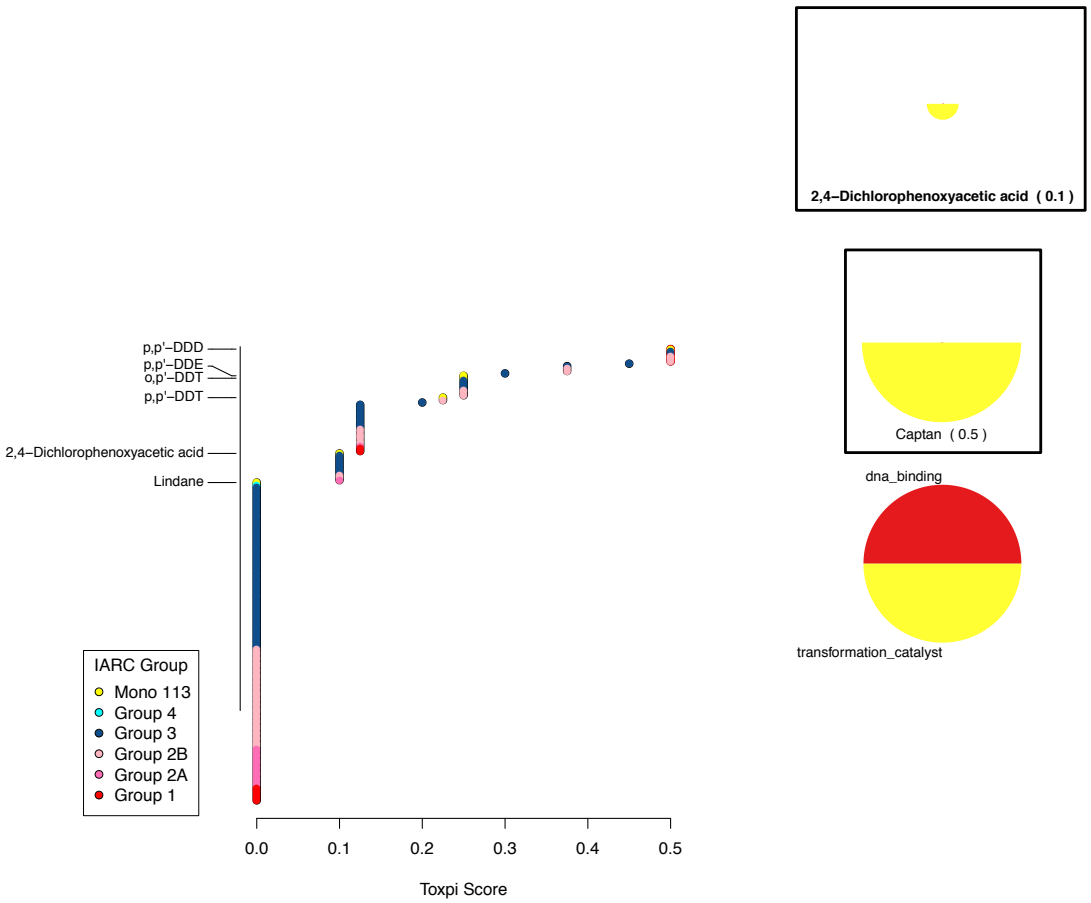


On the left-hand side, the relative rank of 2,4-D is shown (*y*-axis) with respect to its ToxPi score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* Volume 113) and the 181 chemicals previously evaluated by IARC. The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the highest-ranked chemical (in this case, malathion) and the target chemical(s) (2,4-D) are shown with their respective ToxPi score in parentheses.

Characteristic (8) *Modulates receptor-mediated effects:* 2,4-D was tested for all 92 assay end-points mapped to this characteristic, and was active for 1 out of 12 PPAR assay end-points. In comparison, the highest-ranked chemical, clomiphene citrate (IARC Group 3; IARC, 1979), was active for 5 out of 11 androgen-receptor assay end-points, 13 out of 18 estrogen-receptor assay end-points, 3 out of 7 FXR assay end-points, 6 out of 29 other nuclear-receptor assay end-points, 2 out of 12 PPAR assay end-points, 5 out of 7 PXR_VDR assay end-points, and 1 out of 6 RAR assay end-points (Fig. 4.5).

Characteristic (10) *Alters cell proliferation, cell death, or nutrient supply:* 2,4-D was tested for 67 out of 68 assay end-points mapped to this characteristic, and was active for 1 of the 41 assay end-points related to cytotoxicity. In comparison, the highest-ranked chemical, ziram (IARC Group 3; IARC, 1991), was active for 2 out of 16 cell-cycle assay end-points,

Fig. 4.2 ToxPi ranking for 2,4-dichlorophenoxyacetic acid (2,4-D) using ToxCast assay end-points mapped to epigenetic alterations



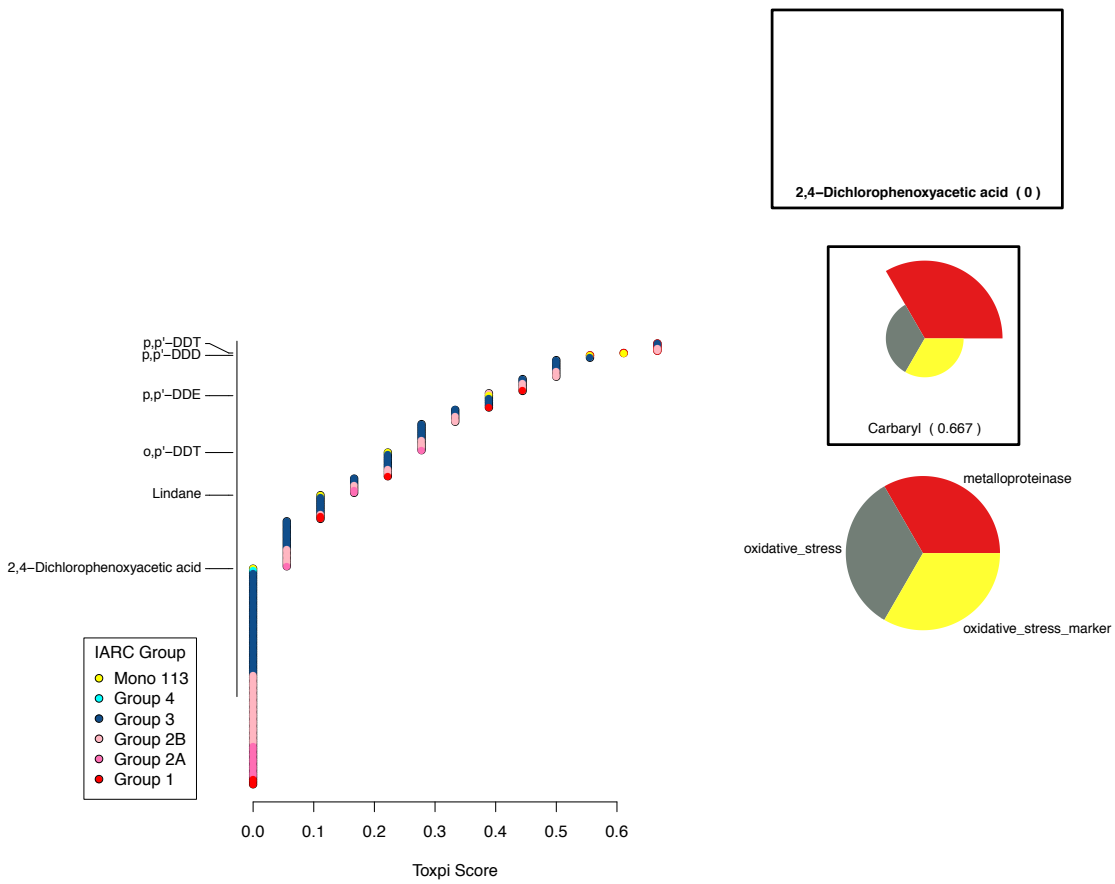
On the left-hand side, the relative rank of 2,4-D is shown (*y*-axis) with respect to their ToxPi score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* Volume 113) and the 181 chemicals previously evaluated by IARC. The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the highest-ranked chemical (in this case, captan) and the target chemical(s) (2,4-D) are shown with their respective ToxPi score in parentheses.

33 out of 41 cytotoxicity assay end-points, and 2 out of 7 mitochondrial-toxicity assay end-points (Fig. 4.6).

4.3.4 Summary of all effects across the "key characteristics" based on data from screening in vitro

As a high-level summary of activity, data were recombined into six ToxPi slices, where each slice represents activity across all component assays mapped to a given characteristic. In the figure (Fig. 4.7), slices are labelled "metabolism" (*Is electrophilic or can undergo metabolic activation*), "epigenetic" (*Induces epigenetic alterations*), "stress" (*Induces oxidative stress*), "inflammation" (*Induces chronic inflammation*), "receptor" (*Modulates receptor-mediated effects*), and "cellular" (*Alters cell proliferation, cell death, or nutrient supply*). Overall, 2,4-D was active in four of the assays. In comparison, the highest-ranked chemical, clomiphene citrate (IARC Group 3; IARC, 1979), was active for 104 assay end-points.

Fig. 4.3 ToxPi ranking for 2,4-dichlorophenoxyacetic acid (2,4-D) using ToxCast assay end-points mapped to oxidative stress markers



On the left-hand side, the relative rank of 2,4-D is shown (*y*-axis) with respect to its ToxPi score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* Volume 113) and the 181 chemicals previously evaluated by IARC. The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the highest-ranked chemical (in this case, carbaryl) and the target chemical(s) (2,4-D) are shown with their respective ToxPi score in parentheses.

4.4 Cancer susceptibility data

Data were not available to the Working Group concerning differential susceptibility due to toxicokinetic or mechanistic factors in humans or experimental systems.

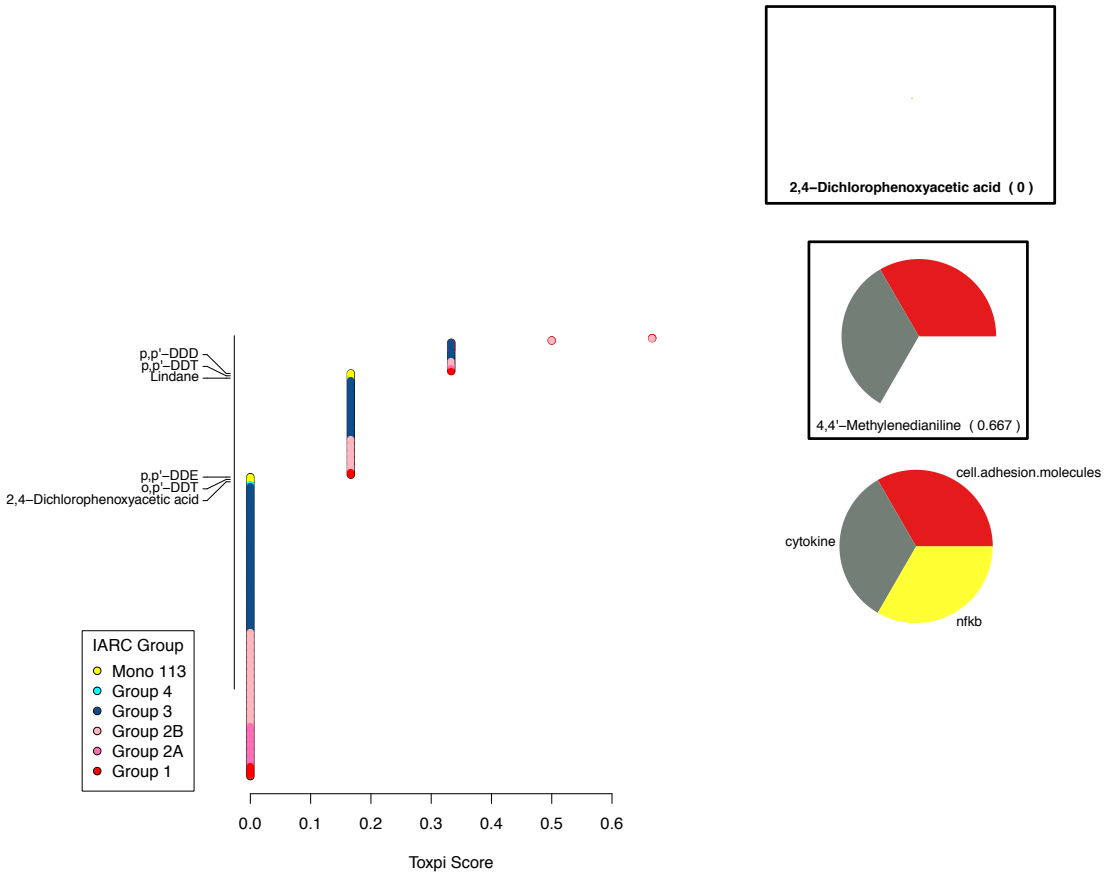
4.5 Other adverse effects

Other adverse effects not addressed in sections 4.1–4.4 that may be relevant to cancer hazard identification for 2,4-D include toxicity in the liver, the lympho-haematopoietic system, or the male reproductive tract.

4.5.1 Humans

One study reported acute hepatitis in a man exposed to 2,4-D through habitual licking of golf balls (Leonard et al., 1997). The patient's liver enzyme levels returned to normal after cessation of exposure, deteriorated again when the behaviour resumed, and then returned back to normal once exposure stopped again.

Fig. 4.4 ToxPi ranking for 2,4-dichlorophenoxyacetic acid (2,4-D) using ToxCast assay end-points mapped to chronic inflammation



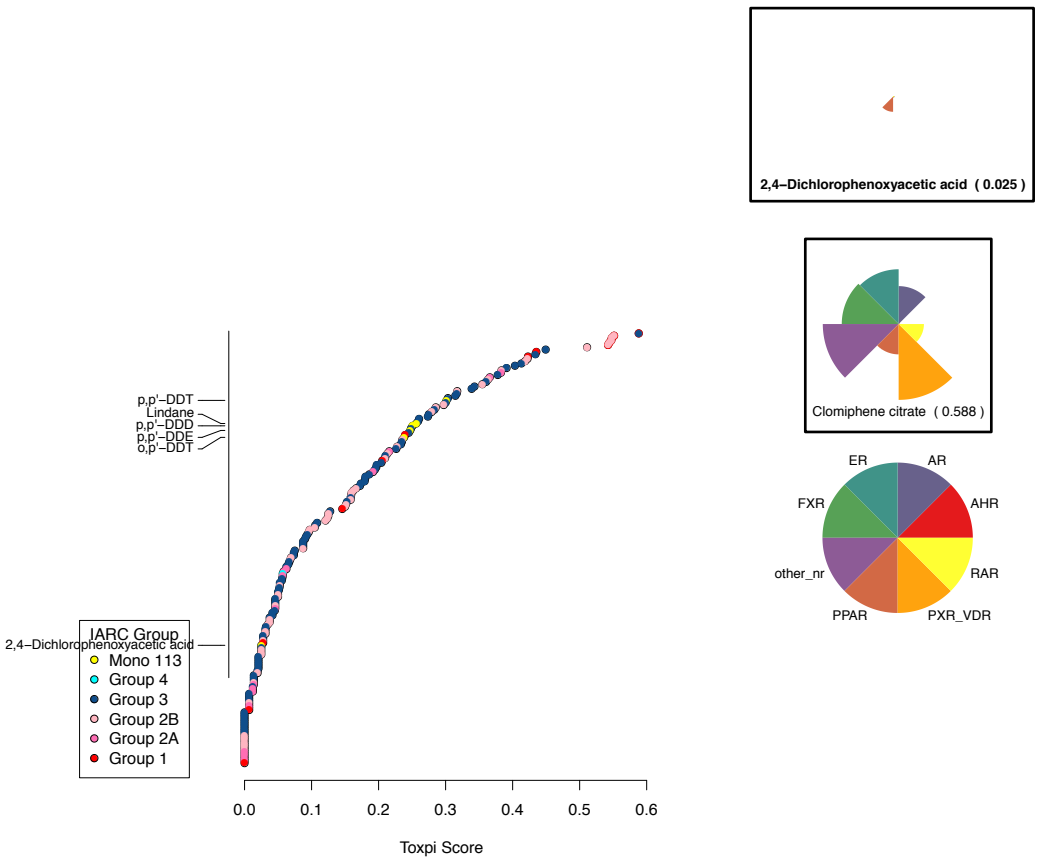
On the left-hand side, the relative rank of 2,4-D is shown (*y*-axis) with respect to its ToxPi score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* Volume 113) and the 181 chemicals previously evaluated by IARC. The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the highest-ranked chemical (in this case, 4,4' -methylenedianiline) and the target chemical(s) (2,4-D) are shown with their respective ToxPi score in parentheses.

4.5.2 Experimental systems

Increased liver weights were reported in a short-term study of toxicity with 2,4-D (Charles et al., 1996) and in a short-term study of toxicity with a 2,4-D-based formulation (Tayeb et al., 2010) in rats, but not in single-dose or short-term exposures to mice (Borzelleca et al., 1985). In the short-term study in rats, histological changes, including hepatic cord disruption, focal necrosis, vessel dilation, and pyknotic nuclei, were also reported, with severity increasing with dose (Tayeb et al., 2010). Increases in alkaline phosphatase activity were reported in female mice exposed for 90 days (Borzelleca et al., 1985). No changes in the liver or in any other organs were reported in a long-term study in rats and dogs (Hansen et al., 1971).

