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International Agency for Research on Cancer

World Health Organization

DIAZINON

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 333-41-5

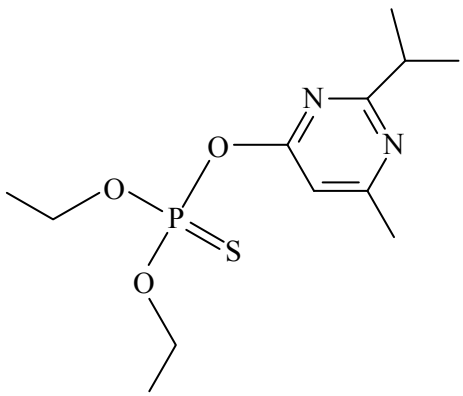
Chem. Abstr. Serv. Name: O,O-diethyl *O*-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] phosphorothioate

Preferred IUPAC Name: O,O-diethyl *O*-[6-methyl-2-(propan-2-yl)pyrimidin-4-yl] phosphorothioate

Synonyms: Bazudine, Diazinon, Dimpylate, Neocidol, Neotsidol

Trade Names: Diazinon products have been sold in various countries under numerous trade names, including, for example, Basudin; Cekuzinon; Dianon; Diazol; Dragon; Kayazinon; Knox Out; Neocidol; Spectracide; Terminator (Farm Chemicals International, 2014; NCBI, 2015)

1.1.2 Structural and molecular formulae, and relative molecular mass



Molecular formula: $C_{12}H_{21}N_2O_3PS$

Relative molecular mass: 304.35

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

1.1.3 Chemical and physical properties of the pure substance

Description: The pure form is a colourless oily liquid. The technical grade is light amber to dark brown in colour, and the insecticide formulation is a colourless liquid with a faint ester-like odour (NIOSH, 2010; NCBI, 2015).

Solubility: Slightly soluble in water at 60 mg/L (NCBI, 2015) at 20 °C. Completely miscible with common organic solvents, e.g. ethers, alcohols, benzene, toluene, hexane, cyclohexane, dichloromethane, acetone, petroleum oils (NCBI, 2015)

Volatility: Vapour pressure, 9.01×10^{-5} mm Hg (25 °C); low vapour pressure suggests that little volatilization from soil would be expected (NCBI, 2015).

Stability: More stable in alkaline formulations than at neutral or acid pH (NCBI, 2015)

Reactivity: Susceptible to oxidation above 100 °C (Tomlin, 2000)

Octanol/water partition coefficient (P): log K_{ow} 3.81 (NCBI, 2015)

Henry's law: 1.13×10^{-7} atm m^3 mol^{-1}; the low Henry's law constant suggests that little volatilization from water surfaces would be expected (NCBI, 2015).

Conversion factor: Assuming normal temperature (25 °C) and pressure (101 kPa), $mg/m^3 = 12.4 \times ppm$.

1.1.4 Technical products and impurities

Concentrations of *O*,*O*,*O*',*O*'-tetraethyl thiopyrophosphate (*O*,*S*-TEPP) and *O*,*O*,*O*',*O*'-tetraethyl dithiopyrophosphate (*S*,*S*-TEPP) are limited to 0.2 and 2.5 g/kg, respectively (WHO, 1999). Some diazinon formulations may contain other pesticides such as pyrethrins, lindane (gamma-hexachlorocyclohexane), and disulfoton (EXTOXNET, 2015).

1.2 Production and use

1.2.1 Production

Production and usage figures for diazinon are not available for most parts of the world. In the USA, the production volume of diazinon in 1990 was 4670 tonnes (Davies et al., 1996). The USA exported an estimated 2600 tonnes of diazinon between 1997 and 2000 (ATSDR, 2008). From 1987 until 1997, annual usage of diazinon in the USA was more than 5900 tonnes, with about 70% for outdoor residential uses (ATSDR, 2008). Total use of diazinon in the USA decreased from 2000–3000 tonnes in 2001 (diazinon was ranked third among organophosphate insecticides) to < 500 tonnes in 2007 (diazinon was ranked eighth) as a result of regulatory action (EPA, 2011).

Diazinon is reported to be manufactured by 46 producers in 11 countries, including 22 in China, six in India, five in the USA, four in Singapore, three in the United Kingdom, and one each in Canada, Israel, Japan, Mexico, Taiwan (China), and Thailand (Farm Chemicals International, 2015).

1.2.2 Uses

Diazinon is a wide-ranging non-systemic insecticide, miticide, and nematicide with contact, stomach, and respiratory action. It is effective against flying insects, crawling insects, mites, ticks, and spiders (IPCS, 1998). It has been employed since the early 1950s (IPCS, 1998) for uses including control of sucking and chewing insects and mites on a wide range of fruit, vegetables, and forage and field crops; on ranges, pastures, grasslands, and ornamentals; against ticks on cattle, blowflies and mites on sheep, and flies in greenhouses and mushroom houses; against grubs and nematodes in turf, and in seed treatment (Tomlin, 2000; EPA, 2006). Diazinon has also been used for general-purpose gardening and for indoor pest control against cockroaches, silverfish, ants, and scorpions, and in flea collars for pets (IPCS, 1998).

Diazinon has been produced in various commercial formulations, including liquids and concentrates, wettable powders, granules, dusts, and impregnated materials (EPA, 2006). Liquid formulations of diazinon can be sprayed by several application methods, including backpack and hand-held sprayers, and by aircraft; granular diazinon can be applied using manual or mechanized spreaders or grinders (EPA, 2006).