With respect to male reproductive toxicity, Charles et al. (1996) reported decreased testes weights in rats after short-term exposure to 2,4-D. In an F1-extended one-generation study of reproductive toxicity with 2,4-D, Marty et al. (2013) reported reduced testicular weights

Fig. 4.5 ToxPi ranking for 2,4-dichlorophenoxyacetic acid (2,4-D) using ToxCast assay end-points mapped to modulation of receptor-mediated effects



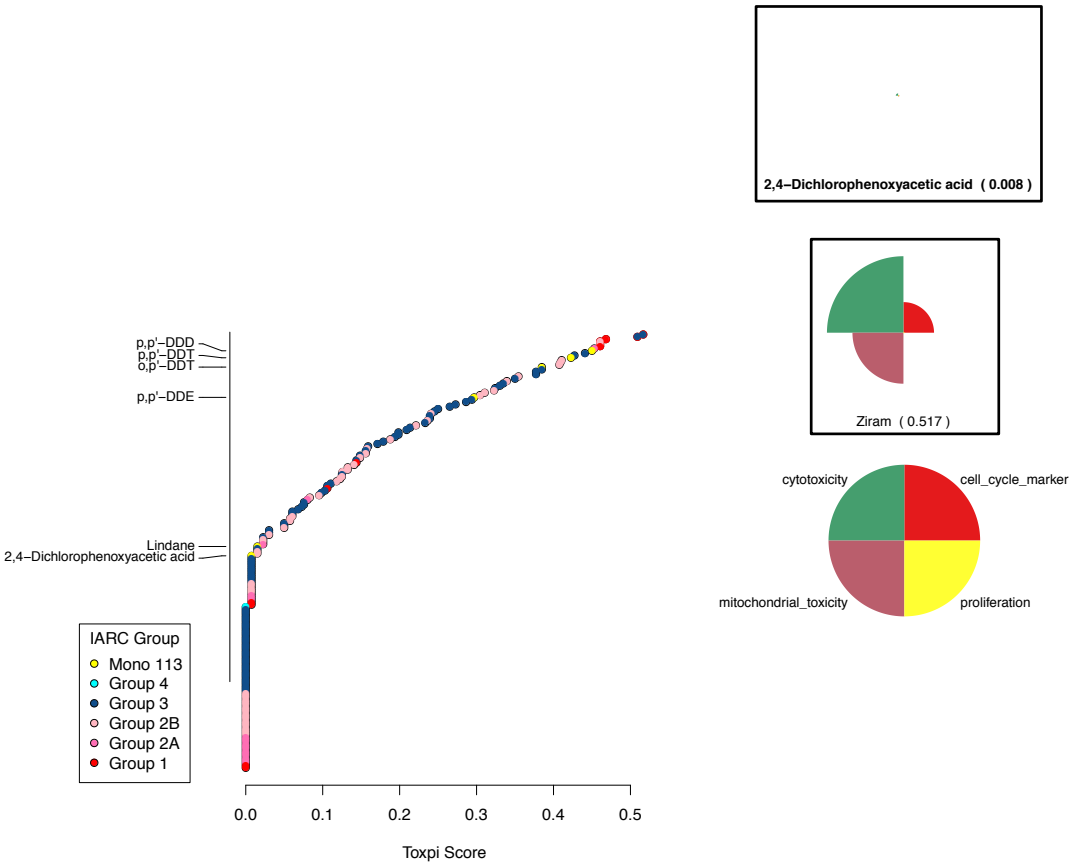
On the left-hand side, the relative rank of 2,4-D is shown (*y*-axis) with respect to its ToxPi score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* Volume 113) and the 181 chemicals previously evaluated by IARC. The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the highest-ranked chemical (in this case, clomiphene citrate) and the target chemical(s) (2,4-D) are shown with their respective ToxPi score in parentheses.

accompanied by decreased body weights in weanling rats, and decreased seminal vesicle weights, but no decreases in testicular weights in P1 rats. In rats exposed to a herbicide formulation containing 2,4-D and picloram, Oakes et al. (2002) reported reduced testes weight, shrunken tubules, and germ-cell depletion.

Data were extracted from the Toxicity Reference Database (ToxRefDB), EPA, which contains information on long-term and short-term studies of cancer, developmental and reproductive toxicity in vivo on hundreds of chemicals (Martin et al., 2009). All source files are publicly available from the October 2014 data release (https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data).

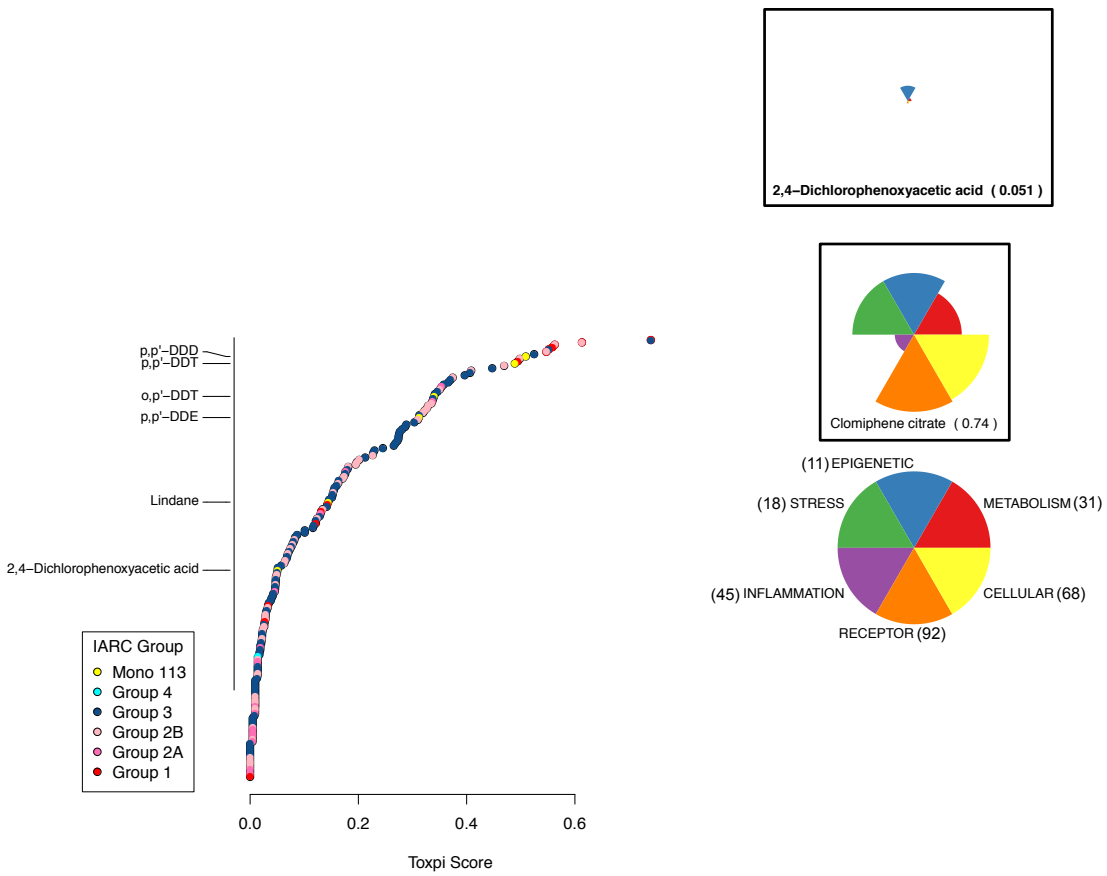
The end-point effects associated with administration of 2,4-D are presented by study type in Table 4.6.

Fig. 4.6 ToxPi ranking for 2,4-dichlorophenoxyacetic acid (2,4-D) using ToxCast assay end-points mapped to metabolic activation



On the left-hand side, the relative rank of 2,4-D is shown (*y*-axis) with respect to its ToxPi score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* Volume 113) and the 181 chemicals previously evaluated by IARC. The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the highest-ranked chemical (in this case, ziram) and the target chemical (2,4-D) are shown with its respective ToxPi score in parentheses.

Fig. 4.7 ToxPi ranking for 2,4-dichlorophenoxyacetic acid (2,4-D) using ToxCast assay end-points: summary of key characteristics



The figure represents a high-level summary of the activity of 2,4-D for end-points covering the seven key characteristics for which it was tested. On the left-hand side, the relative rank of 2,4-D is shown (*y*-axis) with respect to its ToxPi score (*x*-axis) compared with the other chemicals evaluated in the present volume (*IARC Monographs* Volume 113) and the 181 chemicals previously evaluated by IARC. The inset in the scatter plot gives the subcategories and colour coding for the subcategories of the assays. On the right-hand side, the ToxPi charts of the highest-ranked chemical (in this case, clomiphene citrate) and the target chemical (2,4-D) are shown with its respective ToxPi score in parentheses.

Table 4.6 End-point effects associated with administration of 2,4-D reported in the Toxicity Reference Database of the United States Environmental Protection Agency

Target organ	End-point effect		
	13-week studies of toxicity[a]	Long-term studies of toxicity or carcinogenicity[b]	Multigenerational studies of reproductive toxicity in vivo
Adipose tissue		Atrophy in male and female rats	
Adrenal gland	Weight changes in male and female dogs and rats Pathology in male and female rats		
Bone marrow	Haematopoietic hypocellularity in male and female rats	Haematopoietic cell proliferation in female rats	
Brain	Weight changes in male and female rats	Weight changes in male and female dogs	
Eye	Pathology in male and female rats	Pathology in male and female rats	
Heart	Weight changes in male and female dogs and rats		
Kidney	Weight changes and pathology in male and female dogs and rats	Pathology in male and female dogs, rats, and mice Weight changes in male and female mice	Pathology in male rats
Liver	Weight changes in male and female rats and dogs Pathology in male and female rats	Pathology in male and female rats and dogs	
Lung	Pathology in male and female rats	Pathology in male and female rats	
Ovary	Weight changes in rats	Weight changes in dogs and rats	
Pituitary	Weight changes in male and female rats		
Prostate	Inflammation in dogs	Inflammation in dogs	
Spleen	Atrophy in male and female rats		
Testes	Weight changes and pathology in dogs and rats Reduced epididymis size in male rats;	Weight changes and pathology in dogs and rats	
Thymus	Weight changes in male and female rats Atrophy in female rats		
Thyroid	Weight changes in female dogs and male and female rats Pathology in female rats	Pathology and weight changes in male and female rats	

[a] See also Schulze (1991) and EPA (1993)
[b] See also EPA (1995a) and EPA (1995b)
[c] See also Tasker (1985)
2,4-D, 2,4-dichlorophenoxyacetic acid
From EPA (2015c)

5. Summary of data reported

5.1 Exposure data

The common name for 2,4-dichlorophenoxyacetic acid is 2,4-D. In addition to 2,4-D, several 2,4-D ester and salt compounds have been manufactured and used in herbicide products. 2,4-D was commercially introduced in 1944, and has been in continuous production and use worldwide since that time. It is one of the most widely used herbicides around the world for the control of broadleaf weeds and plants in agriculture, forestry, right-of-way (e.g. roadside, rail track, power line), lawn or turf, and aquatic weed control. 2,4-D salts and esters are not persistent under most environmental conditions and are expected to degrade rapidly (within days) to the acid form. Occupational exposures to 2,4-D can result from product manufacturing, and from its use as a herbicide, and occurs primarily via dermal and inhalation routes. Indirect or para-occupational exposure may occur in some populations as a result of take-home and drift exposure pathways, and occurs through dermal absorption, inhalation, and indirect ingestion. The general population may be exposed as a result of the presence of 2,4-D in house dust, food, air, water, and soil. In some areas, residential exposures may be related to use of 2,4-D on lawns. Exposures of the general population may occur through inhalation, dermal absorption, and ingestion. Occupational exposures are often found to be one to three orders of magnitude higher than those in the general population.

5.2 Human carcinogenicity data

Exposure to 2,4-D has been evaluated in relation to cancer risk in population-based case–control studies and in several cohorts of agricultural workers, pesticide applicators, and pesticide manufacturers in the USA, Canada, and Europe. The Working Group also reviewed studies of workers and military personnel who had been exposed to phenoxy herbicides as a class, or to herbicides containing dioxin, but determined that the majority of these studies were uninformative as they did not provide specific risk estimates for 2,4-D. In the studies that were considered to be informative, exposure to 2,4-D was largely assessed by questionnaire, sometimes with expert assessment of work activities and crops grown, and in the industrial cohorts by linkage of work histories to company records of exposure levels. Data were reported in the cohorts for a wide variety of cancers, and in the case–control studies with more detail for non-Hodgkin-lymphoma (NHL), leukaemia, soft tissue sarcoma, and glioma.

More than 10 studies evaluated exposure to 2,4-D in relation to risk of NHL, with NHL classified according to either traditional systems or the more recent WHO classification, which defines lymphoid neoplasms as a broad category that includes lymphoid leukaemia and multiple myeloma. A nested case–control study of NHL within an international cohort of herbicide-manufacturing and -spraying workers, and follow-up of 2,4-D–manufacturing workers in the USA did not observe any strong or consistent increases in risk of NHL in relation to 2,4-D exposure, although the highest risks were observed in the manufacturing cohort in the USA in the highest categories of duration and intensity. A strongly increased risk of NHL, but not leukaemia, was associated with exposure to 2,4-D in a case–control study nested within a cohort of members of a farmworker labour union; nevertheless, the semi-ecological exposure assessment in this study limited the inferences that could be made. Population-based case–control studies of exposure to 2,4-D in relation to risk of lymphoma and leukaemia provided mixed results. Studies in North America found positive associations with exposure to 2,4-D, with evidence for an exposure–response relationship with increasing

frequency of use in two studies, but not in a third. Four other studies in the USA and Europe found largely null results. In two studies and a pooled analysis of three studies, associations with 2,4-D were reduced toward the null after adjustment for other pesticides. Studies in which exposure assessment was based on measurement of 2,4-D in house dust did not find an association with risk of NHL or childhood acute lymphocytic lymphoma; however,the validity of dust measurement to reflect exposure during a time frame of etiological interest is unclear.

Two meta-analyses of exposure to 2,4-D and risk of NHL have been published; one included five studies and showed a moderate, statistically significant increase in risk; the other included nine studies and showed no association. The Working Group carried out an additional meta-analysis for exposure to 2,4-D and risk of NHL that included 11 studies, and also showed no association for ever-exposure to 2,4-D. However, sensitivity analyses showed positive associations when risk estimates that were adjusted for other pesticides were replaced by risk estimates from the same studies that were not adjusted for other pesticides, including replacing the pooled analysis that adjusted for multiple pesticides with estimates that were not adjusted for other pesticides from the primary studies.

Risk of soft tissue sarcoma was evaluated in relation to exposure to 2,4-D in three studies. A strong association was found in an international nested case–control study, although the number of cases was small; two case–control studies in the USA reported that they found no association between exposure to 2,4-D and risk of soft tissue sarcoma. There were very few studies examining other cancer sites (e.g. cancers of the prostate, lung, stomach, breast, and melanoma, and glioma), and their findings were inconsistent.

5.3. Animal carcinogenicity data

2,4-D was tested for carcinogenicity by oral administration in two feeding studies in male and female mice, and one study (gavage followed by feeding) in two strains of male and female mice; by single subcutaneous injection in one study in two strains of male and female mice; by oral (drinking-water) administration in two coadministration studies of commercial amine formulations of 2,4-D and the known carcinogen urethane in mice; by oral administration (gavage followed by feeding) in three studies and by single subcutaneous injection of the isopropyl, butyl or isooctyl esters of 2,4-D in three studies in two strains of male and female mice; by oral administration in three feeding studies in male and female rats; and in one epidemiological study in pet dogs.

In mice, subcutaneous administration of the isooctyl ester of 2,4-D resulted in a significantly increased incidence of reticulum cell sarcomas (histiocytic sarcoma/mixed cell malignant lymphoma) in one strain of female mice. This study had limitations in study design, such that a relationship between exposure to 2,4-D and the occurrence of reticulum cell sarcoma could not be established clearly. In one co-carcinogenicity study in male mice, administration of a commercial amine formulation of 2,4-D in drinking-water increased the multiplicity of urethane-induced pulmonary adenoma. The other studies in mice reported negative results.

In one study in rats, oral administration (feeding) of 2,4-D resulted in a significant positive trend in the incidence of astrocytoma of the brain in males. In a second feeding study using higher doses, the incidence and trend in the incidence of astrocytoma of the brain was not increased. The third feeding study reported negative results.

Results of the epidemiological case study in dogs that examined the association between environmental exposure to 2,4-D and risk of canine

malignant lymphoma were difficult to evaluate due to potential exposure misclassification.

5.4 Mechanistic and other relevant data

2,4-D, and its salts and esters, are readily absorbed via all routes of exposure. 2,4-D is widely distributed in the body by blood circulation, and is bound reversibly to plasma proteins. The elimination half-life of 2,4-D after cessation of exposure is on the order of 1 day. 2,4-D is largely eliminated unchanged via the urine, with some also eliminated as 2,4-D conjugates. Renal organic anion transporter 1 (OAT1) is involved in the excretion of 2,4-D via the kidneys. Most studies in humans and experimental mammalian systems have reported no evidence of metabolism other than conjugation, but metabolism to 2,4-DCP was reported in human CYP3A4-transfected yeast exposed to 2,4-D.

With respect to the key characteristics of human carcinogens, adequate data to evaluate 2,4-D were available only for oxidative stress, genotoxicity, immunosuppression, receptor-mediated effects, and altered cell proliferation or death.

The evidence that 2,4-D induces oxidative stress that can operate in human is *strong*. In human erythrocytes, 2,4-D induces oxidative stress in vitro. In rats exposed in utero and postnatally through milk, 2,4-D induced oxidative stress in the prostate, ovaries, and mammary tissue up to 90 days after birth. 2,4-D also caused oxidative stress in several regions of the brain in rat pups exposed exclusively via the milk of exposed mothers. 2,4-D increased hepatic oxidative stress in dams and fetuses; this was partially counteracted by co-administration of vitamin E. In yeast, 2,4-D induced hydroxyl-radical formation, and this effect was stronger in superoxide dismutase-deficient mutants. Although there was only one study in human cells in vitro, the results were consistent with available data in rats and yeast.

The evidence that 2,4-D is genotoxic is *weak*. Many of the studies that reported positive results involved mixtures or formulations from which the specific effect of 2,4-D could not be discerned. Several studies in exposed humans found no association between genotoxic effects and exposure to 2,4-D. Evidence for induction of chromosomal aberration, micronucleus formation, and sister-chromatid exchange in human lymphocytes or in vitro was mixed, with experiments using pure 2,4-D giving largely negative results. Data from experimental mammals and non-mammals were mixed. Some studies have reported induction of mutagenic effects in *Drosophila*. 2,4-D does not induce point mutations in bacteria, although positive results have been reported in fish, yeast, and plant systems.

The evidence that 2,4-D causes immunosuppression is *moderate*. There are contradictory results regarding the effects of 2,4-D on lymphocyte proliferation in exposed humans in longitudinal studies; both suppressive and stimulatory effects have been demonstrated, depending on levels of exposure and formulation. In cultures of isolated human lymphocytes exposed to 2,4-D, the lymphocyte proliferation replicative index showed both increases and decreases, depending on the dose and preparation of 2,4-D used. 2,4-D significantly decreased the number of bone-marrow plasma cells in a study in mice, demonstrating suppression of humoral immunity. However, mixed results have been reported in some other studies in rats and mice.

The evidence that 2,4-D modulates receptor activity is *weak*. One study in exposed humans reported a correlation between urinary concentrations of 2,4-D and serum concentrations of luteinizing hormone and testosterone; however, the study subjects were exposed to multiple pesticides and herbicides. Studies in human cells in vitro showed potentiation of androgenic action. In the rat, a single dose of 2,4-D has been shown

to interfere with thyroid hormone transport, and to reduce levels of thyroid hormones. Estrogenic activity has been reported in rainbow trout exposed to 2,4-D, according to the vitellogenin assay. 2,4-D causes proliferation of peroxisomes in mouse and rat liver.

The evidence that 2,4-D alters cell proliferation or death is *weak*.

There is inadequate evidence to evaluate whether 2,4-D causes chronic inflammation; however, 2,4-D caused allergy-like hypersensitivity effects in mice.

For the other key characteristics of human carcinogens, the data were insufficient for evaluation.

In high-throughput testing in the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes of the government of the USA, 2,4-D gave positive results for 4 assay end-points, including 1 for peroxisome proliferator-activated receptor (PPAR)-related activity, of the 265 assay end-points relevant to the key characteristics of human carcinogens.

There were few data on cancer susceptibility.