(a) Agriculture

Important agricultural applications of diazinon have been in rice, fruit, vineyards, sugar cane, corn, tobacco, potatoes, horticultural crops, and dips and sprays fror animals (IPCS, 1998). Diazinon has been used as the active pesticide ingredient topically applied (e.g. as aerosols, sprays, dips, ear tags) on livestock to control biting insects or ectoparasites (ATSDR, 2008). In the United Kingdom, dipping of sheep in baths containing diazinon to control a mite that causes sheep scab was compulsory until 1992 (Watterson, 1999; HSE, 2010). Diazinon has also been registered for incorporation into compost to control flies in mushroom cultivation (Shamshad, 2010).

(b) Residential use

Diazinon has been widely employed in residential settings, with such uses representing about 70% of total use of diazinon in the USA in 1987–1997 (ATSDR, 2008) Diazinon reportedly represented about 30% of all the homeowner-related insecticide use in the USA before 2004, when all remaining authorized indoor and outdoor residential uses of diazinon were cancelled (Stone et al., 2009). Diazinon was used for the control of household insects, lawn and garden insects, and insects on pets. Residential application methods included aerosol cans, spray equipment, and granular spreaders (ATSDR, 2008).

(c) Public health

In the USA, diazinon is currently permitted for the control of fire ants, and for the control of plague-infected fleas on squirrels (EPA, 2004).

(d) Regulation

In the 1980s, both the USA and Canada suspended the use of diazinon for control of grubs and nematodes on golf courses and sod farms, due to deaths of migratory waterfowl (ATSDR, 2008). In the USA, about 30% of agricultural uses (including most granular, aerial, and foliar applications) were cancelled at the end of 2002, and remaining uses were restricted to trained, certified applicators (EPA, 2001). All indoor residential and non-residential uses of diazinon, as well as outdoor residential lawn and garden products, were phased out of use in the USA by 2004 (EPA, 2006).

Withdrawal of authorizations for use of diazinon-containing products on crops and animals was finalized by the Health and Consumer Protection Directorate General of the European Commission in 2006 (European Commission, 2006). In France in 2012, the Agence Nationale du Médicament Vétérinaire withdrew permission to sell flea collars that contain organophosphates, including diazinon and tetrachlorvinphos (ANSES, 2012).

Occupational exposure limits for diazinon ranging from 0.01 mg/m^3 to 0.3 mg/m^3 have been been established in several countries (IFA, 2015).

1.3 Measurement and analysis

Representative methods of chemical analysis for diazinon and its specific metabolite 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY) are listed in Table 1.1.

1.4 Occurrence and exposure

1.4.1 Exposures

(a) Occupational exposure

Occupational exposure may occur in workers involved in the manufacture of diazinon and formulations containing diazinon, applicators who spray or mix diazinon, farm workers engaged in re-entry tasks, sheep farmers and other livestock workers, vector-control workers, and veterinarians.

Table 1.1 Methods for the analysis of diazinon

Sample matrix	Assay procedure	Limit of detection	Reference
Air	GC-MS	0.3 ng/m³	Elflein et al. (2003)
Water	GC-FPD (with 526 nm filter)	0.01 µg/L	EPA (1992b)
	GC-MS (selected-ion monitoring mode)	0.01 µg/L	Zaugg et al. (1995)
Urine	GC-MS-ECNI-SIM	1 µg/L (as IMPY)	Bouchard et al. (2006)
Fruits and vegetables	GC-MS	0.02 mg/kg	Fillion et al. (2000)
Dust	GC-MS	2 ng/g	Harnly et al. (2009)

GC-FID, gas chromatography-flame ionization detection; GC-FPD, gas chromatography-flame photometric detection; GC-MS, gas chromatography-mass spectrometry; GC-MS-ECNI-SIM, gas chromatography-mass spectrometry with electron capture negative ionization in single-ion monitoring mode; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine

No data on exposure of workers involved in the production of diazinon were available to the Working Group.

(i) Air

Concentrations of diazinon in air were measured in a greenhouse during and after spraying and cold fogging operations (Lenhart & Kawamoto, 1994). The personal exposure of an applicator during spraying was 226 µg/m³, resulting in an 8-hour, time-weighted average (TWA) exposure of 25 µg/m³. Area measurements of diazinon concentrations were similar during spraying, but considerably higher (up to 3030 µg/m³) during cold fogging. TWA concentrations declined after both types of operation, but diazinon was still detectable after 4 days (Lenhart & Kawamoto, 1994).

(ii) Skin

In agricultural workers and pesticide applicators, skin contact is the most important route of exposure. Davis et al. (1983) estimated that dermal exposure in applicators spraying diazinon was 5500–29 000 µg/hour, depending on the activity, spraying method, and type of clothing worn, while exposure by the respiratory route was 1.9–7.4 µg/hour.

(iii) Biological markers

Several studies have reported metabolites of diazinon in the urine of exposed workers (Table 1.2). The highest mean urinary concentration of IMPY was reported in banana-plantation workers from Nicaragua, and was related to the volume of diazinon used, inappropriate application methods, and poor protection and hygiene of the workers (Rodríguez et al., 2006).

Diazinon has also been detected in saliva and blood of banana-plantation workers (Lu et al., 2006).

(b) Community exposure

(i) Air and dust

Diazinon and its metabolite, diazoxon, have been detected in urban and agricultural settings in the USA in the past, but levels are expected to have been reduced due to the implementation of regulations (EPA, 2004).