2,4-D has been associated with liver effects in a human case report, and in rats and mice, and with reproductive toxicity in males in some studies in rats.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of 2,4-dichlorophenoxyacetic acid (2,4-D)

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of 2,4-dichlorophenoxyacetic acid (2,4-D)

6.3 Overall evaluation

2,4-Dichlorophenoxyacetic acid (2,4-D) is *possibly carcinogenic to humans* (*Group 2B*)

References

Abbott IM, Bonsall JL, Chester G, Hart TB, Turnbull GJ (1987). Worker exposure to a herbicide applied with ground sprayers in the United Kingdom. *Am Ind Hyg Assoc J*, 48(2):167–75. doi:10.1080/15298668791384571 PMID:3565271

Acquavella JF, Alexander BH, Mandel JS, Burns CJ, Gustin C (2006). Exposure misclassification in studies of agricultural pesticides: insights from biomonitoring. *Epidemiology*, 17(1):69–74. doi:10.1097/01.ede.0000190603.52867.22 PMID:16357597

Adhikari N, Grover IS (1988). Genotoxic effects of some systemic pesticides: in vivo chromosomal aberrations in bone marrow cells in rats. *Environ Mol Mutagen*, 12(2):235–42. doi:10.1002/em.2860120209 PMID:3409877

Ahmadi F, Bakhshandeh F (2009). In vitro study of damaging effects of 2,4-dichlorophenoxyacetic acid on DNA structure by spectroscopic and voltammetric techniques. *DNA Cell Biol*, 28(10):527–33. doi:10.1089/dna.2009.0892 PMID:19563251

Alavanja MC, Dosemeci M, Samanic C, Lubin J, Lynch CF, Knott C et al. (2004). Pesticides and lung cancer risk in the Agricultural Health Study cohort. *Am J Epidemiol*, 160(9):876–85. doi:10.1093/aje/kwh290 PMID:15496540

Alexander BH, Mandel JS, Baker BA, Burns CJ, Bartels MJ, Acquavella JF et al. (2007). Biomonitoring of 2,4-dichlorophenoxyacetic acid exposure and dose in farm families. *Environ Health Perspect*, 115(3):370–6. doi:10.1289/ehp.8869 PMID:17431485

Amer SM, Ali EM (1974). Cytological effects of pesticides V. Effects of some herbicides on *Vicia faba*. *Cytologia (Tokyo)*, 39:633–43.

Amer SM, Aly FA (2001). Genotoxic effect of 2,4-dichlorophenoxy acetic acid and its metabolite 2,4-dichlorophenol in mouse. *Mutat Res*, 494(1–2):1–12. doi:10.1016/S1383-5718(01)00146-2 PMID:11423340

Arbuckle TE, Burnett R, Cole D, Teschke K, Dosemeci M, Bancej C et al. (2002). Predictors of herbicide exposure in farm applicators. *Int Arch Occup Environ Health*, 75(6):406–14. doi:10.1007/s00420-002-0323-7 PMID:12070637

Arbuckle TE, Cole DC, Ritter L, Ripley BD (2004). Farm children's exposure to herbicides: comparison of biomonitoring and questionnaire data. *Epidemiology*, 15(2):187–94. doi:10.1097/01.ede.0000112212.01931.81 PMID:15127911

Arbuckle TE, Cole DC, Ritter L, Ripley BD (2005). Biomonitoring of herbicides in Ontario farm applicators. *Scand J Work Environ Health*, 31(Suppl 1):90–7, discussion 63–5. PMID:16190154

Arbuckle TE, Ritter L (2005). Phenoxyacetic acid herbicide exposure for women on Ontario farms. *J Toxicol Environ Health A*, 68(15):1359–70. doi:10.1080/15287390590953635 PMID:16020195

Arcury TA, Grzywacz JG, Barr DB, Tapia J, Chen H, Quandt SA (2007). Pesticide urinary metabolite levels of children in eastern North Carolina farmworker households. *Environ Health Perspect*, 115(8):1254–60. doi:10.1289/ehp.9975 PMID:17687456

Arias E (2003). Sister chromatid exchange induction by the herbicide 2,4-dichlorophenoxyacetic acid in chick embryos. *Ecotoxicol Environ Saf*, 55(3):338–43. doi:10.1016/S0147-6513(02)00131-8 PMID:12798768

Arias E (2007). Cytogenetic effects of short- and long-term exposure of chick embryos to the phenoxyherbicide 2,4-D. *Environ Mol Mutagen*, 48(6):462–6. doi:10.1002/em.20301 PMID:17372986

Arnold EK, Beasley VR (1989). The pharmacokinetics of chlorinated phenoxy acid herbicides: a literature review. *Vet Hum Toxicol*, 31(2):121–5. PMID:2648672

Ateeq B, Abul Farah M, Ahmad W (2005). Detection of DNA damage by alkaline single cell gel electrophoresis in 2,4-dichlorophenoxyacetic-acid- and butachlor-exposed erythrocytes of Clarias batrachus. *Ecotoxicol Environ Saf*, 62(3):348–54. doi:10.1016/j.ecoenv.2004.12.011 PMID:16216628

Ateeq B, Abul Farah M, Niamat Ali M, Ahmad W (2002a). Clastogenicity of pentachlorophenol, 2,4-D and butachlor evaluated by *Allium* root tip test. *Mutat Res*, 514(1–2):105–13. doi:10.1016/S1383-5718(01)00327-8 PMID:11815249

Ateeq B, Abul farah M, Niamat Ali M, Ahmad W (2002b). Induction of micronuclei and erythrocyte alterations in the catfish *Clarias batrachus* by 2,4-dichlorophenoxyacetic acid and butachlor. *Mutat Res*, 518(2):135–44. doi:10.1016/S1383-5718(02)00075-X PMID:12113764

Aulagnier F, Poissant L, Brunet D, Beauvais C, Pilote M, Deblois C et al. (2008). Pesticides measured in air and precipitation in the Yamaska Basin (Québec): occurrence and concentrations in 2004. *Sci Total Environ*, 394(2-3):338–48. doi:10.1016/j.scitotenv.2008.01.042 PMID:18325567

Aydin H, Ozdemir N, Uzunören N (2005). Investigation of the accumulation of 2,4-dichlorophenoxyacetic acid (2,4-D) in rat kidneys. *Forensic Sci Int*, 153(1):53–7. doi:10.1016/j.forsciint.2005.04.018 PMID:15935583

Badawi AF, Cavalieri EL, Rogan EG (2000). Effect of chlorinated hydrocarbons on expression of cytochrome P450 1A1, 1A2 and 1B1 and 2- and 4-hydroxylation of 17beta-estradiol in female Sprague-Dawley rats. *Carcinogenesis*, 21(8):1593–9. doi:10.1093/carcin/21.8.1593 PMID:10910964

Baharuddin MR, Sahid IB, Noor MA, Sulaiman N, Othman F (2011). Pesticide risk assessment: A study on inhalation and dermal exposure to 2,4-D and paraquat among Malaysian paddy farmers. *J Environ Sci Health B*, 46(7):600–7. doi:10.1080/03601234.2011.589309 PMID:21749249

Baker SE, Barr DB, Driskell WJ, Beeson MD, Needham LL (2000). Quantification of selected pesticide metabolites in human urine using isotope dilution high-performance liquid chromatography/tandem mass spectrometry. *J Expo Anal Environ Epidemiol*, 10(6 Pt 2):789–98. doi:10.1038/sj.jea.7500123 PMID:11138671

Bakke B, De Roos AJ, Barr DB, Stewart PA, Blair A, Freeman LB et al. (2009). Exposure to atrazine and selected non-persistent pesticides among corn farmers during a growing season. *J Expo Sci Environ Epidemiol*, 19(6):544–54. doi:10.1038/jes.2008.53 PMID:19052531

Band PR, Abanto Z, Bert J, Lang B, Fang R, Gallagher RP et al. (2011). Prostate cancer risk and exposure to pesticides in British Columbia farmers. *Prostate*, 71(2):168–83. doi:10.1002/pros.21232 PMID:20799287

Baraud L, Tessier D, Aaron JJ, Quisefit JP, Pinart J (2003). A multi-residue method for characterization and determination of atmospheric pesticides measured at two French urban and rural sampling sites. *Anal Bioanal Chem*, 377(7-8):1148–52. doi:10.1007/s00216-003-2196-3 PMID:13680058

Barnekow DE, Hamburg AW, Puvanesarajah V, Guo M (2001). Metabolism of 2,4-dichlorophenoxyacetic acid in laying hens and lactating goats. *J Agric Food Chem*, 49(1):156–63. doi:10.1021/jf000119r PMID:11170571

Becher H, Flesch-Janys D, Kauppinen T, Kogevinas M, Steindorf K, Manz A et al. (1996). Cancer mortality in German male workers exposed to phenoxy herbicides and dioxins. *Cancer Causes Control*, 7(3):312–21. doi:10.1007/BF00052936 PMID:8734824

Bharadwaj L, Dhami K, Schneberger D, Stevens M, Renaud C, Ali A (2005). Altered gene expression in human hepatoma HepG2 cells exposed to low-level 2,4-dichlorophenoxyacetic acid and potassium nitrate. *Toxicol In Vitro*, 19(5):603–19. doi:10.1016/j.tiv.2005.03.011 PMID:15878651

Bhatti P, Blair A, Bell EM, Rothman N, Lan Q, Barr DB et al. (2010). Predictors of 2,4-dichlorophenoxyacetic acid exposure among herbicide applicators. *J Expo Sci*

Environ Epidemiol, 20(2):160–8. doi:10.1038/jes.2009.14 PMID:19319162

Blair A, Tarone R, Sandler D, Lynch C, Rowland A, Wintersteen W et al. (2000). Reliability of reporting on lifestyle and agricultural factors by a sample of participants in the agricultural health study from iowa. *Ann Epidemiol*, 10(7):478 doi:10.1016/S1047-2797(00)00113-7 PMID:11018423

Blakley BR (1986). The effect of oral exposure to the n-butylester of 2,4-dichlorophenoxyacetic acid on the immune response in mice. *Int J Immunopharmacol*, 8(1):93–9. doi:10.1016/0192-0561(86)90077-9 PMID:3485585

Blakley BR (1997). Effect of roundup and tordon 202C herbicides on antibody production in mice. *Vet Hum Toxicol*, 39(4):204–6. PMID:9251167

Blakley BR, Blakley PM (1986). The effect of prenatal exposure to the n-butylester of 2,4-dichlorophenoxyacetic acid (2,4-D) on the immune response in mice. *Teratology*, 33(1):15–20. doi:10.1002/tera.1420330104 PMID:3488604

Blakley BR, Gagnon JM, Rousseaux CG (1992). The effect of a commercial 2,4-D formulation on chemical- and viral-induced tumor production in mice. *J Appl Toxicol*, 12(4):245–9. doi:10.1002/jat.2550120406 PMID:1430774

Blakley BR, Schiefer BH (1986). The effect of topically applied n-butylester of 2,4-dichlorophenoxyacetic acid on the immune response in mice. *J Appl Toxicol*, 6(4):291–5. doi:10.1002/jat.2550060411 PMID:3760456

Blakley BR, Yole MJ, Brousseau P, Boermans H, Fournier M (1998). Effect of 2,4-dicholorophenoxyacetic acid, trifluralin and triallate herbicides on immune function. *Vet Hum Toxicol*, 40(1):5–10. PMID:9467199

Bloemen LJ, Mandel JS, Bond GG, Pollock AF, Vitek RP, Cook RR (1993). An update of mortality among chemical workers potentially exposed to the herbicide 2,4-dichlorophenoxyacetic acid and its derivatives. *J Occup Med*, 35(12):1208–12. PMID:8113924

Boers D, Portengen L, Bueno-de-Mesquita HB, Heederik D, Vermeulen R (2010). Cause-specific mortality of Dutch chlorophenoxy herbicide manufacturing workers. *Occup Environ Med*, 67(1):24–31. doi:10.1136/oem.2008.044222 PMID:19736176

Bokán K, Syberg K, Jensen K, Rank J (2013). Genotoxic potential of two herbicides and their active ingredients assessed with comet assay on a fish cell line, epithelioma papillosum cyprini (EPC). *J Toxicol Environ Health A*, 76(20):1129–37. doi:10.1080/15287394.2013.843068 PMID:24279814

Bond GG, Wetterstroem NH, Roush GJ, McLaren EA, Lipps TE, Cook RR (1988). Cause specific mortality among employees engaged in the manufacture, formulation, or packaging of 2,4-dichlorophenoxyacetic acid and related salts. *Br J Ind Med*, 45(2):98–105. PMID:3342201

Borzelleca JF, Hayes JR, Condie LW, Egle JL Jr (1985). Acute and subchronic toxicity of 2,4-dichlorophenol in CD-1 mice. *Fundam Appl Toxicol*, 5(3):478–86. doi:10.1016/0272-0590(85)90095-8 PMID:4007306

Brand RM, Charron AR, Sandler VL, Jendrzejewski JL (2007a). Moisturizing lotions can increase transdermal absorption of the herbicide 2,4-dichlorophenoxacetic acid across hairless mouse skin. *Cutan Ocul Toxicol*, 26(1):15–23. doi:10.1080/15569520601182791 PMID:17464745

Brand RM, Jendrzejewski JL, Charron AR (2007b). Potential mechanisms by which a single drink of alcohol can increase transdermal absorption of topically applied chemicals. *Toxicology*, 235(3):141–9. doi:10.1016/j.tox.2007.03.008 PMID:17467136

Brand RM, McMahon L, Jendrzejewski JL, Charron AR (2007c). Transdermal absorption of the herbicide 2,4-dichlorophenoxyacetic acid is enhanced by both ethanol consumption and sunscreen application. *Food Chem Toxicol*, 45(1):93–7. doi:10.1016/j.fct.2006.08.005 PMID:17030379

Brand RM, Pike J, Wilson RM, Charron AR (2003). Sunscreens containing physical UV blockers can increase transdermal absorption of pesticides. *Toxicol Ind Health*, 19(1):9–16. doi:10.1191/0748233703th169oa PMID:15462532

Brown LM, Blair A, Gibson R, Everett GD, Cantor KP, Schuman LM et al. (1990). Pesticide exposures and other agricultural risk factors for leukemia among men in Iowa and Minnesota. *Cancer Res*, 50(20):6585–91. PMID:2208120

Brown LM, Burmeister LF, Everett GD, Blair A (1993). Pesticide exposures and multiple myeloma in Iowa men. *Cancer Causes Control*, 4(2):153–6. doi:10.1007/BF00053156 PMID:8481493

Bueno de Mesquita HB, Doornbos G, Van der Kuip DA, Kogevinas M, Winkelmann R (1993). Occupational exposure to phenoxy herbicides and chlorophenols and cancer mortality in The Netherlands. *Am J Ind Med*, 23(2):289–300. doi:10.1002/ajim.4700230206 PMID:8427257

Bukowska B (2003). Effects of 2,4-D and its metabolite 2,4-dichlorophenol on antioxidant enzymes and level of glutathione in human erythrocytes. *Comp Biochem Physiol C Toxicol Pharmacol*, 135(4):435–41. doi:10.1016/S1532-0456(03)00151-0 PMID:12965188

Bukowska B, Rychlik B, Krokosz A, Michałowicz J (2008). Phenoxyherbicides induce production of free radicals in human erythrocytes: oxidation of dichlorodihydrofluorescine and dihydrorhodamine 123 by 2,4-D-Na and MCPA-Na. *Food Chem Toxicol*, 46(1):359–67. doi:10.1016/j.fct.2007.08.011 PMID:17889420

Burckhardt G, Wolff NA (2000). Structure of renal organic anion and cation transporters. *Am J Physiol Renal Physiol*, 278(6):F853–66. PMID:10836973

Burns C, Bodner K, Swaen G, Collins J, Beard K, Lee M (2011). Cancer incidence of 2,4-D production workers. *Int J Environ Res Public Health*, 8(9):3579–90. doi:10.3390/ijerph8093579 PMID:22016704

Burns CJ, Beard KK, Cartmill JB (2001). Mortality in chemical workers potentially exposed to 2,4-dichlorophenoxyacetic acid (2,4-D) 1945–94: an update. *Occup Environ Med*, 58(1):24–30. doi:10.1136/oem.58.1.24 PMID:11119631

Burns CJ, Swaen GM (2012). Review of 2,4-dichlorophenoxyacetic acid (2,4-D) biomonitoring and epidemiology. *Crit Rev Toxicol*, 42(9):768–86. doi:10.3109/10408444.2012.710576 PMID:22876750

Burton JA, Gardiner TH, Schanker LS (1974). Absorption of herbicides from the rat lung. *Arch Environ Health*, 29(1):31–3. doi:10.1080/00039896.1974.10666523 PMID:4841458

Cantor KP, Blair A, Everett G, Gibson R, Burmeister LF, Brown LM et al. (1992). Pesticides and other agricultural risk factors for non-Hodgkin's lymphoma among men in Iowa and Minnesota. *Cancer Res*, 52(9):2447–55. PMID:1568215

CAREX Canada (2009). 2,4-D. Agents cancérogènes. British Columbia: CAREX Canada. Available from: http://www.carexcanada.ca/fr/2,4-d.pdf.

Carlo GL, Cole P, Miller AB, Munro IC, Solomon KR, Squire RA (1992). Review of a study reporting an association between 2,4-dichlorophenoxyacetic acid and canine malignant lymphoma: report of an expert panel. *Regul Toxicol Pharmacol*, 16(3):245–52. doi:10.1016/0273-2300(92)90004-S PMID:1293641

CDC (2015). Fourth national report on human exposure to environmental chemicals, February 2015. Updated tables. Atlanta (GA): Centers for Disease Control and Prevention. Available from: http://www.cdc.gov/biomonitoring/pdf/FourthReport_UpdatedTables_Feb2015.pdf, accessed 9 April 2015.

Cenkci S, Yildiz M, Ciğerci IH, Bozdağ A, Terzi H, Terzi ES (2010). Evaluation of 2,4-D and Dicamba genotoxicity in bean seedlings using comet and RAPD assays. *Ecotoxicol Environ Saf*, 73(7):1558–64. doi:10.1016/j.ecoenv.2010.07.033 PMID:20797789

Charles JM, Bond DM, Jeffries TK, Yano BL, Stott WT, Johnson KA et al. (1996a). Chronic dietary toxicity/oncogenicity studies on 2,4-dichlorophenoxyacetic acid in rodents. *Fundam Appl Toxicol*, 33(2):166–72. doi:10.1006/faat.1996.0154 PMID:8921335

Charles JM, Cunny HC, Wilson RD, Bus JS (1996). Comparative subchronic studies on 2,4-dichlorophenoxyacetic acid, amine, and ester in rats. *Fundam Appl Toxicol*, 33(2):161–5. doi:10.1006/faat.1996.0153 PMID:8921334

Charles JM, Cunny HC, Wilson RD, Bus JS, Lawlor TE, Cifone MA et al. (1999a). Ames assays and unscheduled DNA synthesis assays on 2, 4-dichlorophenoxyacetic acid and its derivatives. *Mutat Res*, 444(1):207–16. doi:10.1016/S1383-5718(99)00074-1 PMID:10477356

Charles JM, Cunny HC, Wilson RD, Ivett JL, Murli H, Bus JS et al. (1999b). In vivo micronucleus assays on 2,4-dichlorophenoxyacetic acid and its derivatives. *Mutat Res*, 444(1):227–34. doi:10.1016/S1383-5718(99)00076-5 PMID:10477358

Charles JM, Hanley TR Jr, Wilson RD, van Ravenzwaay B, Bus JS (2001). Developmental toxicity studies in rats and rabbits on 2,4-dichlorophenoxyacetic acid and its forms. *Toxicol Sci*, 60(1):121–31. doi:10.1093/toxsci/60.1.121 PMID:11222879

Clausen M, Leier G, Witte I (1990). Comparison of the cytotoxicity and DNA-damaging properties of 2,4-D and U 46 D fluid (dimethylammonium salt of 2,4-D). *Arch Toxicol*, 64(6):497–501. doi:10.1007/BF01977633 PMID:2275605

Coady K, Marino T, Thomas J, Sosinski L, Neal B, Hammond L (2013). An evaluation of 2,4-dichlorophenoxyacetic acid in the Amphibian Metamorphosis Assay and the Fish Short-Term Reproduction Assay. *Ecotoxicol Environ Saf*, 90:143–50. doi:10.1016/j.ecoenv.2012.12.025 PMID:23357564

Coady KK, Kan HL, Schisler MR, Gollapudi BB, Neal B, Williams A et al. (2014). Evaluation of potential endocrine activity of 2,4-dichlorophenoxyacetic acid using in vitro assays. *Toxicol In Vitro*, 28(5):1018–25. doi:10.1016/j.tiv.2014.04.006 PMID:24815817

Coble J, Arbuckle T, Lee W, Alavanja M, Dosemeci M (2005). The validation of a pesticide exposure algorithm using biological monitoring results. *J Occup Environ Hyg*, 2(3):194–201. doi:10.1080/15459620590923343 PMID:15764542

Cocco P, Satta G, D'Andrea I, Nonne T, Udas G, Zucca M et al. (2013b). Lymphoma risk in livestock farmers: results of the Epilymph study. *Int J Cancer*, 132(11):2613–8. doi:10.1002/ijc.27908 PMID:23065666

Cocco P, Satta G, Dubois S, Pili C, Pilleri M, Zucca M et al. (2013a). Lymphoma risk and occupational exposure to pesticides: results of the Epilymph study. *Occup Environ Med*, 70(2):91–8. doi:10.1136/oemed-2012-100845 PMID:23117219

Coggon D, Ntani G, Harris EC, Jayakody N, Palmer KT (2015). Soft tissue sarcoma, non-Hodgkin's lymphoma and chronic lymphocytic leukaemia in workers exposed to phenoxy herbicides: extended follow-up of a UK cohort. *Occup Environ Med*, 72(6):435-41. doi:10.1136/oemed-2014-102654 PMID:25694496

Colt JS, Gunier RB, Metayer C, Nishioka MG, Bell EM, Reynolds P et al. (2008). Household vacuum cleaners vs. the high-volume surface sampler for collection of carpet dust samples in epidemiologic studies of children. *Environ Health*, 7(1):6. doi:10.1186/1476-069X-7-6 PMID:18291036

Curwin BD, Hein MJ, Sanderson WT, Barr DB, Heederik D, Reynolds SJ et al. (2005a). Urinary and hand wipe

pesticide levels among farmers and nonfarmers in Iowa. *J Expo Anal Environ Epidemiol*, 15(6):500–8. doi:10.1038/sj.jea.7500428 PMID:15841098

Curwin BD, Hein MJ, Sanderson WT, Nishioka MG, Reynolds SJ, Ward EM et al. (2005b). Pesticide contamination inside farm and nonfarm homes. *J Occup Environ Hyg*, 2(7):357–67. doi:10.1080/15459620591001606 PMID:16020099

Cushman JR, Street JC (1982). Allergic hypersensitivity to the herbicide 2,4-D in BALB/c mice. *J Toxicol Environ Health*, 10(4–5):729–41. doi:10.1080/15287398209530291 PMID:7161824

de la Rosa P, Barnett J, Schafer R (2003). Loss of pre-B and IgM(+) B cells in the bone marrow after exposure to a mixture of herbicides. *J Toxicol Environ Health A*, 66(24):2299–313. doi:10.1080/716100638 PMID:14630522

de la Rosa P, Barnett JB, Schafer R (2005). Characterization of thymic atrophy and the mechanism of thymocyte depletion after in vivo exposure to a mixture of herbicides. *J Toxicol Environ Health A*, 68(2):81–98. doi:10.1080/15287390590886072 PMID:15762548

De Moliner KL, Evangelista de Duffard AM, Soto E, Duffard R, Adamo AM (2002). Induction of apoptosis in cerebellar granule cells by 2,4-dichlorophenoxyacetic acid. *Neurochem Res*, 27(11):1439–46. doi:10.1023/A:1021665720446 PMID:12512947

De Roos AJ, Zahm SH, Cantor KP, Weisenburger DD, Holmes FF, Burmeister LF et al. (2003). Integrative assessment of multiple pesticides as risk factors for non-Hodgkin's lymphoma among men. *Occup Environ Med*, 60(9):E11. doi:10.1136/oem.60.9.e11 PMID:12937207

Dennis LK, Lynch CF, Sandler DP, Alavanja MC (2010). Pesticide use and cutaneous melanoma in pesticide applicators in the agricultural heath study. *Environ Health Perspect*, 118(6):812–7. doi:10.1289/ehp.0901518 PMID:20164001

Deregowski K, Sulik M, Kemona A, Stefańska-Sulik E (1990). [Distribution and elimination of C-14–2,4-dichlorophenoxyacetic acid (2,4 D) in rat tissues in acute poisoning]. *Rocz Panstw Zakl Hig*, 41(1-2):71–4. PMID:2244176

Deziel NC, Colt JS, Kent EE, Gunier RB, Reynolds P, Booth B et al. (2015). Associations between self-reported pest treatments and pesticide concentrations in carpet dust. *Environ Health*, 14(1):27. doi:10.1186/s12940-015-0015-x PMID:25889489

Dickow LM, Gerken DF, Sams RA, Ashcraft SM (2001). Simultaneous determination of 2,4-D and MCPA in canine plasma and urine by HPLC with fluorescence detection using 9-anthryldiazomethane (ADAM). *J Anal Toxicol*, 25(1):35–9. doi:10.1093/jat/25.1.35 PMID:11215998

Doleźel J, Lucretti S, Novák FJ (1987). The influence of 2,4-dichlorophenoxyacetic acid on cell cycle kinetics and sister-chromatid exchange frequency in garlic (*Allium sativum* L.) meristem cells. *Biol Plant*, 29(4):253–7. doi:10.1007/BF02892785

Dosemeci M, Alavanja MC, Rowland AS, Mage D, Zahm SH, Rothman N et al. (2002). A quantitative approach for estimating exposure to pesticides in the Agricultural Health Study. *Ann Occup Hyg*, 46(2):245–60. doi:10.1093/annhyg/mef011 PMID:12074034

Draper WM (1982). A multiresidue procedure for the determination and confirmation of acidic herbicide residues in human urine. *J Agric Food Chem*, 30(2):227–31. doi:10.1021/jf00110a004 PMID:6853852

Draper WM, Street JC (1982). Applicator exposure to 2,4-D, dicamba, and a dicamba isomer. *J Environ Sci Health B*, 17(4):321–39. doi:10.1080/03601238209372324 PMID:7108143

Duff RM, Kissel JC (1996). Effect of soil loading on dermal absorption efficiency from contaminated soils. *J Toxicol Environ Health*, 48(1):93–106. doi:10.1080/009841096161492 PMID:8637061

Duyzer JH, Vonk AW (2003). Atmospheric deposition of pesticides, PAHs and PCBs in the Netherlands (translation of R2002/606). TNO-report R 2003/255. Available from: http://repository.tudelft.nl/view/tno/uuid%3A9f2f89da-c2e8-4eb1-8c5a-61858d53b9e4/, accessed 23 January 2016.

Edwards MD, Pazzi KA, Gumerlock PH, Madewell BR (1993). c-N-ras is activated infrequently in canine malignant lymphoma. *Toxicol Pathol*, 21(3):288–91. doi:10.1177/019262339302100304 PMID:8248717

EFSA (2011). Reasoned opinion. Review of the existing maximum residue levels (MRLs) for 2,4-D according to Article 12 of Regulation (EC) No 396/2005. *EFSA Journal*, 9(11):2431. Available fromhttp://www.efsa.europa.eu/fr/efsajournal/doc/2431.pdf.

EFSA (2015). The 2013 European Union report on pesticide residues in food. *EFSA Journal*, 13(3):4038.

Ekström AM, Eriksson M, Hansson LE, Lindgren A, Signorello LB, Nyrén O et al. (1999). Occupational exposures and risk of gastric cancer in a population-based case-control study. *Cancer Res*, 59(23):5932–7. PMID:10606238

EPA (1990a).Toxicology data in response to testing requirements of the 2,4-D registration standard - TXR008183. EPA Identification Data. No.0073 *Record No.*, 261:253. Washington (DC): United States Environmental Protection Agency.

EPA (1990b).Toxicology data in response to testing requirements of the 2,4-D registration standard - TXR008183. EPA Identification Data. No.0073 *Record No.*, 261:251. Washington (DC): United States Environmental Protection Agency.

EPA (1993). 13-Week dietary toxicity study of 2,4-D in dogs: Final Report. Lab Project Number: HWA 2184-125. Unpublished study prepared by Hazleton Washington, Inc by Dalgard D. Washington (DC): United States

Environmental Protection Agency. Available from: http://archive.epa.gov/pesticides/chemicalsearch/chemical/foia/web/pdf/030001/030001-1994-04-25a.pdf.

EPA (1995a). Review of Jeffries T, Yano B, Ormand J, Battjes JE. 2,4-Dichlorophenoxyacetic acid: chronic toxicity/oncogenicity study in Fischer 344 rats: Final report. Lab Project Number: K/002372/064. Unpublished study prepared by The Dow Chemical Co., Health and Environment. Washington (DC): United States Environmental Protection Agency.

EPA (1995b). Review of Stott W, Johnson K, Gilbert K, Battjes JE. 2,4-Dichlorophenoxyacetic acid: dietary oncogenicity study in male B6C3F1 mice--Two year final report. Lab Project No: K-002372-063M: 33475: 913. Unpublished study prepared by Dow Chemical Co. Washington (DC): United States Environmental Protection Agency. Available from: http://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-030001_23-May-96_a.pdf.

EPA (1997). Carcinogenicity peer review (4th) of 2,4-dichlorophenoxyacetic acid (2,4-D). Washington (DC): Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency.

EPA (2000). Determination of chlorinated acids in drinking water by liquid-liquid microextraction, derivatization, and fast gas chromatography with electron capture detection. Revision 1.0. Method 515.4. Cincinatti (OH): Technical Support Centre, Office of Ground Water and Drinking Water, United States Environmental Protection Agency. Available from: http://nepis.epa.gov/Exe/ZyPDF.cgi/P1008MC8.PDF?Dockey=P1008MC8.PDF, accessed 18 February 2015.

EPA (2005). Reregistration eligibility decision for 2,4-D. EPA 738-R-05–002. Washington (DC): Prevention, pesticides, and Toxic Substances, United States Environmental Protection Agency. Available from: http://archive.epa.gov/pesticides/reregistration/web/pdf/24d_red.pdf.

EPA (2011). Pesticides industry sales and usage, 2006 and 2007 market estimates. Washington (DC): Office of Chemical Safety and Pollution Prevention, United States Environmental Protection Agency.

EPA (2014). Enlist Duo herbicide fact sheet. Washington (DC): United States Environmental Protection Agency. Available from: https://www.epa.gov/sites/production/files/2014-09/documents/enlist-duo-herbicide-fact-sheet.pdf.

EPA (2015a). Interactive Chemical Safety for Sustainability (iCSS) Dashboard. Washington (DC): United States Environmental Protection Agency. Available from: www.actor.epa.gov/dashboard.

EPA (2015b). ToxCastTM Data. Washington (DC): United States Environmental Protection Agency. Available from: http://epa.gov/ncct/toxcast/data.html. Online database

EPA (2015c). Animal toxicity studies: Effects and end-points (Toxicity Reference Database - ToxCast ToxRefDB files). Washington (DC): United States Environmental Protection Agency. Available from: https://www3.epa.gov/research/COMPTOX/animal_toxicity_data.html.

Epstein SS, Arnold E, Andrea J, Bass W, Bishop Y (1972). Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol Appl Pharmacol*, 23(2):288–325. doi:10.1016/0041-008X(72)90192-5 PMID:5074577

Eriksson M, Hardell L, Berg NO, Möller T, Axelson O (1981). Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. *Br J Ind Med*, 38(1):27–33. PMID:7470401

Eriksson M, Hardell L, Carlberg M, Akerman M (2008). Pesticide exposure as risk factor for non-Hodgkin lymphoma including histopathological subgroup analysis. *Int J Cancer*, 123(7):1657–63. doi:10.1002/ijc.23589 PMID:18623080

Eriksson M, Karlsson M (1992). Occupational and other environmental factors and multiple myeloma: a population based case-control study. *Br J Ind Med*, 49(2):95–103. PMID:1536825

Erne K (1966). Distribution and elimination of chlorinated phenoxyacetic acids in animals. *Acta Vet Scand*, 7(3):240–56. PMID:5959182

Estevam EC, Nakano E, Kawano T, de Bragança Pereira CA, Amancio FF, de Albuquerque Melo AMM (2006). Dominant lethal effects of 2,4-D in Biomphalaria glabrata. *Mutat Res*, 611(1–2):83–8. doi:10.1016/j.mrgentox.2006.07.001 PMID:16973407

European Commission (1991). Council Directive of 15 July 1991 concerning the placing of plant protection products on the market (91/414/EEC). Official Journal of the European Communities, No. L230/1–32. Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31991L0414&from=EN, accessed 18 February 2016.

European Commission (2001). Review report for the active substance 2,4-D. Report No. 7599/V1/97-final. Commission working document. Brussels: Health & Consumer Protection Directorate-General, Directorate E1 – Food safety, European Commission. Available from: http://24d.org/govtrev/ec20011001.pdf.

Fahrig R (1974). Comparative mutagenicity studies with pesticides. *IARC Sci Publ*, 10:161–81.

Fang SC, Lindstrom FT (1980). In vitro binding of 14C-labeled acidic compounds to serum albumin and their tissue distribution in the rat. *J Pharmacokinet Biopharm*, 8(6):583–97. doi:10.1007/BF01060055 PMID:7229910

Farah MA, Ateeq B, Ahmad W (2006). Antimutagenic effect of neem leaves extract in freshwater fish, Channa punctatus evaluated by cytogenetic tests.

Sci Total Environ, 364(1–3):200–14. doi:10.1016/j.scitotenv.2005.07.008 PMID:16169061

Farah MA, Ateeq B, Ali MN, Ahmad W (2003). Evaluation of genotoxicity of PCP and 2,4-D by micronucleus test in freshwater fish Channa punctatus. *Ecotoxicol Environ Saf*, 54(1):25–9. doi:10.1016/S0147-6513(02)00037-4 PMID:12547631

Faustini A, Settimi L, Pacifici R, Fano V, Zuccaro P, Forastiere F (1996). Immunological changes among farmers exposed to phenoxy herbicides: preliminary observations. *Occup Environ Med*, 53(9):583–5. doi:10.1136/oem.53.9.583 PMID:8882113

Feldmann RJ, Maibach HI (1974). Percutaneous penetration of some pesticides and herbicides in man. *Toxicol Appl Pharmacol*, 28(1):126–32. doi:10.1016/0041-008X(74)90137-9 PMID:4853576

Félix-Cañedo TE, Durán-Álvarez JC, Jiménez-Cisneros B (2013). The occurrence and distribution of a group of organic micropollutants in Mexico City's water sources. *Sci Total Environ*, 454-455:109–18. doi:10.1016/j.scitotenv.2013.02.088 PMID:23542484

Ferri A, Duffard R, de Duffard AM (2007). Selective oxidative stress in brain areas of neonate rats exposed to 2,4-Dichlorophenoxyacetic acid through mother's milk. *Drug Chem Toxicol*, 30(1):17–30. doi:10.1080/01480540601017629 PMID:17364861

Figgs LW, Holland NT, Rothmann N, Zahm SH, Tarone RE, Hill R et al. (2000). Increased lymphocyte replicative index following 2,4-dichlorophenoxyacetic acid herbicide exposure. *Cancer Causes Control*, 11(4):373–80. doi:10.1023/A:1008925824242 PMID:10843448

Filer D, Patisaul HB, Schug T, Reif D, Thayer K (2014). Test driving ToxCast: endocrine profiling for 1858 chemicals included in phase II. *Curr Opin Pharmacol*, 19:145–52. doi:10.1016/j.coph.2014.09.021 PMID:25460227

Filkowski J, Besplug J, Burke P, Kovalchuk I, Kovalchuk O (2003). Genotoxicity of 2,4-D and dicamba revealed by transgenic Arabidopsis thaliana plants harboring recombination and point mutation markers. *Mutat Res*, 542(1–2):23–32. doi:10.1016/j.mrgentox.2003.07.008 PMID:14644350

Flower KB, Hoppin JA, Lynch CF, Blair A, Knott C, Shore DL et al. (2004). Cancer risk and parental pesticide application in children of Agricultural Health Study participants. *Environ Health Perspect*, 112(5):631–5. doi:10.1289/ehp.6586 PMID:15064173

Fontana A, Picoco C, Masala G, Prastaro C, Vineis P (1998). Incidence rates of lymphomas and environmental measurements of phenoxy herbicides: ecological analysis and case-control study. *Arch Environ Health*, 53(6):384–7. doi:10.1080/00039899809605725 PMID:9886156

Fritschi L, Benke G, Hughes AM, Kricker A, Turner J, Vajdic CM et al. (2005). Occupational exposure to pesticides and risk of non-Hodgkin's lymphoma. *Am J Epidemiol*, 162(9):849–57. doi:10.1093/aje/kwi292 PMID:16177143

Fukuyama T, Kosaka T, Tajima Y, Ueda H, Hayashi K, Shutoh Y et al. (2010). Prior exposure to organophosphorus and organochlorine pesticides increases the allergic potential of environmental chemical allergens in a local lymph node assay. *Toxicol Lett*, 199(3):347–56. doi:10.1016/j.toxlet.2010.09.018 PMID:20920556

Fukuyama T, Tajima Y, Ueda H, Hayashi K, Shutoh Y, Harada T et al. (2009). Allergic reaction induced by dermal and/or respiratory exposure to low-dose phenoxyacetic acid, organophosphorus, and carbamate pesticides. *Toxicology*, 261(3):152–61. doi:10.1016/j.tox.2009.05.014 PMID:19467290