Available reports of diazinon concentrations in outdoor air ranged from not detected to a mean of 0.42 µg/m³ (Carey & Kutz 1985; Zabik & Seiber 1993; Whitmore et al. 1994; Majewski et al., 1998; Morgan et al., 2014). In indoor air, mean concentrations of diazinon ranged from 0.001 to 6 µg/m³, with the highest concentrations reported in studies in homes of pregnant women in New York, USA (Whitmore et al., 1994; Whyatt et al., 2005; Morgan et al., 2014). Diazinon may be transported in the atmosphere, with concentrations declining with distance from the source (Aggarwal et al., 2013).

Table 1.2 Concentrations of diazinon metabolites in the urine of occupationally exposed workers

Country, year	Job/process	Results	Comments/additional data	Reference
Canada, 2003	Greenhouse; 18 workers	IMPY, < LOD	IMPY not detected in 54 samples from 18 workers at an horticultural greenhouse (LOD, 1 µg/L)	Bouchard et al. (2006)
Nicaragua, 2003		IMPY: Geometric mean, 1.3–168 µg/L for two plantations Range for individual workers, ND to 412 µg/L	IMPY was detected in 79% of samples. Concentrations declined 45–75% after 24 hours	Rodríguez et al. (2006)
USA, 2002	Flea-control operations; 5 workers	DEP range, < LOD to 16.2 µg/L DETP range, < LOD to 44.6 µg/L	DEP and DETP are non-specific metabolites of organophosphate pesticides, but only diazinon was used by the workers	Gerry et al. (2005)
USA, 2010	Migrant farmworkers; 371 men	IMPY, ≥ LOD in 15% of samples	Geometric mean, NR	Raymer et al. (2014)

DEP, diethyl phosphate; DETP, diethyl thiophosphate; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine (specific metabolite of diazinon); LOD, limit of detection; ND, not detected; NR, not reported

Residues of diazinon in domestic dust ranged from not detected to 11 µg/g in urban and agricultural settings, with higher maximum concentrations in urban areas (Gunier et al., 2011; Quirós-Alcalá et al., 2011; Morgan et al., 2014).

(ii) Water

Diazinon is released into water directly by drift during application and runoff from rural and urban areas (ATSDR, 2008). It is moderately mobile in some soil types, and therefore has the potential to leach into groundwater (Fenlon et al., 2011). Diazinon has been reported in groundwater, drinking-water, main streams, and rural ponds in regions close to cultivation areas. Table 1.3 summarizes concentrations of diazinon reported in surface water in largely agricultural areas in the USA, Canada, and the Islamic Republic of Iran; concentrations ranged from not detected to 491.6 µg/L (Carey & Kutz, 1985; Frank & Logan, 1988; Frank et al., 1990; Maguire & Tkacz, 1993; Hall, 2003; Banks et al., 2005; Shayeghi et al., 2007; Zhang et al., 2012).

(iii) Soil

Morgan et al. (2014) reported detectable concentrations of diazinon in soil samples from 18% of 129 homes with children (range, not detected to 5.5 µg/g), and none of 13 day-care centres sampled in North Carolina, USA.

Diazinon is considered to be moderately mobile in soil. Microbiological degradation in soil and water is the main manner by which diazinon dissipates in the environment. In microbially active soils, diazinon is degraded rapidly (Bondarenko et al., 2004; Fenlon et al., 2011).

(iv) Household exposure

In a survey of 259 households in California, USA, 12% were found to be storing a product containing diazinon (Guha et al., 2013).

(v) Residues in food, and dietary intake

Several studies have reported small amounts of diazinon in a variety of food items, including fruits, vegetables, grains, meat, milk, and oils sold to consumers in several countries (Túri et al. 2000; Quintero et al., 2008; Zhang et al., 2008; Cho et al., 2009; Fuentes et al., 2010; Riederer

Table 1.3 Concentration of diazinon in surface water

Country Year of sampling	Number of samples/setting	Results	Comments/additional data	Reference
Ontario, Canada 1981–1985	446 samples from three rivers in agricultural areas	Detected in 1 out of 446 samples Concentration, 0.21 µg/L		Frank & Logan (1988)
Quebec, Canada 1986–1987	Number, NR; surface water	Range, 0.002–0.027 µg/L		Maguire & Tkacz (1993)
Islamic Republic of Iran Year, NR	Four stations near agricultural areas; samples were taken 1 day, 1 week, 2 weeks, 1 month, 2 months, and 3 months after spraying	Range, ND–491.6 µg/L	Maximum concentrations detected 1 day after application Diazinon residues decreased with increasing distance and time since spraying	Shayeghi et al. (2007)
USA 1976–1980	Number, NR; surface water	Detected in 1.2% of samples		Carey & Kutz (1985)
California, USA 1991–2001	27 sites; surface water	Range, ND–6.84 µg/L	90th percentile range, 0.01–14.90 µg/L	Hall (2003)
Texas, USA 2001–2004	1243 samples from 70 sites in agricultural areas	Range of mean concentrations, 0.04–0.32 µg/L	Concentrations decreased significantly between 2001 and 2004	Banks et al. (2005)
California, USA 2005–2010	3638 samples from 251 sites in 5 agricultural areas	Diazinon detection frequencies ranged from 10% to 90% Range of maximum concentrations, 1.0–24 µg/L		Zhang et al. (2012)
Washington, USA Year, NR	211 rural ponds in agricultural areas	Mean, 1.2 µg/L Range, < 0.002 to 1.7 µg/L	Found in two ponds contaminated by spill	Frank et al. (1990)

ND, not detected; NR, not reported

et al., 2010; EFSA, 2011; Srivastava et al., 2011; USDA, 2014). The highest concentration reported (3.8 mg/kg) was found in vegetables in the Republic of Korea (Cho et al., 2009). Many of the concentrations recorded in industrialized countries were below the reported limit of detection. [The Working Group noted the wide range of detection limits reported.]