Gao B, Meier PJ (2001). Organic anion transport across the choroid plexus. *Microsc Res Tech*, 52(1):60–4. doi:10.1002/1097-0029(20010101)52:1<60::AID-JEMT8>3.0.CO;2-C PMID:11135449

Garabrant DH, Philbert MA (2002). Review of 2,4-dichlorophenoxyacetic acid (2,4-D) epidemiology and toxicology. *Crit Rev Toxicol*, 32(4):233–57. doi:10.1080/20024091064237 PMID:12184504

Garaj-Vrhovac V, Zeljezic D (2000). Evaluation of DNA damage in workers occupationally exposed to pesticides using single-cell gel electrophoresis (SCGE) assay. Pesticide genotoxicity revealed by comet assay. *Mutat Res*, 469(2):279–85. doi:10.1016/S1383-5718(00)00092-9 PMID:10984689

Garaj-Vrhovac V, Zeljezic D (2001). Cytogenetic monitoring of croatian population occupationally exposed to a complex mixture of pesticides. *Toxicology*, 165(2–3):153–62. doi:10.1016/S0300-483X(01)00419-X PMID:11522373

Garaj-Vrhovac V, Zeljezic D (2002). Assessment of genome damage in a population of Croatian workers employed in pesticide production by chromosomal aberration analysis, micronucleus assay and Comet assay. *J Appl Toxicol*, 22(4):249–55. doi:10.1002/jat.855 PMID:12210542

Gardner M, Spruill-McCombs M, Beach J, Michael L, Thomas K, Helburn RS (2005). Quantification of 2,4-D on solid-phase exposure sampling media by LC-MS-MS. *J Anal Toxicol*, 29(3):188–92. doi:10.1093/jat/29.3.188 PMID:15842762

Garry VF, Tarone RE, Kirsch IR, Abdallah JM, Lombardi DP, Long LK et al. (2001). Biomarker correlations of urinary 2,4-D levels in foresters: genomic instability and endocrine disruption. *Environ Health Perspect*, 109(5):495–500. doi:10.1289/ehp.01109495 PMID:11401761

Glozier NE, Struger J, Cessna AJ, Gledhill M, Rondeau M, Ernst WR et al. (2012). Occurrence of glyphosate and acidic herbicides in select urban rivers and streams in Canada, 2007. *Environ Sci Pollut Res Int*, 19(3):821–34. doi:10.1007/s11356-011-0600-7 PMID:21948131

Goldner WS, Sandler DP, Yu F, Shostrom V, Hoppin JA, Kamel F et al. (2013). Hypothyroidism and pesticide use among male private pesticide applicators in the agricultural health study. *J Occup Environ Gar*, 55(10):1171–8. doi:10.1097/JOM.0b013e31829b290b PMID:24064777

Gollapudi BB, Charles JM, Linscombe VA, Day SJ, Bus JS (1999). Evaluation of the genotoxicity of 2,4-dichlorophenoxyacetic acid and its derivatives in mammalian cell cultures. *Mutat Res*, 444(1):217–25. doi:10.1016/S1383-5718(99)00075-3 PMID:10477357

González M, Soloneski S, Reigosa MA, Larramendy ML (2005). Genotoxicity of the herbicide 2,4-dichlorophenoxyacetic and a commercial formulation, 2,4-dichlorophenoxyacetic acid dimethylamine salt. I. Evaluation of DNA damage and cytogenetic endpoints in Chinese Hamster ovary (CHO) cells. *Toxicol In Vitro*, 19(2):289–97. doi:10.1016/j.tiv.2004.10.004 PMID:15649642

Goodman JE, Loftus CT, Zu K (2015). 2,4-Dichlorophenoxyacetic Acid and Non-Hodgkin's Lymphoma, Gastric Cancer, and Prostate Cancer: Meta-analyses of the Published Literature *Ann Epidemiol*, doi:10.1016/j.annepidem.2015.04.002

Grabińska-Sota E, Kalka J, Wiśniowska E (2000). Biodegradation and genotoxicity of some chemical plant protection products. *Cent Eur J Public Health*, 8(Suppl):93–4. PMID:10943490

Grabińska-Sota E, Wiśniowska E, Kalka J, Scieranka B (2002). Genotoxicological effects of some phenoxyherbicides and their metabolites on Bacillus subtilis M45 Rec- and H17 Rec+ strains. *Chemosphere*, 47(1):81–5. doi:10.1016/S0045-6535(01)00211-9 PMID:11996139

Graf U, Würgler FE (1996). The somatic white-ivory eye spot test does not detect the same spectrum of genotoxic events as the wing somatic mutation and recombination test in Drosophila melanogaster. *Environ Mol Mutagen*, 27(3):219–26. doi:10.1002/(SICI)1098-2280(1996)27:3<219::AID-EM7>3.0.CO;2-9 PMID:8625958

Griffin RJ, Godfrey VB, Kim YC, Burka LT (1997). Sex-dependent differences in the disposition of 2,4-dichlorophenoxyacetic acid in Sprague-Dawley rats, B6C3F1 mice, and Syrian hamsters. *Drug Metab Dispos*, 25(9):1065–71. PMID:9311622

Grissom RE Jr, Brownie C, Guthrie FE (1985). Dermal absorption of pesticides in mice. *Pestic Biochem Physiol*, 24(1):119–23. doi:10.1016/0048-3575(85)90121-X

Grover R, Franklin CA, Muir NI, Cessna AJ, Riedel D (1986). Dermal exposure and urinary metabolite excretion in farmers repeatedly exposed to 2,4-D amine. *Toxicol Lett*, 33(1-3):73–83. doi:10.1016/0378-4274(86)90072-X PMID:3775823

Hansen WH, Quaife ML, Habermann RT, Fitzhugh OG (1971). Chronic toxicity of 2,4-dichlorophenoxyacetic acid in rats and dogs. *Toxicol Appl Pharmacol*, 20(1):122–9. doi:10.1016/0041-008X(71)90096-2 PMID:5110820

Hardell L, Eriksson M, Degerman A (1994). Exposure to phenoxyacetic acids, chlorophenols, or organic solvents in relation to histopathology, stage, and anatomical localization of non-Hodgkin's lymphoma. *Cancer Res*, 54(9):2386–9. PMID:8162585

Hardell L, Johansson B, Axelson O (1982). Epidemiological study of nasal and nasopharyngeal cancer and their relation to phenoxy acid or chlorophenol exposure. *Am J Ind Med*, 3(3):247–57. doi:10.1002/ajim.4700030304 PMID:6303119

Hardell L, Sandström A (1979). Case-control study: soft-tissue sarcomas and exposure to phenoxyacetic acids or chlorophenols. *Br J Cancer*, 39(6):711–7. doi:10.1038/bjc.1979.125 PMID:444410

Harris SA, Solomon KR (1992). Percutaneous penetration of 2,4-dichlorophenoxyacetic acid and 2,4-D dimethylamine salt in human volunteers. *J Toxicol Environ Health*, 36(3):233–40. doi:10.1080/15287399209531634 PMID:1629934

Harris SA, Solomon KR, Stephenson GR (1992). Exposure of homeowners and bystanders to 2,4-dichlorophenoxyacetic acid (2,4-D). *J Environ Sci Health B*, 27(1):23–38. doi:10.1080/03601239209372765 PMID:1556388

Harris SA, Villeneuve PJ, Crawley CD, Mays JE, Yeary RA, Hurto KA et al. (2010). National study of exposure to pesticides among professional applicators: an investigation based on urinary biomarkers. *J Agric Food Chem*, 58(18):10253–61. doi:10.1021/jf101209g PMID:20799690

Hartge P, Colt JS, Severson RK, Cerhan JR, Cozen W, Camann D et al. (2005). Residential herbicide use and risk of non-Hodgkin lymphoma. *Cancer Epidemiol Biomarkers Prev*, 14(4):934–7. doi:10.1158/1055-9965.EPI-04-0730 PMID:15824166

Hayes HM, Tarone RE, Cantor KP (1995). On the association between canine malignant lymphoma and opportunity for exposure to 2,4-dichlorophenoxyacetic acid. *Environ Res*, 70(2):119–25. doi:10.1006/enrs.1995.1056 PMID:8674480

Hayes HM, Tarone RE, Cantor KP, Jessen CR, McCurnin DM, Richardson RC (1991). Case-control study of canine malignant lymphoma: positive association with dog owner's use of 2,4-dichlorophenoxyacetic acid herbicides. *J Natl Cancer Inst*, 83(17):1226–31. doi:10.1093/jnci/83.17.1226 PMID:1870148

Health Canada (2010). Report on human biomonitoring of environmental chemicals in Canada. Results of the Canadian Health Measures Survey Cycle 1 (2007–2009). Ottawa (ON): Health Canada. Available from: http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/chms-ecms/report-rapport-eng.pdf.

Herrero-Hernández E, Rodríguez-Gonzalo E, Andrades MS, Sánchez-González S, Carabias-Martínez R (2013).

Occurrence of phenols and phenoxyacid herbicides in environmental waters using an imprinted polymer as a selective sorbent. *Sci Total Environ*, 454-455:299–306. doi:10.1016/j.scitotenv.2013.03.029 PMID:23562684

Hines CJ, Deddens JA, Striley CAF, Biagini RE, Shoemaker DA, Brown KK et al. (2003). Biological monitoring for selected herbicide biomarkers in the urine of exposed custom applicators: application of mixed-effect models. *Ann Occup Hyg*, 47(6):503–17. doi:10.1093/annhyg/meg067 PMID:12890659

Hines CJ, Deddens JA, Tucker SP, Hornung RW (2001). Distributions and determinants of pre-emergent herbicide exposures among custom applicators. *Ann Occup Hyg*, 45(3):227–39. doi:10.1093/annhyg/45.3.227 PMID:11295146

Hoar SK, Blair A, Holmes FF, Boysen CD, Robel RJ, Hoover R et al. (1986). Agricultural herbicide use and risk of lymphoma and soft-tissue sarcoma. *JAMA*, 256(9):1141–7. doi:10.1001/jama.1986.03380090081023 PMID:3801091

Hohenadel K, Harris SA, McLaughlin JR, Spinelli JJ, Pahwa P, Dosman JA et al. (2011). Exposure to multiple pesticides and risk of non-Hodgkin lymphoma in men from six Canadian provinces. *Int J Environ Res Public Health*, 8(6):2320–30. doi:10.3390/ijerph8062320 PMID:21776232

Holland NT, Duramad P, Rothman N, Figgs LW, Blair A, Hubbard A et al. (2002). Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-dichlorophenoxyacetic acid in vitro and in vivo. *Mutat Res*, 521(1–2):165–78. doi:10.1016/S1383-5718(02)00237-1 PMID:12438013

Hooiveld M, Heederik DJ, Kogevinas M, Boffetta P, Needham LL, Patterson DG Jr et al. (1998). Second follow-up of a Dutch cohort occupationally exposed to phenoxy herbicides, chlorophenols, and contaminants. *Am J Epidemiol*, 147(9):891–901. doi:10.1093/oxfordjournals.aje.a009543 PMID:9583720

Hoppin JA, Yucel F, Dosemeci M, Sandler DP (2002). Accuracy of self-reported pesticide use duration information from licensed pesticide applicators in the Agricultural Health Study. *J Expo Anal Environ Epidemiol*, 12(5):313–8. doi:10.1038/sj.jea.7500232 PMID:12198579

Hou L, Andreotti G, Baccarelli AA, Savage S, Hoppin JA, Sandler DP et al. (2013). Lifetime pesticide use and telomere shortening among male pesticide applicators in the Agricultural Health Study. *Environ Health Perspect*, 121(8):919–24. doi:10.1289/ehp.1206432 PMID:23774483

IARC (1976). Some carbamates, thiocarbamates and carbazides. *IARC Monogr Eval Carcinog Risk Chem Man*, 12:1–282. Available from: http://monographs.iarc.fr/ENG/Monographs/vol1-42/mono12.pdf PMID:188751

IARC (1977). Some fumigants, the herbicides 2,4-D and 2,4,5-T, chlorinated dibenzodioxins and miscellaneous industrial chemicals. *IARC Monogr Eval Carcinog Risk Chem Man*, 15:1–354. Available from: http://monographs.iarc.fr/ENG/Monographs/vol1-42/mono15.pdf PMID:330387

IARC (1979). Sex hormones (II). *IARC Monogr Eval Carcinog Risk Chem Hum*, 21:1–583. Available from: http://monographs.iarc.fr/ENG/Monographs/vol1-42/mono21.pdf.

IARC (1983). Miscellaneous pesticides. *IARC Monogr Eval Carcinog Risk Chem Hum*, 30:1–424. Available from: http://monographs.iarc.fr/ENG/Monographs/vol1-42/mono30.pdf PMID:6578175

IARC (1986). Some chemicals used in plastics and elastomers. *IARC Monogr Eval Carcinog Risk Chem Hum*, 39:1–403. Available from: http://monographs.iarc.fr/ENG/Monographs/vol1-42/mono39.pdf.

IARC (1987). Overall evaluations of carcinogenicity: an updating of *IARC Monographs* volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum,* Suppl 7:1–440. PMID:3482203

IARC (1991). Occupational exposures in insecticide application, and some pesticides. *IARC Monogr Eval Carcinog Risks Hum*, 53:1–612. Available from: http://monographs.iarc.fr/ENG/Monographs/vol53/index.php.

IARC (2017). Malathion. In: Some organophosphate insecticides and herbicides. *IARC Monogr Eval Carcinog Risks Hum*, 112:1–452. Available from http://monographs.iarc.fr/ENG/Monographs/vol112/mono112-07.pdf.

IARC (2016). List of ToxCast/Tox21 assay endpoints. In: Supplemental Material to *IARC Monographs* Volume 113. IARC, Lyon. Available from: http://monographs.iarc.fr/ENG/Monographs/vol113/113-Annex1.pdf.

ILO (1999). 2,4-D. International Chemical Safety Cards. Geneva: International Labour Organization. Available from: http://siri.org/msds/mf/cards/file/0033.html, accessed 23 January 2016

Imaoka T, Kusuhara H, Adachi-Akahane S, Hasegawa M, Morita N, Endou H et al. (2004). The renal-specific transporter mediates facilitative transport of organic anions at the brush border membrane of mouse renal tubules. *J Am Soc Nephrol*, 15(8):2012–22. doi:10.1097/01.ASN.0000135049.20420.E5 PMID:15284287

Imel'baeva EA, Teplova SN, Kamilov FKh, Kaiumova AF (1999). [The effect of the amine salt of 2,4-dichlorophenoxyacetic acid on the cluster- and colony-forming capacity of the bone marrow and on the mononuclear phagocyte system] *Zh Mikrobiol Epidemiol Immunobiol*, (6):67–70. PMID:10876855

Innes JR, Ulland BM, Valerio MG, Petrucelli L, Fishbein L, Hart ER et al. (1969). Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. *J Natl Cancer Inst*, 42(6):1101–14. PMID:5793189

IPCS/ICSC (2015). 2,4-D. ICSC 0034. International Chemical Safety Card. Geneva: International Programme of Chemical Safety, World Health Organization. Available from: http://www.inchem.org/documents/icsc/icsc/eics0033.htm, accessed 6 November 2015.

Jacobi H, Metzger J, Witte I (1992). Synergistic effects of Cu(II) and dimethylammonium 2,4-dichlorophenoxyacetate (U46 D fluid) on PM2 DNA and mechanism of DNA damage. *Free Radic Res Commun*, 16(2):123–30. doi:10.3109/10715769209049165 PMID:1628858

Jenssen D, Renberg L (1976). Distribution and cytogenetic test of 2,4-D and 2,4,5-T phenoxyacetic acids in mouse blood tissues. *Chem Biol Interact*, 14(3–4):279–89. PMID:954145

JMPR (1996). 2,4-Dichlorophenoxyacetic acid (2,4-D). Geneva: Joint FAO/WHO Meeting on Pesticide Residues World Health Organization. Available from: http://www.inchem.org/documents/jmpr/jmpmono/v96pr04.htm.

Jurewicz J, Hanke W, Sobala W, Ligocka D (2012). Exposure to phenoxyacetic acid herbicides and predictors of exposure among spouses of farmers. *Ann Agric Environ Med*, 19(1):51–6. PMID:22462445

Kahn PC, Gochfeld M, Nygren M, Hansson M, Rappe C, Velez H et al. (1988). Dioxins and dibenzofurans in blood and adipose tissue of Agent Orange-exposed Vietnam veterans and matched controls. *JAMA*, 259(11):1661–7. doi:10.1001/jama.1988.03720110023029 PMID:3343772

Kaioumova D, Kaioumov F, Opelz G, Süsal C (2001b). Toxic effects of the herbicide 2,4-dichlorophenoxyacetic acid on lymphoid organs of the rat. *Chemosphere*, 43(4–7):801–5. doi:10.1016/S0045-6535(00)00436-7 PMID:11372868

Kaioumova D, Süsal C, Opelz G (2001a). Induction of apoptosis in human lymphocytes by the herbicide 2,4-dichlorophenoxyacetic acid. *Hum Immunol*, 62(1):64–74. doi:10.1016/S0198-8859(00)00229-9 PMID:11165716

Kaioumova DF, Khabutdinova LK (1998). Cytogenetic characteristics of herbicide production workers in Ufa. *Chemosphere*, 37(9–12):1755–9. doi:10.1016/S0045-6535(98)00240-9 PMID:9828303

Kale PG, Petty BT Jr, Walker S, Ford JB, Dehkordi N, Tarasia S et al. (1995). Mutagenicity testing of nine herbicides and pesticides currently used in agriculture. *Environ Mol Mutagen*, 25(2):148–53. doi:10.1002/em.2850250208 PMID:7698107

Kaneene JB, Miller R (1999). Re-analysis of 2,4-D use and the occurrence of canine malignant lymphoma. *Vet Hum Toxicol*, 41(3):164–70. PMID:10349709

Kavlock R, Chandler K, Houck K, Hunter S, Judson R, Kleinstreuer N et al. (2012). Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*, 25(7):1287–302. doi:10.1021/tx3000939 PMID:22519603

Kawashima Y, Katoh H, Nakajima S, Kozuka H, Uchiyama M (1984). Effects of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid on peroxisomal enzymes in rat liver. *Biochem Pharmacol*, 33(2):241–5. doi:10.1016/0006-2952(84)90481-7 PMID:6704149

Kaya B, Yanikoglu A, Marcos R (1999). Genotoxicity studies on the phenoxyacetates 2,4-D and 4-CPA in the Drosophila wing spot test. *Teratog Carcinog Mutagen*, 19(4):305–12. doi:10.1002/(SICI)1520-6866(1999)19:4<305::AID-TCM7>3.0.CO;2-X PMID:10406894

Kenigsberg IaE (1975). [Relation of the immune response in rats to the state of the lysosomes and intensity of protein synthesis] *Zh Mikrobiol Epidemiol Immunobiol*, (6):32–5. PMID:1098336

Kim CS, Binienda Z, Sandberg JA (1996). Construction of a physiologically based pharmacokinetic model for 2,4-dichlorophenoxyacetic acid dosimetry in the developing rabbit brain. *Toxicol Appl Pharmacol*, 136(2):250–9. doi:10.1006/taap.1996.0032 PMID:8619233