(vi) Biological markers

Exposure to diazinon in the general population has been assessed by the presence of IMPY in urine samples, and diazinon in blood and saliva. IMPY was detected in 55% of urine samples from 60 farmworkers' children in North Carolina, USA, with a creatinine-adjusted geometric mean of 0.70 µg/g (Arcury et al., 2007). IMPY was detected in 5% of urine samples, and diazinon was found in 41% of saliva samples from 10 children of banana-plantation workers in Nicaragua (Lu et al., 2006; Rodríguez et al., 2006).

1.4.2 Exposure assessment

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

2. Cancer in Humans

2.1 Summary of frequently cited epidemiological studies

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

2.2 Cohort studies

Three cohort studies were identified that reported relative risk estimates for the association between diazinon exposure and cancer outcomes: the Florida Pest Control Worker Study (Section 2.2.1), the United Farm Workers of America cohort study (Section 2.2.2), and the Agricultural Health Study (AHS) (Section 2.2.3). The studies were conducted among farm workers (United Farm Workers of America), and professional pesticide users (Florida Pest Control Worker Study; AHS) and their spouses (AHS) in the USA (see Table 2.1).

2.2.1 Florida Pest Control Worker Study

Pesatori et al. (1994) conducted a case–control study nested within the cohort of the Florida Pest Control Worker Study cohort and included 65 deceased cases of cancer of the lung and 294 controls (deceased, 122; living, 172) (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Proxy interviews were completed for 65 cases deceased between 1965–1982, and for 122 deceased and 172 living controls randomly selected from cohort members matched on year of birth and death. Telephone interviews covered tobacco use, diet, and occupations. For each occupation involving pesticide use, information on specific chemicals used was collected. Ever versus never use of diazinon was associated with an odds ratio of 2.0 (95% CI, 0.7–5.5) when comparing with deceased controls, and 1.3 (95% CI, 0.6–3.1) when comparing with living controls, after adjusting for age and smoking (see Table 2.1). [The Working Group noted substantial limitations to the pesticide exposure assessment based on proxy interviews, and the potential for differential exposure misclassification.]

Table 2.1 Cohort studies of cancer and exposure to diazinon

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
Pesatori et al. (1994) Florida, USA Enrolment, 1965–66; follow-up until 1982 Nested case–control study	Cases: 65 (response rate, 83%; percentage of surrogate respondents that could be located and interviewed) Controls: 294 (122 deceased, 172 living) (response rates: deceased controls, 80%, living controls, 75%); 16 living controls were interviewed directly because next-of-kin was not located Exposure assessment method: questionnaire; information collected from proxies at time of interview	Lung	Diazinon (using deceased controls) Diazinon (using living controls)	17 17	2 (0.7–5.5) 1.3 (0.6–3.1)	Age, smoking Age, smoking	Florida Pest Control Worker Study [Strengths: population of pesticide applicators with high exposure prevalence. Use of both deceased and living controls. Limitations: exposure assessment to specific pesticides based on interview with proxies (mostly wives) (possible information bias); small number of cases in the cohort; possible healthy worker effect]

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. (2005a) California, USA 1988–2001 Nested case–control study	Cases: 131 (response rate, NR) identified by linking the cohort to the California Cancer Registry for 1988–2001 Controls: 651 (response rate, NR) from the United Farm Workers of America cohort Exposure assessment method: union records to identify farms where the worker had worked (work histories collected); link to obtain potential exposure to pesticides from the California Department of Pesticide Regulation (pesticide databank)	Total leukaemia	High vs low	NR	1.32 (0.65–2.65)	Age, sex, length of union affiliation, date of first union affiliation	United Farm Workers of America [Strengths: the study was conducted among farm workers (as opposed to pesticide applicators); the study included women; objective exposure assessment using a historical databank of pesticide use in the region – this method reduced recall bias. Limitations: number of cases was small; number of cases and controls exposed was not reported; the exposure assessment was based on regional pesticide use data and does not take personal use of pesticides or tasks into account, leading to possible exposure misclassification]
			High vs low (men)	NR	0.9 (0.37–2.19)		
			High vs low (women)	NR	2.7 (0.8–9.13)		
		Total NHL	High vs low	NR	1.39 (0.76–2.53)		
			High vs low (men)	NR	1.97 (0.97–4.00)		
			High vs low (women)	NR	0.8 (0.23–2.81)		
		Lymphocytic leukaemia	High vs low	NR	1.42 (0.46–4.43)		
		Granulocytic leukaemia	High vs low	NR	1.94 (0.66–5.72)		
		NHL, nodal	High vs low	NR	1.26 (0.60–2.66)		
		NHL, extranodal	High vs low	NR	1.57 (0.57–4.32)		

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 & Yang (2005b) California, USA 1988–2001 Nested case–control study	Cases: 128 (response rate, NR); identified by linking the cohort to the California Cancer Registry for 1988–2001 Controls: 640 (response rate, NR); five controls for each case from the cohort who had not been diagnosed with any cancer and matched on sex, 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 period before cancer diagnosis; classified "high exposure" can be interpreted as having worked in an area with high use	Breast (diagnosed 1988–1994)	No diazinon use (ref.)	12	1	Age, date of first union affiliation, fertility, socioeconomic level	United Farm Workers of America cohort [Strengths: the study was conducted among farm workers (as opposed to pesticide applicators); the study included women; objective exposure assessment using a historical databank of pesticide use in the region – this method reduced recall bias. Limitations: number of cases was small; number of cases and controls exposed was not reported; the exposure assessment was based on regional pesticide use data and does not take personal use of pesticides or tasks into account, leading to possible exposure misclassification; surrogate variables for reproductive histories: county level measures of fertility and socioeconomic status]
			Low	9	0.78 (0.12–4.84)		
			Medium	17	1.54 (0.22–10.68)		
			High	10	1.50 (0.18–12.35)		
		Breast (diagnosed 1995–2001)	No diazinon use (ref.)	26	1		
			Low	20	1.18 (0.27–5.20)		
			Medium	21	1.42 (0.30–6.81)		
			High	13	0.76 (0.15–3.92)		

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
Alavanja et al. (2004) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 31 December 2001	57 284 licensed pesticide applicators and 32 333 spouses with no history of lung cancer at enrolment Incident cancers were identified from enrolment (i.e. 1993–1997) until 31 December 2001. Study subjects alive but no longer residing in Iowa or North Carolina (n = 875) were identified through personal contacts with the study subject, motor vehicle records, pesticide registration records, and the current address records of the Internal Revenue Service, and they were censored in the year they left the state Exposure assessment method: questionnaire	Lung	None (ref.) LED < 20.0 LED 20.0–108.5 LED > 108.5 Trend-test P value: 0.008	65 10 11 7	1 0.93 (0.50–1.80) 1.40 (0.70–2.70) 2.70 (1.20–6.10)	Age, sex, smoking, total days of any pesticide application	AHS [Strengths: large study of highly exposed workers. Limitations: very low study power for female cohort members (only 3 cases of cancer of the lung)]

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
Beane Freeman et al. (2005) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, until 2002	23 106 male applicators who completed the take-home questionnaire that included questions on duration and frequency of diazinon use Exposure assessment method: take-home self-administered questionnaire	All neoplasms (ICD-9 140–208)	No diazinon (ref.)	722	1	Age, smoking, education, family history of cancer, state of residence, total days of any pesticide application	AHS This publication from the AHS is on cancer risk associated with diazinon specifically. Exposure metrics were LED and IW-LED, with analyses using either unexposed referents or the lowest exposure category as referents, essentially showing the same results. Results were reported for the cancer sites: colorectum, lung, prostate, melanoma, lympho-haematopoietic, NHL, and leukaemia. This paper overlaps with Lee et al. (2007) [Strengths: large size; licensed pesticide applicators only, resulting in high exposure prevalence (21.5% for diazinon) and good quality reporting of use of specific pesticides; complete follow-up; detailed exposure assessment based on questionnaire completed at time of enrolment, before disease outcome; different approaches for quantification of lifetime exposure enabling dose–response analyses. Limitations: results are for men only]
			LED < 20	106	1.12 (0.91–1.38)		
			LED 20.0–38.8	64	1.08 (0.83–1.40)		
			LED > 38.8	77	1.39 (1.09–1.78)		
			Trend-test *P* value: 0.009				
		All neoplasms	No diazinon (ref.)	722	1		
			LED < 20	106	1.12 (0.91–1.38)		
			LED 20.0–38.8	64	1.08 (0.83–1.39)		
			LED 38.9–108.8	45	1.28 (0.93–1.73)		
			> 108.8	32	1.58 (1.10–2.28)		
			Trend-test *P* value: 0.007				
		All neoplasms	IW-LED				
			No diazinon (ref.)	722	1		
			Tertile 1	85	1.10 (0.95–1.49)		
			Tertile 2	81	1.09 (0.86–1.38)		
			Tertile 3, low	39	1.16 (0.84–1.62)		
			Tertile 3, high	42	1.41 (1.03–1.95)		
			Trend-test *P* value: 0.033				
		All neoplasms	IW-LED				
			No diazinon (ref.)	722	1		
			Tertile 1	85	1.19 (0.95–1.50)		
			Tertile 2	81	1.09 (0.86–1.38)		
			Tertile 3	81	1.28 (1.01–1.63)		
			Trend-test *P* value: 0.05				
		Lung	No diazinon (ref.)	57	1		
			LED < 20	9	1.01 (0.48–2.15)		
			LED 20.0–38.8	3	0.54 (0.17–1.75)		
			LED > 38.8	15	2.41 (1.31–4.43)		
			Trend-test *P* value: 0.005				

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
Beane Freeman et al. (2005) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up, until 2002 (cont.)		Prostate	No diazinon (ref.)	299	1		
			LED < 20	56	1.41 (1.05–1.88)		
			LED 20.0–38.8	32	1.28 (0.88–1.85)		
			LED > 38.8	26	1.19 (0.79–1.81)		
			Trend-test *P* value: 0.34				
		Lympho-haematopoietic (ICD-9 200–208)	No diazinon (ref.)	67	1		
			LED < 20	10	1.17 (0.60–2.29)		
			LED 20.0–38.8	7	1.31 (0.60–2.90)		
			LED > 38.8	9	1.84 (0.89–3.82)		
			Trend-test *P* value: 0.094				
		NHL (ICD-9 200 & 202)	No diazinon (ref.)	26	1		
			LED < 20	6	1.76 (0.72–4.35)		
			LED 20.0–38.8	3	1.36 (0.40–4.56)		
			LED > 38.8	2	0.92 (0.21–4.05)		
			Trend-test *P* value: 0.95				
		Leukaemia (ICD-9 204–208)	No diazinon (ref.)	21	1		
			LED < 20	3	1.1 (0.32–3.72)		
			LED 20.0–38.8	4	2.62 (0.88–7.82)		
			LED > 38.8	4	3.36 (1.08–10.49)		
			Trend-test *P* value: 0.026				
		Colorectum	No diazinon (ref.)	57	1		
			LED < 20	6	0.92 (0.39–2.15)		
			LED 20.0–38.8	6	1.53 (0.65–3.59)		
			LED > 38.8	4	1.21 (0.43–3.45)		
			Trend-test *P* value: 0.61				
		Melanoma	No diazinon (ref.)	31	1		
			LED < 20	7	1.67 (0.73–3.87)		
			LED 20.0–38.8	2	0.75 (0.18–3.15)		
			LED > 38.8	2	0.71 (0.16–3.04)		
			Trend-test *P* value: 0.59				