Kim CS, Gargas ML, Andersen ME (1994). Pharmacokinetic modeling of 2,4-dichlorophenoxyacetic acid (2,4-D) in rat and in rabbit brain following single dose administration. *Toxicol Lett*, 74(3):189–201. doi:10.1016/0378-4274(94)90078-7 PMID:7871543

Kim CS, Keizer RF, Pritchard JB (1988). 2,4-Dichlorophenoxyacetic acid intoxication increases its accumulation within the brain. *Brain Res*, 440(2):216–26. doi:10.1016/0006-8993(88)90989-4 PMID:3359212

Kim CS, O'Tuama LA, Mann JD, Roe CR (1983). Saturable accumulation of the anionic herbicide, 2,4-dichlorophenoxyacetic acid (2,4-D), by rabbit choroid plexus: early developmental origin and interaction with salicylates. *J Pharmacol Exp Ther*, 225(3):699–704. PMID:6864528

Kim H-J, Park YI, Dong M-S (2005). Effects of 2,4-D and DCP on the DHT-induced androgenic action in human prostate cancer cells. *Toxicol Sci*, 88(1):52–9. doi:10.1093/toxsci/kfi287 PMID:16107550

King KW, Balogh JC (2010). Chlorothalonil and 2,4-D losses in surface water discharge from a managed turf watershed. *J Environ Monit*, 12(8):1601–12. doi:10.1039/c0em00030b PMID:20526481

Knapp GW, Setzer RW, Fuscoe JC (2003). Quantitation of aberrant interlocus T-cell receptor rearrangements in mouse thymocytes and the effect of the herbicide 2,4-dichlorophenoxyacetic acid. *Environ Mol Mutagen*, 42(1):37–43. doi:10.1002/em.10168 PMID:12874811

Knopp D (1994). Assessment of exposure to 2,4-dichlorophenoxyacetic acid in the chemical industry: results of a five year biological monitoring study. *Occup*

Environ Med, 51(3):152–9. doi:10.1136/oem.51.3.152 PMID:8130842

Knopp D, Schiller F (1992). Oral and dermal application of 2,4-dichlorophenoxyacetic acid sodium and dimethylamine salts to male rats: investigations on absorption and excretion as well as induction of hepatic mixed-function oxidase activities. *Arch Toxicol*, 66(3):170–4. doi:10.1007/BF01974010 PMID:1497479

Kogevinas M, Becher H, Benn T, Bertazzi PA, Boffetta P, Bueno-de-Mesquita HB et al. (1997). Cancer mortality in workers exposed to phenoxy herbicides, chlorophenols, and dioxins. An expanded and updated international cohort study. *Am J Epidemiol*, 145(12):1061–75. doi:10.1093/oxfordjournals.aje.a009069 PMID:9199536

Kogevinas M, Kauppinen T, Winkelmann R, Becher H, Bertazzi PA, Bueno-de-Mesquita HB et al. (1995). Soft tissue sarcoma and non-Hodgkin's lymphoma in workers exposed to phenoxy herbicides, chlorophenols, and dioxins: two nested case-control studies. *Epidemiology*, 6(4):396–402. doi:10.1097/00001648-199507000-00012 PMID:7548348

Kohli JD, Khanna RN, Gupta BN, Dhar MM, Tandon JS, Sircar KP (1974). Absorption and excretion of 2,4-dichlorophenoxyacetic acid in man. *Xenobiotica*, 4(2):97–100. doi:10.3109/00498257409049349 PMID:4828800

Kolmodin-Hedman B, Erne K (1980). Estimation of occupational exposure to phenoxy acids (2,4-D and 2,4,5-T). *Arch Toxicol Suppl*, 4:318–21. doi:10.1007/978-3-642-67729-8_65 PMID:6933926

Kolmodin-Hedman B, Höglund S, Akerblom M (1983). Studies on phenoxy acid herbicides. I. Field study. Occupational exposure to phenoxy acid herbicides (MCPA, dichlorprop, mecoprop and 2,4-D) in agriculture. *Arch Toxicol*, 54(4):257–65. doi:10.1007/BF01234478 PMID:6667116

Konstantinou IK, Hela DG, Albanis TA (2006). The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. *Environ Pollut*, 141(3):555–70. doi:10.1016/j.envpol.2005.07.024 PMID:16226830

Kornuta N, Bagley E, Nedopitanskaya N (1996). Genotoxic effects of pesticides. *J Environ Pathol Toxicol Oncol*, 15(2–4):75–8. PMID:9216788

Korte C, Jalal SM (1982). 2,4-D induced clastogenicity and elevated rates of sister chromatid exchanges in cultured human lymphocytes. *J Hered*, 73(3):224–6. PMID:7096985

Koutros S, Beane Freeman LE, Lubin JH, Heltshe SL, Andreotti G, Barry KH et al. (2013). Risk of total and aggressive prostate cancer and pesticide use in the Agricultural Health Study. *Am J Epidemiol*, 177(1):59–74. doi:10.1093/aje/kws225 PMID:23171882

Kumari TS, Vaidyanath K (1989). Testing of genotoxic effects of 2,4-dichlorophenoxyacetic acid (2,4-D) using multiple genetic assay systems of plants. *Mutat Res*, 226(4):235–8. doi:10.1016/0165-7992(89)90075-4 PMID:2761564

Lamb JC 4th, Marks TA, Gladen BC, Allen JW, Moore JA (1981). Male fertility, sister chromatid exchange, and germ cell toxicity following exposure to mixtures of chlorinated phenoxy acids containing 2,3,7,8-tetrachlorodibenzo-p-dioxin. *J Toxicol Environ Health*, 8(5–6):825–34. doi:10.1080/15287398109530118 PMID:7338944

Lavy TL, Mattice JD, Marx DB, Norris LA (1987). Exposure of forestry ground workers to 2,4-D, picloram and dichlorprop. *Environ Toxicol Chem*, 6(3):209–24. doi:10.1002/etc.5620060306

Lavy TL, Walstad JD, Flynn RR, Mattice JD (1982). (2,4-Dichlorophenoxy)acetic acid exposure received by aerial application crews during forest spray operations. *J Agric Food Chem*, 30(2):375–81. doi:10.1021/jf00110a042

Lee K, Johnson VJ, Blakley BR (2000). The effect of exposure to a commercial 2,4-D herbicide formulation during gestation on urethan-induced lung adenoma formation in CD-1 mice. *Vet Hum Toxicol*, 42(3):129–32. PMID:10839313

Lee K, Johnson VL, Blakley BR (2001). The effect of exposure to a commercial 2,4-D formulation during gestation on the immune response in CD-1 mice. *Toxicology*, 165(1):39–49. doi:10.1016/S0300-483X(01)00403-6 PMID:11551430

Lee WJ, Lijinsky W, Heineman EF, Markin RS, Weisenburger DD, Ward MH (2004). Agricultural pesticide use and adenocarcinomas of the stomach and oesophagus. *Occup Environ Med*, 61(9):743–9. doi:10.1136/oem.2003.011858 PMID:15317914

Leljak-Levanić D, Bauer N, Mihaljević S, Jelaska S (2004). Changes in DNA methylation during somatic embryogenesis in Cucurbita pepo L. *Plant Cell Rep*, 23(3):120–7. doi:10.1007/s00299-004-0819-6 PMID:15221278

Leonard C, Burke CM, O'Keane C, Doyle JS (1997). "Golf ball liver": agent orange hepatitis. *Gut*, 40(5):687–8. doi:10.1136/gut.40.5.687 PMID:9203952

Lewis RC, Cantonwine DE, Anzalota Del Toro LV, Calafat AM, Valentin-Blasini L, Davis MD et al. (2014). Urinary biomarkers of exposure to insecticides, herbicides, and one insect repellent among pregnant women in Puerto Rico. *Environ Health*, 13(1):97 doi:10.1186/1476-069X-13-97 PMID:25409771

Libich S, To JC, Frank R, Sirons GJ (1984). Occupational exposure of herbicide applicators to herbicides used along electric power transmission line right-of-way. *Am Ind Hyg Assoc J*, 45(1):56–62. doi:10.1080/15298668491399370 PMID:6702600

Lindh CH, Littorin M, Amilon A, Jönsson BA (2008). Analysis of phenoxyacetic acid herbicides as biomarkers in human urine using liquid chromatography/triple quadrupole mass spectrometry. *Rapid Commun*

Mass Spectrom, 22(2):143–50. doi:10.1002/rcm.3348 PMID:18059043

Linnainmaa K (1983). Sister chromatid exchanges among workers occupationally exposed to phenoxy acid herbicides 2,4-D and MCPA. *Teratog Carcinog Mutagen*, 3(3):269–79. doi:10.1002/1520-6866(1990)3:3<269::AID-TCM1770030306>3.0.CO;2-F PMID:6137083

Linnainmaa K (1984). Induction of sister chromatid exchanges by the peroxisome proliferators 2,4-D, MCPA, and clofibrate in vivo and in vitro. *Carcinogenesis*, 5(6):703–7. doi:10.1093/carcin/5.6.703 PMID:6722984

Liu W, Li H, Tao F, Li S, Tian Z, Xie H (2013). Formation and contamination of PCDD/Fs, PCBs, PeCBz, HxCBz and polychlorophenols in the production of 2,4-D products. *Chemosphere*, 92(3):304–8. doi:10.1016/j.chemosphere.2013.03.031 PMID:23601123

Loos R, Gawlik BM, Locoro G, Rimaviciute E, Contini S, Bidoglio G (2009). EU-wide survey of polar organic persistent pollutants in European river waters. *Environ Pollut*, 157(2):561–8. doi:10.1016/j.envpol.2008.09.020 PMID:18952330

Loos R, Locoro G, Comero S, Contini S, Schwesig D, Werres F et al. (2010a). Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water. *Water Res*, 44(14):4115–26. doi:10.1016/j.watres.2010.05.032 PMID:20554303

Loos R, Locoro G, Contini S (2010b). Occurrence of polar organic contaminants in the dissolved water phase of the Danube River and its major tributaries using SPE-LC-MS(2) analysis. *Water Res*, 44(7):2325–35. doi:10.1016/j.watres.2009.12.035 PMID:20074769

Lundgren B, Meijer J, DePierre JW (1987). Induction of cytosolic and microsomal epoxide hydrolases and proliferation of peroxisomes and mitochondria in mouse liver after dietary exposure to p-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. *Biochem Pharmacol*, 36(6):815–21. doi:10.1016/0006-2952(87)90169-9 PMID:3032197

Lynge E (1985). A follow-up study of cancer incidence among workers in manufacture of phenoxy herbicides in Denmark. *Br J Cancer*, 52(2):259–70. doi:10.1038/bjc.1985.186 PMID:4027168

Lynge E (1998). Cancer incidence in Danish phenoxy herbicide workers, 1947–1993. *Environ Health Perspect*, 106(Suppl 2):683–8. doi:10.1289/ehp.98106683 PMID:9599717

Madrigal-Bujaidar E, Hernández-Ceruelos A, Chamorro G (2001). Induction of sister chromatid exchanges by 2,4-dichlorophenoxyacetic acid in somatic and germ cells of mice exposed in vivo. *Food Chem Toxicol*, 39(9):941–6. doi:10.1016/S0278-6915(01)00037-0 PMID:11498271

Maire MA, Rast C, Landkocz Y, Vasseur P (2007). 2,4-Dichlorophenoxyacetic acid: effects on Syrian hamster embryo (SHE) cell transformation, c-Myc expression, DNA damage and apoptosis. *Mutat Res*, 631(2):124–36. doi:10.1016/j.mrgentox.2007.03.008 PMID:17540612

Maloney EK, Waxman DJ (1999). trans-Activation of PPARalpha and PPARgamma by structurally diverse environmental chemicals. *Toxicol Appl Pharmacol*, 161(2):209–18. doi:10.1006/taap.1999.8809 PMID:10581215

Malysheva LN, Zhavoronkov AA (1997). Morphological and histochemical changes in the thyroid gland after a single exposure to 2,4-DA herbicide. *Bull Exp Biol Med*, 124(6):1223–4. doi:10.1007/BF02445126

Martin MT, Judson RS, Reif DM, Kavlock RJ, Dix DJ (2009). Profiling chemicals based on chronic toxicity results from the U.S. EPA ToxRef Database. *Environ Health Perspect*, 117(3):392–9. doi:10.1289/ehp.0800074 PMID:19337514

Martínez-Tabche L, Madrigal-Bujaidar E, Negrete T (2004). Genotoxicity and lipoperoxidation produced by paraquat and 2,4-dichlorophenoxyacetic acid in the gills of rainbow trout (Oncorhynchus mikiss). *Bull Environ Contam Toxicol*, 73(1):146–52. doi:10.1007/s00128-004-0406-0 PMID:15386085

Marty MS, Neal BH, Zablotny CL, Yano BL, Andrus AK, Woolhiser MR et al. (2013). An F1-extended one-generation reproductive toxicity study in Crl:CD(SD) rats with 2,4-dichlorophenoxyacetic acid. *Toxicol Sci*, 136(2):527–47. doi:10.1093/toxsci/kft213 PMID:24072463

Mason RW (1975). Binding of some phenoxyalkanoic acids to bovine serum albumin in vitro. *Pharmacology*, 13(2):177–86. doi:10.1159/000136898 PMID:1170578

Mazhar FM, Moawad KM, El-Dakdoky MH, Am AS (2014). Fetotoxicity of 2,4-dichlorophenoxyacetic acid in rats and the protective role of vitamin E. *Toxicol Ind Health*, 30(5):480–8. doi:10.1177/0748233712459915 PMID:22949405

McDuffie HH, Pahwa P, McLaughlin JR, Spinelli JJ, Fincham S, Dosman JA et al. (2001). Non-Hodgkin's lymphoma and specific pesticide exposures in men: cross-Canada study of pesticides and health. *Cancer Epidemiol Biomarkers Prev*, 10(11):1155–63. PMID:11700263

McDuffie HH, Pahwa P, Robson D, Dosman JA, Fincham S, Spinelli JJ et al. (2005). Insect repellents, phenoxyherbicide exposure, and non-Hodgkin's lymphoma. *J Occup Environ Med*, 47(8):806–16. PMID:16093930

McManus SL, Moloney M, Richards KG, Coxon CE, Danaher M (2014). Determination and occurrence of phenoxyacetic acid herbicides and their transformation products in groundwater using ultra high performance liquid chromatography coupled to tandem mass spectrometry. *Molecules*, 19(12):20627–49. doi:10.3390/molecules191220627 PMID:25514054

Mehmood Z, Williamson MP, Kelly DE, Kelly SL (1996). Human cytochrome P450 3A4 is involved in the biotransformation of the herbicide 2,4-dichlorophenoxyacetic acid. *Environ Toxicol Pharmacol*, 2(4):397–401. doi:10.1016/S1382-6689(96)00077-4 PMID:21781748

Metayer C, Colt JS, Buffler PA, Reed HD, Selvin S, Crouse V et al. (2013). Exposure to herbicides in house dust and risk of childhood acute lymphoblastic leukemia. *J Expo Sci Environ Epidemiol*, 23(4):363–70. doi:10.1038/jes.2012.115 PMID:23321862

Miassod R, Cecchini JP (1979). Partial base-methylation and other structural differences in the 17 S ribosomal RNA of sycamore cells during growth in cell culture. *Biochim Biophys Acta*, 562(2):292–301. doi:10.1016/0005-2787(79)90174-6 PMID:444529

Miligi L, Costantini AS, Bolejack V, Veraldi A, Benvenuti A, Nanni O et al. (2003). Non-Hodgkin's lymphoma, leukemia, and exposures in agriculture: results from the Italian multicenter case-control study. *Am J Ind Med*, 44(6):627–36. doi:10.1002/ajim.10289 PMID:14635239

Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P (2006b). Living in a Chemical World: Framing the Future in Light of the Past. Volume 1076. Oxford, UK: Blackwell Publishing. pp. 366–377.

Miligi L, Costantini AS, Veraldi A, Benvenuti A, Vineis P; WILL (2006a). Cancer and pesticides: an overview and some results of the Italian multicenter case-control study on hematolymphopoietic malignancies. *Ann N Y Acad Sci*, 1076(1):366–77. doi:10.1196/annals.1371.036 PMID:17119216

Mills PK, Yang R, Riordan D (2005). Lymphohematopoietic cancers in the United Farm Workers of America (UFW), 1988–2001. *Cancer Causes Control*, 16(7):823–30. doi:10.1007/s10552-005-2703-2 PMID:16132792

Mills PK, Yang RC (2007). Agricultural exposures and gastric cancer risk in Hispanic farm workers in California. *Environ Res*, 104(2):282–9. doi:10.1016/j.envres.2006.11.008 PMID:17196584

Mohandas T, Grant WF (1972). Cytogenetic effects of 2,4-D and amitrole in relation to nuclear volume and DNA content in some higher plants. *Can J Genet Cytol*, 14(4):773–83. doi:10.1139/g72-095

Moody RP, Franklin CA, Ritter L, Maibach HI (1990). Dermal absorption of the phenoxy herbicides 2,4-D, 2,4-D amine, 2,4-D isooctyl, and 2,4,5-T in rabbits, rats, rhesus monkeys, and humans: a cross-species comparison. *J Toxicol Environ Health*, 29(3):237–45. doi:10.1080/15287399009531387 PMID:2313737

Moody RP, Wester RC, Melendres JL, Maibach HI (1992). Dermal absorption of the phenoxy herbicide 2,4-D dimethylamine in humans: effect of DEET and anatomic site. *J Toxicol Environ Health*, 36(3):241–50. doi:10.1080/15287399209531635 PMID:1629935

Morgan MK, Sheldon LS, Croghan CW, Chuang JC, Lordo RA, Wilson NK, et al. (2004). A pilot study of children's total exposure to persistent pesticides and other persistent organic pollutants (CTEPP). United States Environmental Protection Agency. EPA/600/R-041/193.