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
Engel et al. (2005) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2000	30 454 wives of licensed pesticide applicators with no history of breast cancer at enrolment Exposure assessment method: questionnaire	Breast	Wife's use (direct exposure)	31	1.0 (0.7–1.5)	Age, race (white/ other), state of residence	AHS [Strengths: large study; female study population; mostly farmers' wives with high exposure prevalence (10% of all wives used diazinon); focus on direct and indirect exposure (24% of wives who never used pesticides themselves were indirectly exposed to diazinon); collection of detailed exposure information at enrolment, before disease outcome. Limitations: few cases who used diazinon themselves based on self-reported exposure]
			Wife's use (direct exposure), Iowa	18	0.9 (0.6–1.5)		
			Wife's use (direct exposure), North Carolina	13	1.2 (0.7–2.1)		
			Wife's use (direct exposure), premenopausal women	8	0.8 (0.4–1.6)		
			Wife's use (direct exposure), postmenopausal women	19	1.1 (0.7–1.8)		
			Wife's use (direct exposure), family history of breast cancer	13	1.7 (0.9–3.2)		
			Wife's use (direct exposure), no family history of breast cancer	17	0.8 (0.5–1.2)		
			Husband's use (indirect exposure)	39	1.4 (0.9–2.0)		
			Husband's use (indirect exposure), Iowa	19	1.6 (0.9–2.6)		

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
Engel et al. (2005) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up to 2000 (cont.)			Husband's use (indirect exposure), North Carolina	20	1.2 (0.7–20)		
			Husband's use (indirect exposure), premenopausal women	10	1.5 (0.7–3.2)		
			Husband's use (indirect exposure), postmenopausal women	28	1.5 (0.9–2.3)		

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 December 31 2007	54 412 licensed private pesticide applicators (Iowa and North Carolina) and 4916 licensed commercial applicators (Iowa); 1962 incident cases including 919 aggressive cancers Exposure assessment method: questionnaire	Prostate, total cancers	Not exposed to diazinon (ref.)	727	1	Age, state, family history of prostate cancer, smoking, fruit servings, leisure time physical activity in winter, race	AHS A prior AHS publication already reported on diazinon and prostate cancer (Beane Freeman et al., 2005), but here 5 years additional follow-up were included [Strengths: large cohort study in agricultural population with high exposure prevalence, good exposure assessment; large number of prostate cancers, subanalysis of aggressive tumours, defined on histological and clinical parameters; adjustments for other pesticides]
			LED quartile 1	66	1.30 (1.01–1.68)		
			LED quartile 2	63	1.15 (0.88–1.49)		
			LED quartile 3	66	1.04 (0.81–1.35)		
			LED quartile 4	63	0.94 (0.72–1.24)		
			Trend-test *P* value: 0.59				
		Prostate, aggressive cancers	Not exposed to diazinon (ref.)	343	1	Age, state, family history of prostate cancer, smoking, fruit servings, leisure time physical activity in winter, race	
			LED quartile 1	31	1.24 (0.84–1.85)		
			LED quartile 2	29	1.00 (0.67–1.48)		
			LED quartile 3	30	0.89 (0.59–1.34)		
			LED quartile 4	30	1.31 (0.87–1.96)		
			Trend-test *P* value: 0.27				
		Prostate (no family history)	Not exposed to diazinon (ref.)	531	1	Age, state, smoking, fruit servings, leisure time physical activity in winter, race	
			LED quartile 1	51	1.34 (1.00–1.79)		
			LED quartile 2	49	1.20 (0.89–1.61)		
			LED quartile 3	45	0.96 (0.71–1.31)		
			LED quartile 4	48	1.08 (0.79–1.47)		
			Trend-test *P* value: 0.78				
		Prostate (family history)	Not exposed to diazinon (ref.)	121	1	Age, state, smoking, fruit servings, leisure time physical activity in winter, race	
			LED quartile 1	11	1.15 (0.62–2.14)		
			LED quartile 2	9	0.93 (0.46–1.86)		
			LED quartile 3	15	1.26 (0.72–2.20)		
			LED quartile 4	8	0.88 (0.42–1.83)		
			Trend-test *P* value: 0.82				