Morgan MK, Sheldon LS, Thomas KW, Egeghy PP, Croghan CW, Jones PA et al. (2008). Adult and children's exposure to 2,4-D from multiple sources and pathways. *J Expo Sci Environ Epidemiol*, 18(5):486–94. doi:10.1038/sj.jes.7500641 PMID:18167507

Morgan MK, Wilson NK, Chuang JC (2014). Exposures of 129 preschool children to organochlorines, organophosphates, pyrethroids, and acid herbicides at their homes and daycares in North Carolina. *Int J Environ Res Public Health*, 11(4):3743–64. doi:10.3390/ijerph110403743 PMID:24705361

Moriya M, Ohta T, Watanabe K, Miyazawa T, Kato K, Shirasu Y (1983). Further mutagenicity studies on pesticides in bacterial reversion assay systems. *Mutat Res*, 116(3–4):185–216. doi:10.1016/0165-1218(83)90059-9 PMID:6339892

Mortelmans K, Haworth S, Speck W, Zeiger E (1984). Mutagenicity testing of agent orange components and related chemicals. *Toxicol Appl Pharmacol*, 75(1):137–46. doi:10.1016/0041-008X(84)90084-X PMID:6379990

Mufazalova NA, Medvedev IuA, Basyrova NK (2001). [Corrective effects of tocopherol on changes in indices of the protective activity of phagocytes under the action of the herbicide 2,4-DA] *Gig Sanit*, (6):61–3. PMID:11810914

Murata M (1989). Effects of auxin and cytokinin on induction of sister chromatid exchanges in cultured cells of wheat (Triticum aestivum L.). *Theor Appl Genet*, 78(4):521–4. doi:10.1007/BF00290836 PMID:24225679

Mustonen R, Kangas J, Vuojolahti P, Linnainmaa K (1986). Effects of phenoxyacetic acids on the induction of chromosome aberrations in vitro and in vivo. *Mutagenesis*, 1(4):241–5. doi:10.1093/mutage/1.4.241 PMID:3331666

Nakbi A, Tayeb W, Grissa A, Issaoui M, Dabbou S, Chargui I et al. (2010). Effects of olive oil and its fractions on oxidative stress and the liver's fatty acid composition in 2,4-Dichlorophenoxyacetic acid-treated rats. *Nutr Metab (Lond)*, 7(1):80 doi:10.1186/1743-7075-7-80 PMID:21034436

Navaranjan G, Hohenadel K, Blair A, Demers PA, Spinelli JJ, Pahwa P et al. (2013). Exposures to multiple pesticides and the risk of Hodgkin lymphoma in Canadian men. *Cancer Causes Control*, 24(9):1661–73. doi:10.1007/s10552-013-0240-y PMID:23756639

NCBI (2015). Compound summary for CID 1486. 2,4-dichlorophenoxyacetic acid. PubChem Open Chemistry Database. Bethesda (MD): National Center for Biotechnology Information. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/1486#section=3D-Conformer, accessed 5 March 2015.

NIOSH (1994). 2,4-D. Method 5001, Issue 2, dated 15 August 1994. In: NIOSH Manual of Analytical Methods, Fourth Edition. Atlanta (GA): National Institute for Occupational Safety and Health, Centers for Disease Control and Prevention. Available from: http://www.cdc.gov/niosh/docs/2003-154/pdfs/500124-d.pdf. Accessed May 27, 2015.

Nishioka MG, Lewis RG, Brinkman MC, Burkholder HM, Hines CE, Menkedick JR (2001). Distribution of 2,4-D in air and on surfaces inside residences after lawn applications: comparing exposure estimates from various media for young children. *Environ Health Perspect*, 109(11):1185–91. doi:10.1289/ehp.011091185 PMID:11713005

Nordström M, Hardell L, Magnuson A, Hagberg H, Rask-Andersen A (1998). Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. *Br J Cancer*, 77(11):2048–52. doi:10.1038/bjc.1998.341 PMID:9667691

Nozaki Y, Kusuhara H, Kondo T, Hasegawa M, Shiroyanagi Y, Nakazawa H et al. (2007). Characterization of the uptake of organic anion transporter (OAT) 1 and OAT3 substrates by human kidney slices. *J Pharmacol Exp Ther*, 321(1):362–9. doi:10.1124/jpet.106.113076 PMID:17255469

NPIC (2008). 2,4-D Technical Fact Sheet. National Pesticide Information Center, Oregon State University. Available from: http://npic.orst.edu/factsheets/archive/2,4-DTech.html, accessed 18 February 2016.

NTIS (1968). Evaluation of carcinogenic, teratogenic and mutagenic activities of selected pesticides and industrial chemicals. Volume I. Carcinogenic study. Prepared by Bionetics Research Labs, Incorporated. Report No. PB 223 159, Washington (DC): National Technical Information Service, United States Department of Commerce. Available from: http://www.nal.usda.gov/exhibits/speccoll/files/original/81a28fea12a8e39a2c7fa354bf29737d.pdf.

Oakes DJ, Webster WS, Brown-Woodman PD, Ritchie HE (2002). Testicular changes induced by chronic exposure to the herbicide formulation, Tordon 75D (2,4-dichlorophenoxyacetic acid and picloram) in rats. *Reprod Toxicol*, 16(3):281–9. doi:10.1016/S0890-6238(02)00015-1 PMID:12128102

Örberg J (1980). Observations on the 2,4-dichlorophenoxyacetic acid (2,4-D) excretion in the goat. *Acta Pharmacol Toxicol (Copenh)*, 46(1):78–80. doi:10.1111/j.1600-0773.1980.tb02424.x PMID:7361563

Orsi L, Delabre L, Monnereau A, Delval P, Berthou C, Fenaux P et al. (2009). Occupational exposure to pesticides and lymphoid neoplasms among men: results of a French case-control study. *Occup Environ Med*, 66(5):291–8. doi:10.1136/oem.2008.040972 PMID:19017688

OSHA (2015). 2,4-D. Chemical sampling information. Washington (DC): Occupational Safety and Health Administration, United States Department of Labor. Available from: https://www.osha.gov/dts/chemicalsampling/data/CH_231150.html.

Ozaki K, Mahler JF, Haseman JK, Moomaw CR, Nicolette ML, Nyska A (2001). Unique renal tubule changes induced in rats and mice by the peroxisome proliferators 2,4-dichlorophenoxyacetic acid (2,4-D) and WY-14643. *Toxicol Pathol*, 29(4):440–50. doi:10.1080/01926230152499791 PMID:11560249

Ozcan Oruc E, Sevgiler Y, Uner N (2004). Tissue-specific oxidative stress responses in fish exposed to 2,4-D and azinphosmethyl. *Comp Biochem Physiol C Toxicol Pharmacol*, 137(1):43–51. doi:10.1016/j.cca.2003.11.006 PMID:14984703

Pahwa M, Harris SA, Hohenadel K, McLaughlin JR, Spinelli JJ, Pahwa P et al. (2012). Pesticide use, immunologic conditions, and risk of non-Hodgkin lymphoma in Canadian men in six provinces. *Int J Cancer*, 131(11):2650–9. doi:10.1002/ijc.27522 PMID:22396152

Pahwa P, McDuffie HH, Dosman JA, McLaughlin JR, Spinelli JJ, Robson D et al. (2006). Hodgkin lymphoma, multiple myeloma, soft tissue sarcomas, insect repellents, and phenoxyherbicides. *J Occup Environ Med*, 48(3):264–74. doi:10.1097/01.jom.0000183539.20100.06 PMID:16531830

Palmeira CM, Moreno AJ, Madeira VMC (1995). Thiols metabolism is altered by the herbicides paraquat, dinoseb and 2,4-D: a study in isolated hepatocytes. *Toxicol Lett*, 81(2–3):115–23. doi:10.1016/0378-4274(95)03414-5 PMID:8553365

Panuwet P, Prapamontol T, Chantara S, Barr DB (2009). Urinary pesticide metabolites in school students from northern Thailand. *Int J Hyg Environ Health*, 212(3):288–97. doi:10.1016/j.ijheh.2008.07.002 PMID:18760967

Panuwet P, Prapamontol T, Chantara S, Thavornyuthikarn P, Montesano MA, Whitehead RD Jr et al. (2008). Concentrations of urinary pesticide metabolites in small-scale farmers in Chiang Mai Province, Thailand. *Sci Total Environ*, 407(1):655–68. doi:10.1016/j.scitotenv.2008.08.044 PMID:18954893

Pavlica M, Papes D, Nagy B (1991). 2,4-Dichlorophenoxyacetic acid causes chromatin and chromosome abnormalities in plant cells and mutation in cultured mammalian cells. *Mutat Res*, 263(2):77–81. doi:10.1016/0165-7992(91)90063-A PMID:2046706

Pearce N (1989). Phenoxy herbicides and non-Hodgkin's lymphoma in New Zealand: frequency and duration of herbicide use. *Br J Ind Med*, 46(2):143–4. PMID:2923826

Pearce NE, Sheppard RA, Smith AH, Teague CA (1987). Non-Hodgkin's lymphoma and farming: an expanded case-control study. *Int J Cancer*, 39(2):155–61. doi:10.1002/ijc.2910390206 PMID:3804490

Pearce NE, Smith AH, Howard JK, Sheppard RA, Giles HJ, Teague CA (1986a). Case-control study of multiple

myeloma and farming. *Br J Cancer*, 54(3):493–500. doi:10.1038/bjc.1986.202 PMID:3756085

Pearce NE, Smith AH, Howard JK, Sheppard RA, Giles HJ, Teague CA (1986b). Non-Hodgkin's lymphoma and exposure to phenoxyherbicides, chlorophenols, fencing work, and meat works employment: a case-control study. *Br J Ind Med*, 43(2):75–83. PMID:3753879

Pelletier O, Ritter L, Caron J (1990). Effects of skin preapplication treatments and postapplication cleansing agents on dermal absorption of 2,4-dichlorophenoxyacetic acid dimethylamine by Fischer 344 rats. *J Toxicol Environ Health*, 31(4):247–60. doi:10.1080/15287399009531454 PMID:2254951

Pelletier O, Ritter L, Caron J, Somers D (1989). Disposition of 2,4-dichlorophenoxyacetic acid dimethylamine by Fischer 344 rats dosed orally and dermally. *J Toxicol Environ Health*, 28(2):221–34. doi:10.1080/15287398909531342 PMID:2795703

Persson B, Dahlander AM, Fredriksson M, Brage HN, Ohlson CG, Axelson O (1989). Malignant lymphomas and occupational exposures. *Br J Ind Med*, 46(8):516–20. PMID:2775671

Pest Management Regulatory Agency (Canada) (2013). Re-evaluation update 2,4-D REV2013–02. Ottawa. Available from: http://www.hc-sc.gc.ca/cps-spc/alt_formats/pdf/pubs/pest/decisions/rev2013-02/rev2013-02-eng.pdf.

Phillips PJ, Bode RW (2004). Pesticides in surface water runoff in south-eastern New York State, USA: seasonal and stormflow effects on concentrations. *Pest Manag Sci*, 60(6):531–43. doi:10.1002/ps.879 PMID:15198325

Pilinskaia MA (1974). [The cytogenetic effect of herbicide 2,4-D on human and animal chromosomes]. *Tsitol Genet*, 8(3):202–6. PMID:4464590

Pineau T, Hudgins WR, Liu L, Chen LC, Sher T, Gonzalez FJ et al. (1996). Activation of a human peroxisome proliferator-activated receptor by the antitumor agent phenylacetate and its analogs. *Biochem Pharmacol*, 52(4):659–67. doi:10.1016/0006-2952(96)00340-1 PMID:8759039

Pochettino AA, Bongiovanni B, Duffard RO, Evangelista de Duffard AM (2013). Oxidative stress in ventral prostate, ovary, and breast by 2,4-dichlorophenoxyacetic acid in pre- and postnatal exposed rats. *Environ Toxicol*, 28(1):1–10. doi:10.1002/tox.20690 PMID:21374790

Pont AR, Charron AR, Wilson RM, Brand RM (2003). Effects of active sunscreen ingredient combinations on the topical penetration of the herbicide 2,4-dichlorophenoxyacetic acid. *Toxicol Ind Health*, 19(1):1–8. doi:10.1191/0748233703th172oa PMID:15462531

Pritchard JB (1980). Accumulation of anionic pesticides by rabbit choroid plexus in vitro. *J Pharmacol Exp Ther*, 212(2):354–9. PMID:7351648

PubChem (2015). PubChem Open Chemistry Database. Bethesda (MD): National Center for Biotechnology Information. Available from: http://pubchem.ncbi.nlm.nih.gov, accessed November 2015.

Purcell M, Neault JF, Malonga H, Arakawa H, Carpentier R, Tajmir-Riahi HA (2001). Interactions of atrazine and 2,4-D with human serum albumin studied by gel and capillary electrophoresis, and FTIR spectroscopy. *Biochim Biophys Acta*, 1548(1):129–38. doi:10.1016/S0167-4838(01)00229-1 PMID:11451446

Raftopoulou EK, Dailianis S, Dimitriadis VK, Kaloyianni M (2006). Introduction of cAMP and establishment of neutral lipids alterations as pollution biomarkers using the mussel Mytilus galloprovincialis. Correlation with a battery of biomarkers. *Sci Total Environ*, 368(2–3):597–614. doi:10.1016/j.scitotenv.2006.04.031 PMID:16780930

Raina-Fulton R (2014). A review of methods for the analysis of orphan and difficult pesticides: glyphosate, glufosinate, quaternary ammonium and phenoxy acid herbicides, and dithiocarbamate and phthalimide fungicides. *J AOAC Int*, 97(4):965–77. doi:10.5740/jaoacint.SGERaina-Fulton PMID:25145125

Raymer JH, Studabaker WB, Gardner M, Talton J, Quandt SA, Chen H et al. (2014). Pesticide exposures to migrant farmworkers in Eastern NC: detection of metabolites in farmworker urine associated with housing violations and camp characteristics. *Am J Ind Med*, 57(3):323–37. doi:10.1002/ajim.22284 PMID:24273087

Reif DM, Martin MT, Tan SW, Houck KA, Judson RS, Richard AM et al. (2010). Endocrine profiling and prioritization of environmental chemicals using ToxCast data. *Environ Health Perspect*, 118(12):1714–20. doi:10.1289/ehp.1002180 PMID:20826373

Reif DM, Sypa M, Lock EF, Wright FA, Wilson A, Cathey T et al. (2013). ToxPi GUI: an interactive visualization tool for transparent integration of data from diverse sources of evidence. *Bioinformatics*, 29(3):402–3. doi:10.1093/bioinformatics/bts686 PMID:23202747

Rivarola V, Mori G, Balegno H (1992). 2,4-Dichlorophenoxyacetic acid action on in vitro protein synthesis and its relation to polyamines. *Drug Chem Toxicol*, 15(3):245–57. doi:10.3109/01480549209014154 PMID:1425363

Rivarola VA, Balegno HF (1991). Effects of 2,4-dichlorophenoxyacetic acid on polyamine synthesis in Chinese hamster ovary cells. *Toxicol Lett*, 56(1–2):151–7. doi:10.1016/0378-4274(91)90101-B PMID:2017772

Rivarola VA, Bergesse JR, Balegno HF (1985). DNA and protein synthesis inhibition in Chinese hamster ovary cells by dichlorophenoxyacetic acid. *Toxicol Lett*, 29(2–3):137–44. doi:10.1016/0378-4274(85)90034-7 PMID:4089882

Rodríguez T, van Wendel de Joode B, Lindh CH, Rojas M, Lundberg I, Wesseling C (2012). Assessment of long-term and recent pesticide exposure among rural school children in Nicaragua. *Occup Environ Med*, 69(2):119–25. doi:10.1136/oem.2010.062539 PMID:21725072

Ross JH, Driver JH, Harris SA, Maibach HI (2005). Dermal absorption of 2,4-D: a review of species differences. *Regul Toxicol Pharmacol*, 41(1):82–91. doi:10.1016/j.yrtph.2004.10.001 PMID:15649830

Rosso SB, Gonzalez M, Bagatolli LA, Duffard RO, Fidelio GD (1998). Evidence of a strong interaction of 2,4-dichlorophenoxyacetic acid herbicide with human serum albumin. *Life Sci*, 63(26):2343–51. doi:10.1016/S0024-3205(98)00523-2 PMID:9877224

Saghir SA, Marty MS, Zablotny CL, Passage JK, Perala AW, Neal BH et al. (2013). Life-stage-, sex-, and dose-dependent dietary toxicokinetics and relationship to toxicity of 2,4-dichlorophenoxyacetic acid (2,4-D) in rats: implications for toxicity test dose selection, design, and interpretation. *Toxicol Sci*, 136(2):294–307. doi:10.1093/toxsci/kft212 PMID:24105888

Saghir SA, Mendrala AL, Bartels MJ, Day SJ, Hansen SC, Sushynski JM et al. (2006). Strategies to assess systemic exposure of chemicals in subchronic/chronic diet and drinking water studies. *Toxicol Appl Pharmacol*, 211(3):245–60. doi:10.1016/j.taap.2005.06.010 PMID:16040073

Salazar KD, de la Rosa P, Barnett JB, Schafer R (2005). The polysaccharide antibody response after Streptococcus pneumoniae vaccination is differentially enhanced or suppressed by 3,4-dichloropropionanilide and 2,4-dichlorophenoxyacetic acid. *Toxicol Sci*, 87(1):123–33. doi:10.1093/toxsci/kfi244 PMID:15976183

Sandal S, Yilmaz B (2011). Genotoxic effects of chlorpyrifos, cypermethrin, endosulfan and 2,4-D on human peripheral lymphocytes cultured from smokers and nonsmokers. *Environ Toxicol*, 26(5):433–42. doi:10.1002/tox.20569 PMID:20196147

Sandberg JA, Duhart HM, Lipe G, Binienda Z, Slikker W Jr, Kim CS (1996). Distribution of 2,4-dichlorophenoxyacetic acid (2,4-D) in maternal and fetal rabbits. *J Toxicol Environ Health*, 49(5):497–509. doi:10.1080/009841096160718 PMID:8968410

Santilio A, Stefanelli P, Girolimetti S, Dommarco R (2011). Determination of acidic herbicides in cereals by QuEChERS extraction and LC/MS/MS. *J Environ Sci Health B*, 46(6):535–43. PMID:21726153

Sapin MR, Lebedeva SN, Zhamsaranova SD, Erofeeva LM (2003). [Comparative analysis of disorders in duodenal lymphoid tissue of mice treated with azathioprine and herbicide 2,4-dichlorophenoxyacetic acid and their correction by plant and animal origin remedies] *Morfologiia*, 124(4):70–3. PMID:14628561

Saracci R, Kogevinas M, Bertazzi PA, Bueno de Mesquita BH, Coggon D, Green LM et al. (1991). Cancer mortality in workers exposed to chlorophenoxy herbicides and chlorophenols. *Lancet*, 338(8774):1027–32. doi:10.1016/0140-6736(91)91898-5 PMID:1681353

Sauerhoff MW, Braun WH, Blau GE, Gehring PJ (1977). The fate of 2,4-dichlorophenoxyacetic acid (2,4-D) following oral administration to man. *Toxicology*, 8(1):3–11. doi:10.1016/0300-483X(77)90018-X PMID:929615

Schaner A, Konecny J, Luckey L, Hickes H (2007). Determination of chlorinated acid herbicides in vegetation and soil by liquid chromatography/electrospray-tandem mass spectrometry. *J AOAC Int*, 90(5):1402–10. PMID:17955986

Schinasi L, Leon ME (2014). Non-Hodgkin lymphoma and occupational exposure to agricultural pesticide chemical groups and active ingredients: a systematic review and meta-analysis. *Int J Environ Res Public Health*, 11(4):4449–527. doi:10.3390/ijerph110404449 PMID:24762670

Schop RN, Hardy MH, Goldberg MT (1990). Comparison of the activity of topically applied pesticides and the herbicide 2,4-D in two short-term in vivo assays of genotoxicity in the mouse. *Fundam Appl Toxicol*, 15(4):666–75. doi:10.1016/0272-0590(90)90183-K PMID:2086312

Schulze G (1991). Subchronic Toxicity Study in Rats with 2,4-Di- chlorophenoxyacetic Acid. Lab Project Number: 2184-116. Unpublished study prepared by Hazleton Laboratories America, 529 p.