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
Alavanja et al. (2014a) Iowa and North Carolina, USA Enrolment, 1993–1997; follow up until 31 December 2010 in North Carolina, and 31 December 2011 in Iowa	54 306 licensed pesticide applicators (523 incident cases of NHL) with no prevalent cancer at baseline, not living outside the catchment area of Iowa and North Carolina cancer registries, and with complete data on potential confounders Exposure assessment method: questionnaire	NHL	Ever vs never	144	1.0 (0.8–1.3)	Age, state, race (white/black), tertiles of total herbicide use days (for all except diazinon and lindane for follicular lymphoma)	AHS Additional 8–9 yr of follow-up since Beane Freeman et al. (2005). This paper overlaps with Alavanja et al. (2014b) [Strengths: prospective design; adjustment for other pesticides. Limitations: missing data on specific pesticides were imputed (validation on a subsample)]
			No diazinon (ref.)	187	1		
			Low (LED ≤ 8.75)	28	1.1 (0.7–1.6)		
			Medium (LED > 8.75–25)	19	1 (0.6–1.8)		
			High (LED > 25–457.25)	23	1.2 (0.7–1.9)		
			Trend-test *P* value: 0.52				
		NHL	No diazinon (ref.)	187	1		
			IWED tertile 1	23	1.1 (0.7–1.8)		
			IWED tertile 2	24	0.9 (0.5–1.5)		
			IWED tertile 3	22	1.3 (0.8–2.1)		
			Trend-test *P* value: 0.33				
		SLL, CLL, MCL	Ever vs never	46	1.3 (0.9–1.9)		
		SLL, CLL, MCL	No diazinon (ref.)	53	1		
			Low (LED)	14	1.4 (0.7–2.7)		
			High (LED)	12	1.9 (0.98–3.6)		
			Trend-test *P* value: 0.06				
		DLBC	Ever vs never	30	0.9 (0.6–1.4)		
		DLBC	No diazinon (ref.)	40	1		
			Low (LED)	9	1.5 (0.7–3.2)		
			High (LED)	8	1.1 (0.5–2.4)		
			Trend-test *P* value: 0.72				
		Follicular	Ever use	22	1.3 (0.7–2.3)		
		Follicular	No diazinon (ref.)	15	1		
			Low (LED)	8	2.2 (0.9–5.4)		
			High (LED)	7	3.8 (1.2–11.4)		
			Trend-test *P* value: 0.02				

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
Alavanja et al. (2014a) (cont.)		Follicular	Diazinon & lindane in model concurrently			Age, state, race (white/black), tertiles of total herbicide use days, lindane use (tertiles of LED)	
			No diazinon (ref.)	15	1		
			Low (LED)	8	4.1 (1.5–11.1)		
			High (LED)	7	2.5 (0.9–7.2)		
			Trend-test *P* value: 0.09				
		Multiple myeloma	Ever vs never	27	1 (0.6–1.6)		
		Multiple myeloma	No diazinon (ref.)	41	1		
			Low (LED)	4	0.4 (0.1–1.2)		
			High (LED)	3	0.5 (0.2–1.7)		
			Trend-test *P* value: 0.35				
		Other B-cell	Ever vs never	12	0.8 (0.4–1.6)		
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa)	22 830 male applicators who completed the take home questionnaire and with complete information for LEDs (reported or imputed). Excluded were individuals with prevalent cancer at baseline (n = 622) or who were missing follow-up information (n = 145) and women due to the small number of female applicators (n = 663; 188 of whom reported using diazinon at follow-up)	Lung	LED < 20	32	1.11 (0.75–1.65)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	AHS [Strengths: prospective design; adjustment for other pesticides. Nearly 15 years of follow-up; triple the number of exposed cases of lung cancer since previous AHS report on lung cancer and diazinon; first report on bladder and kidney cancer and diazinon from AHS; separate results for colon and rectal cancer. Limitations: missing data on specific pesticides were imputed (validation on a subsample)]
			LED 20.0–38.8	16	0.76 (0.44–1.3)		
			LED > 38.8	36	1.6 (1.11–2.31)		
			Trend-test *P* value: 0.02				
		Lung, adenocarcinoma	LED < median	9	1.21 (0.57–2.57)	Age, smoking, family history of cancer, state of residence	
			LED ≥ median	14	1.37 (0.75–2.51)		
			Trend-test *P* value: 0.02				
		Lung, squamous cell carcinoma	LED < median	11	1.31 (0.69–2.53)	Age, smoking, family history of cancer, state of residence	
			LED ≥ median	8	0.65 (0.31–1.38)		
			Trend-test *P* value: 0.3				
		Lung, small cell carcinoma	LED < median	4	0.71 (0.25–2.02)	Age, smoking, family history of cancer, state of residence	
			LED ≥ median	11	1.23 (0.62–2.43)		
			Trend-test *P* value: 0.1				

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
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa) (cont.)	Exposure assessment method: questionnaire; lifetime use of diazinon was collected in the take-home survey, and updated during the telephone follow-up interview (phase 2); multiple imputation for applicators who did not participate in follow-up (28%) Linkage to state cancer registries from enrolment until 31 December 2010 for North Carolina and 31 December 2011 for Iowa	Lung, other carcinomas	LED < median	8	0.96 (0.42–2.19)	Age, smoking, family history of cancer, state of residence	
			LED ≥ median	19	1.53 (0.88–2.66)		
			Trend-test *P* value: 0.09				
		Lung	IW-LED < 368	22	1.09 (0.61–1.53)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			IW-LED 369–1800	25	0.99 (0.66–1.52)		
			IW-LED > 1800	37	1.41 (0.98–2.04)		
			Trend-test *P* value: 0.08				
		Lung, adenocarcinoma	IW-LED < median	10	1.17 (0.57–2.39)	Age, smoking, family history of cancer, state of residence	
			IW-LED ≥ median	13	1.43 (0.76–2.69)		
			Trend-test *P* value: 0.14				
		Lung, squamous cell carcinoma	IW-LED < median	9	0.98 (0.48–1.98)	Age, smoking, family history of cancer, state of residence	
			IW-LED ≥ median	10	0.89 (0.45–1.76)		
			Trend-test *P* value: 0.54				
		Lung, small cell carcinoma	IW-LED < median	4	0.63 (0.22–1.76)	Age, smoking, family history of cancer, state of residence	
			IWED ≥ median	11	1.36 (0.68–2.71)		
			Trend-test *P* value: 0.18				
		Lung, other carcinomas	IW-LED < median	10	1.06 (0.49–2.29)	Age, smoking, family history of cancer, state of residence	
			IW-LED ≥ median	17	1.50 (0.84–2.69)		
			Trend-test *P* value: 0.19				
		Bladder	LED < 20	13	0.69 (0.37–1.28)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			LED 20.0–38.8	8	0.72 (0.35–1.48)		
			LED > 38.8	11	0.93 (0.49–1.74)		
			Trend-test *P* value: 0.77				