Shida SS, Nemoto S, Matsuda R (2015). Simultaneous determination of acidic pesticides in vegetables and fruits by liquid chromatography–tandem mass spectrometry. *J Environ Sci Health B*, 50(3):151–62. doi:10.1080/03601234.2015.982381 PMID:25602148

Shirasu Y, Moriya M, Kato K, Furuhashi A, Kada T (1976). Mutagenicity screening of pesticides in the microbial system. *Mutat Res*, 40(1):19–30. doi:10.1016/0165-1218(76)90018-5 PMID:814455

Siebert D, Lemperle E (1974). Genetic effects of herbicides: induction of mitotic gene conversion in Saccharomyces cerevisiae. *Mutat Res*, 22(2):111–20. doi:10.1016/0027-5107(74)90090-6 PMID:4601758

Sipes NS, Martin MT, Kothiya P, Reif DM, Judson RS, Richard AM et al. (2013). Profiling 976 ToxCast chemicals across 331 enzymatic and receptor signalling assays. *Chem Res Toxicol*, 26(6):878–95. doi:10.1021/tx400021f PMID:23611293

Smith MT, Guyton KZ, Gibbons CF, Fritz JM, Portier CJ, Rusyn I et al. (2016). Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis. *Environ Health Perspect*, 124(6):713–21. PMID:26600562

Smith AH, Pearce NE, Fisher DO, Giles HJ, Teague CA, Howard JK (1984). Soft tissue sarcoma and exposure to phenoxyherbicides and chlorophenols in New Zealand. *J Natl Cancer Inst*, 73(5):1111–7. PMID:6593487

Smith JG, Christophers AJ (1992). Phenoxy herbicides and chlorophenols: a case control study on soft tissue sarcoma and malignant lymphoma. *Br J Cancer*, 65(3):442–8. doi:10.1038/bjc.1992.90 PMID:1558802

Soloneski S, González NV, Reigosa MA, Larramendy ML (2007). Herbicide 2,4-dichlorophenoxyacetic

acid (2,4-D)-induced cytogenetic damage in human lymphocytes in vitro in presence of erythrocytes. *Cell Biol Int*, 31(11):1316–22. doi:10.1016/j.cellbi.2007.05.003 PMID:17606385

Song Y (2014). Insight into the mode of action of 2,4-dichlorophenoxyacetic acid (2,4-D) as an herbicide. *J Integr Plant Biol*, 56(2):106–13. doi:10.1111/jipb.12131 PMID:24237670

Sorensen KC, Stucki JW, Warner RE, Wagner ED, Plewa MJ (2005). Modulation of the genotoxicity of pesticides reacted with redox-modified smectite clay. *Environ Mol Mutagen*, 46(3):174–81. doi:10.1002/em.20144 PMID:15920753

Sreekumaran Nair R, Paulmurugan R, Ranjit Singh AJA (2002). Simple radioactive assay for the estimation of DNA breaks. *J Appl Toxicol*, 22(1):19–23. doi:10.1002/jat.807 PMID:11807925

Straif K, Loomis D, Guyton K, Grosse Y, Lauby-Secretan B, El Ghissassi F et al. (2014). Future priorities for *IARC Monographs*. *Lancet Oncol*, 15(7):683–4.

Stürtz N, Bongiovanni B, Rassetto M, Ferri A, de Duffard AM, Duffard R (2006). Detection of 2,4-dichlorophenoxyacetic acid in rat milk of dams exposed during lactation and milk analysis of their major components. *Food Chem Toxicol*, 44(1):8–16. doi:10.1016/j.fct.2005.03.012 PMID:16216402

Stürtz N, Evangelista de Duffard AM, Duffard R (2000). Detection of 2,4-dichlorophenoxyacetic acid (2,4-D) residues in neonates breast-fed by 2,4-D exposed dams. *Neurotoxicology*, 21(1-2):147–54. PMID:10794394

Sun H, Si C, Bian Q, Chen X, Chen L, Wang X (2012). Developing in vitro reporter gene assays to assess the hormone receptor activities of chemicals frequently detected in drinking water. *J Appl Toxicol*, 32(8):635–41. doi:10.1002/jat.1790 PMID:22912978

Surjan A (1989). Analysis of genotoxic activity of 16 compounds and mixtures by the Drosophila mosaic test. *Ann Ist Super Sanita*, 25(4):569–72. PMID:2517189

't Mannetje A, McLean D, Cheng S, Boffetta P, Colin D, Pearce N (2005). Mortality in New Zealand workers exposed to phenoxy herbicides and dioxins. *Occup Environ Med*, 62(1):34–40. doi:10.1136/oem.2004.015776 PMID:15613606

Tagert MLM, Massey JH, Shaw DR (2014). Water quality survey of Mississippi's Upper Pearl River. *Sci Total Environ*, 481:564–73. doi:10.1016/j.scitotenv.2014.02.084 PMID:24631619

Tasker E (1985). A Dietary Two-Generation Reproduction Study in Fischer 344 Rats with 2,4-Dichlorophenoxyacetic Acid: Final Report. Project No. WIL-81137. Unpublished study prepared by Wil Research Laboratories, Inc. 1402 p.

Tayeb W, Nakbi A, Cheraief I, Miled A, Hammami M (2013). Alteration of lipid status and lipid metabolism, induction of oxidative stress and lipid peroxidation by 2,4-dichlorophenoxyacetic herbicide in rat liver. *Toxicol Mech Methods*, 23(6):449–58. doi:10.3109/15376516.2013.780275 PMID:23464821

Tayeb W, Nakbi A, Trabelsi M, Attia N, Miled A, Hammami M (2010). Hepatotoxicity induced by sub-acute exposure of rats to 2,4-Dichlorophenoxyacetic acid based herbicide "Désormone lourd". *J Hazard Mater*, 180(1–3):225–33. doi:10.1016/j.jhazmat.2010.04.018 PMID:20447766

Tayeb W, Nakbi A, Trabelsi M, Miled A, Hammami M (2012). Biochemical and histological evaluation of kidney damage after sub-acute exposure to 2,4-dichlorophenoxyacetic herbicide in rats: involvement of oxidative stress. *Toxicol Mech Methods*, 22(9):696–704. doi:10.3109/15376516.2012.717650 PMID:22894658

Teixeira MC, Telo JP, Duarte NF, Sá-Correia I (2004). The herbicide 2,4-dichlorophenoxyacetic acid induces the generation of free-radicals and associated oxidative stress responses in yeast. *Biochem Biophys Res Commun*, 324(3):1101–7. doi:10.1016/j.bbrc.2004.09.158 PMID:15485668

Thomas KW, Dosemeci M, Coble JB, Hoppin JA, Sheldon LS, Chapa G et al. (2010b). Assessment of a pesticide exposure intensity algorithm in the agricultural health study. *J Expo Sci Environ Epidemiol*, 20(6):559–69. doi:10.1038/jes.2009.54 PMID:19888312

Thomas KW, Dosemeci M, Hoppin JA, Sheldon LS, Croghan CW, Gordon SM et al. (2010a). Urinary biomarker, dermal, and air measurement results for 2,4-D and chlorpyrifos farm applicators in the Agricultural Health Study. *J Expo Sci Environ Epidemiol*, 20(2):119–34. doi:10.1038/jes.2009.6 PMID:19240759

Tice RR, Austin CP, Kavlock RJ, Bucher JR (2013). Improving the human hazard characterization of chemicals: a Tox21 update. *Environ Health Perspect*, 121(7):756–65. doi:10.1289/ehp.1205784 PMID:23603828

Timchalk C (2004). Comparative inter-species pharmacokinetics of phenoxyacetic acid herbicides and related organic acids. evidence that the dog is not a relevant species for evaluation of human health risk. *Toxicology*, 200(1):1–19. doi:10.1016/j.tox.2004.03.005 PMID:15158559

Tripathy NK, Routray PK, Sahu GP, Kumar AA (1993). Genotoxicity of 2,4-dichlorophenoxyacetic acid tested in somatic and germ-line cells of Drosophila. *Mutat Res*, 319(3):237–42. doi:10.1016/0165-1218(93)90083-P PMID:7694145

Troudi A, Ben Amara I, Samet AM, Zeghal N (2012). Oxidative stress induced by 2,4-phenoxyacetic acid in liver of female rats and their progeny: biochemical and histopathological studies. *Environ Toxicol*, 27(3):137–45. doi:10.1002/tox.20624 PMID:20607813

Tuschl H, Schwab C (2003). Cytotoxic effects of the herbicide 2,4-dichlorophenoxyacetic acid in HepG2 cells. *Food Chem Toxicol*, 41(3):385–93. doi:10.1016/S0278-6915(02)00238-7 PMID:12504171

Tuschl H, Schwab CE (2005). The use of flow cytometric methods in acute and long-term in vitro testing. *Toxicol In Vitro*, 19(7):845–52. doi:10.1016/j.tiv.2005.06.026 PMID:16081244

Tyynelä K, Elo HA, Ylitalo P (1990). Distribution of three common chlorophenoxyacetic acid herbicides into the rat brain. *Arch Toxicol*, 64(1):61–5. doi:10.1007/BF01973378 PMID:2306196

USGS (2006). Appendix 7A. Statistical summaries of pesticide compounds in stream water, 1992–2001. In: Pesticides in the nation's streams and ground water, 1992–2001. USGS Circular 1291. United States Geological Survey. Available from: http://water.usgs.gov/nawqa/pnsp/pubs/circ1291/appendix7/7a.html, accessed 26 May 2015.

Vainio H, Nickels J, Linnainmaa K (1982). Phenoxy acid herbicides cause peroxisome proliferation in Chinese hamsters. *Scand J Work Environ Health*, 8(1):70–3. doi:10.5271/sjweh.2494 PMID:7134925

Van den Berg KJ, van Raaij JAGM, Bragt PC, Notten WRF (1991). Interactions of halogenated industrial chemicals with transthyretin and effects on thyroid hormone levels in vivo. *Arch Toxicol*, 65(1):15–9. doi:10.1007/BF01973497 PMID:2043046

van Ravenzwaay B, Hardwick TD, Needham D, Pethen S, Lappin GJ (2003). Comparative metabolism of 2,4-dichlorophenoxyacetic acid (2,4-D) in rat and dog. *Xenobiotica*, 33(8):805–21. doi:10.1080/0049825031000135405 PMID:12936702

Venkov P, Topashka-Ancheva M, Georgieva M, Alexieva V, Karanov E (2000). Genotoxic effect of substituted phenoxyacetic acids. *Arch Toxicol*, 74(9):560–6. doi:10.1007/s002040000147 PMID:11131037

Vineis P, Terracini B, Ciccone G, Cignetti A, Colombo E, Donna A et al. (1987). Phenoxy herbicides and soft-tissue sarcomas in female rice weeders. A population-based case-referent study. *Scand J Work Environ Health*, 13(1):9–17. doi:10.5271/sjweh.2077 PMID:3576149

Vogel E, Chandler JL (1974). Mutagenicity testing of cyclamate and some pesticides in Drosophila melanogaster. *Experientia*, 30(6):621–3. doi:10.1007/BF01921506 PMID:4209504

Vural N, Burgaz S (1984). A gas chromatographic method for determination of 2,4-D residues in urine after occupational exposure. *Bull Environ Contam Toxicol*, 33(5):518–24. doi:10.1007/BF01625578 PMID:6498355

Waite DT, Bailey P, Sproull JF, Quiring DV, Chau DF, Bailey J et al. (2005). Atmospheric concentrations and dry and wet deposits of some herbicides currently used on the Canadian Prairies. *Chemosphere*, 58(6):693–703. doi:10.1016/j.chemosphere.2004.09.105 PMID:15621183

Walters J (1999). Environmental fate of 2,4-dichlorophenoxyacetic acid. Sacramento (CA): California Department of Pesticide Regulation and CalEPA. Available from: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/24-d.pdf, accessed 23 January 2015

Ward MH, Lubin J, Giglierano J, Colt JS, Wolter C, Bekiroglu N et al. (2006). Proximity to crops and residential exposure to agricultural herbicides in iowa. *Environ Health Perspect*, 114(6):893–7. doi:10.1289/ehp.8770 PMID:16759991

Weisenburger DD (1990). Environmental epidemiology of non-Hodgkin's lymphoma in eastern Nebraska. *Am J Ind Med*, 18(3):303–5. doi:10.1002/ajim.4700180310 PMID:2220835

WHO (2003). 2,4-D in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality. Report No. WHO/SDE/WSH/03.04/70. Geneva: World Health Organization. Available from: http://www.who.int/water_sanitation_health/dwq/chemicals/24D.pdf.

WHO (2011). *Guidelines for drinking water quality*. Geneva: Global Malaria Programme, World Health Organization.

Wiklund K, Dich J, Holm LE (1987). Risk of malignant lymphoma in Swedish pesticide appliers. *Br J Cancer*, 56(4):505–8. doi:10.1038/bjc.1987.234 PMID:3689667

Wilson NK, Strauss WJ, Iroz-Elardo N, Chuang JC (2010). Exposures of preschool children to chlorpyrifos, diazinon, pentachlorophenol, and 2,4-dichlorophenoxyacetic acid over 3 years from 2003 to 2005: A longitudinal model. *J Expo Sci Environ Epidemiol*, 20(6):546–58. doi:10.1038/jes.2009.45 PMID:19724304

Witte I, Jacobi H, Juhl-Strauss U (1996). Suitability of different cytotoxicity assays for screening combination effects of environmental chemicals in human fibroblasts. *Toxicol Lett*, 87(1):39–45. doi:10.1016/0378-4274(96)03697-1 PMID:8701443

Woodruff RC, Phillips JP, Irwin D (1983). Pesticide-induced complete and partial chromosome loss in screens with repair-defective females of Drosophila melanogaster. *Environ Mutagen*, 5(6):835–46. doi:10.1002/em.2860050608 PMID:6418539

Woods JS, Polissar L (1989). Non-Hodgkin's lymphoma among phenoxy herbicide-exposed farm workers in western Washington State. *Chemosphere*, 18(1-6):401–6. doi:10.1016/0045-6535(89)90148-3

Woods JS, Polissar L, Severson RK, Heuser LS, Kulander BG (1987). Soft tissue sarcoma and non-Hodgkin's lymphoma in relation to phenoxyherbicide and chlorinated phenol exposure in western Washington. *J Natl Cancer Inst*, 78(5):899–910. PMID:3471999

Woudneh MB, Sekela M, Tuominen T, Gledhill M (2007). Acidic herbicides in surface waters of Lower Fraser Valley, British Columbia, Canada. *J Chromatogr A*, 1139(1):121–9. doi:10.1016/j.chroma.2006.10.081 PMID:17118381

Wright TR, Shan G, Walsh TA, Lira JM, Cui C, Song P et al. (2010). Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase

transgenes. *Proc Natl Acad Sci USA*, 107(47):20240–5. doi:10.1073/pnas.1013154107 PMID:21059954

Xie L, Thrippleton K, Irwin MA, Siemering GS, Mekebri A, Crane D et al. (2005). Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. *Toxicol Sci*, 87(2):391–8. doi:10.1093/toxsci/kfi249 PMID:16049272

Yao Y, Tuduri L, Harner T, Blanchard P, Waite D, Poissant L et al. (2006). Spatial and temporal distribution of pesticide air concentrations in Canadian agricultural regions. *Atmos Environ*, 40(23):4339–51. doi:10.1016/j.atmosenv.2006.03.039

Yiin JH, Ruder AM, Stewart PA, Waters MA, Carreón T, Butler MA et al.; Brain Cancer Collaborative Study Group (2012). The Upper Midwest Health Study: a case-control study of pesticide applicators and risk of glioma. *Environ Health*, 11(1):39. doi:10.1186/1476-069X-11-39 PMID:22691464

Yilmaz HR, Yuksel E (2005). Effect of 2,4-dichlorophenoxyacetic acid on the activities of some metabolic enzymes for generating pyridine nucleotide pool of cells from mouse liver. *Toxicol Ind Health*, 21(9):231–7. doi:10.1191/0748233705th231oa PMID:16342474

Yoder J, Watson M, Benson WW (1973). Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides. *Mutat Res*, 21(6):335–40. doi:10.1016/0165-1161(73)90057-5 PMID:4779319

Zahm SH, Weisenburger DD, Babbitt PA, Saal RC, Vaught JB, Cantor KP et al. (1990). A case-control study of non-Hodgkin's lymphoma and the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) in eastern Nebraska. *Epidemiology*, 1(5):349–56. doi:10.1097/00001648-199009000-00004 PMID:2078610

Zeljezic D, Garaj-Vrhovac V (2001). Chromosomal aberration and single cell gel electrophoresis (Comet) assay in the longitudinal risk assessment of occupational exposure to pesticides. *Mutagenesis*, 16(4):359–63. doi:10.1093/mutage/16.4.359 PMID:11420406

Zeljezic D, Garaj-Vrhovac V (2002). Sister chromatid exchange and proliferative rate index in the longitudinal risk assessment of occupational exposure to pesticides. *Chemosphere*, 46(2):295–303. doi:10.1016/S0045-6535(01)00073-X PMID:11827288

Zeljezic D, Garaj-Vrhovac V (2004). Chromosomal aberrations, micronuclei and nuclear buds induced in human lymphocytes by 2,4-dichlorophenoxyacetic acid pesticide formulation. *Toxicology*, 200(1):39–47. doi:10.1016/j.tox.2004.03.002 PMID:15158562

Zetterberg G, Busk L, Elovson R, Starec-Nordenhammar I, Ryttman H (1977). The influence of pH on the effects of 2,4-D (2,4-dichlorophenoxyacetic acid, Na salt) on Saccharomyces cerevisiae and Salmonella typhimurium. *Mutat Res*, 42(1):3–17. doi:10.1016/S0027-5107(77)80003-1 PMID:15215

Zhamsaranova SD, Lebedeva SN, Liashenko VA (1987). [Immunodepressive effects of the herbicide 2,4-D on mice]. *Gig Sanit*, (5):80–1. PMID:3609786

Zhang X, Acevedo S, Chao Y, Chen Z, Dinoff T, Driver J et al. (2011). Concurrent 2,4-D and triclopyr biomonitoring of backpack applicators, mixer/loader and field supervisor in forestry. *J Environ Sci Health B*, 46(4):281–93. doi:10.1080/03601234.2011.559424 PMID:21500074

Zimmering S, Mason JM, Valencia R, Woodruff RC (1985). Chemical mutagenesis testing in Drosophila. II. Results of 20 coded compounds tested for the National Toxicology Program. *Environ Mutagen*, 7(1):87–100. doi:10.1002/em.2860070105 PMID:3917911

Zychlinski L, Zolnierowicz S (1990). Comparison of uncoupling activities of chlorophenoxy herbicides in rat liver mitochondria. *Toxicol Lett*, 52(1):25–34. doi:10.1016/0378-4274(90)90162-F PMID:2356568