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
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa) (cont.)		Bladder	IW-LED < 368	8	0.58 (0.27–1.24)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			IW-LED 369–1800	11	0.70 (0.37–1.32)		
			IW-LED > 1800	13	1.05 (0.59–1.90)		
			Trend-test *P* value: 0.96				
		Kidney	LED < 20	5	0.53 (0.21–1.31)	Age, smoking, state of residence	
			LED 20.0–38.8	6	0.89 (0.36–2.22)		
			LED > 38.8	10	1.77 (0.9–3.51)		
			Trend-test *P* value: 0.09				
		Kidney	IW-LED < 368	6	0.85 (0.37–1.86)	Age, smoking, state of residence	
			IW-LED 369–1800	6	0.77 (0.33–1.78)		
			IW-LED > 1800	9	1.37 (0.64–2.92)		
			Trend-test *P* value: 0.45				
		Prostate	LED < 20	148	1.10 (0.91–1.32)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence, race	
			LED 20.0–38.8	70	0.89 (0.69–1.17)		
			LED > 38.8	79	1.01 (0.79–1.30)		
			Trend-test *P* value: 0.84				
		Prostate	IW-LED <368	111	1.16 (0.95–1.43)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence, race	
			IW-LED 369–1800	102	0.89 (0.72–1.12)		
			IW-LED > 1800	83	0.99 (0.77–1.28)		
			Trend-test *P* value: 0.64				
		Prostate, aggressive cancers	LED < 20	71	1.08 (0.82–1.41)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence, race	
			LED 20.0–38.8	36	0.98 (0.69–1.39)		
			LED > 38.8	44	1.16 (0.83–1.63)		
			Trend-test *P* value: 0.44				

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
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa) (cont.)		Prostate, aggressive cancers	IW-LED < 368 IW-LED 369–1800 IW-LED > 1800 Trend-test *P* value: 0.22	54 47 50	1.11 (0.82–1.50) 0.90 (0.66–1.23) 1.29 (0.93–1.79)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence, race	
		Colon	LED < 20 LED 20.0–38.8 LED > 38.8 Trend-test *P* value: 0.67	16 14 16	0.84 (0.50–1.41) 1.03 (0.57–1.86) 1.12 (0.63–1.99)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
		Colon	IW-LED < 368 IW-LED 369–1800 IW-LED > 1800 Trend-test *P* value: 0.7	9 19 18	0.63 (0.32–1.24) 1.21 (0.75–1.97) 1.03 (0.56–1.88)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
		Rectum	LED < 20 LED 20.0–38.8 LED > 38.8 Trend-test *P* value: 0.94	5 5 4	0.51 (0.18–1.40) 0.88 (0.32–2.44) 0.94 (0.33–2.66)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
		Rectum	IW-LED < 368 IW-LED 369–1800 IW-LED > 1800 Trend-test *P* value: 0.49	5 2 7	0.67 (0.24–1.85) 0.18 (0.02–1.33) 1.62 (0.71–3.66)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	

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
Jones et al. (2015) Iowa and North Carolina, USA Enrolment, 1993–1997; follow-up until 2010 (North Carolina) 2011 (Iowa) (cont.)		Melanoma	LED < 20	15	0.96 (0.53–1.71)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			LED 20.0–38.8	11	1.22 (0.63–2.36)		
			LED > 38.8	6	0.58 (0.24–1.45)		
			Trend-test *P* value: 0.33				
		Melanoma	IW-LED < 368	14	1.27 (0.71–2.28)	Age, alcohol consumption, smoking, education, family history of cancer, state of residence	
			IW-LED 369–1800	8	0.55 (0.24–1.26)		
			IW-LED > 1800	10	1.00 (0.49–2.02)		
			Trend-test *P* value: 0.69				

IW-LED, intensity-weighted lifetime exposure days; LED, lifetime exposure days; NHL, non-Hodgkin lymphoma

2.2.2 United Farm Workers of America

Mills et al. (2005a) reported on a case–control study of lympho-haematopoietic cancers nested within the United Farm Workers of America cohort (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). The cohort was drawn from the 139 000 ever members of a largely Hispanic farm-workers' union in California between 1973 and 1998 (Mills & Kwong, 2001). 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 years before cancer diagnosis. For the 15 most commonly used pesticides (including diazinon), odds ratios for high versus low use were reported. Odds ratios for high versus low diazinon and total leukaemia (51 cases), lymphocytic leukaemia (23 cases), granulocytic leukaemia (20 cases), total non-Hodgkin lymphoma (NHL) (60 cases), nodal NHL (38 cases), and extranodal NHL (22 cases) were reported. Odds ratios were not reported for multiple myeloma (20 cases). Odds ratios were also reported by sex for all leukaemias (35 males, 16 females) and all NHL (45 males, 15 females). None of the odds ratios reported for diazinon reached statistical significance (see Table 2.1). Results were similar when the odds ratio for each chemical was adjusted for the other 15 chemicals. [The Working Group noted that although some elevated relative risks were observed (see Table 2.1), these were difficult to interpret because the number of exposed cases on which these estimates were based was not reported. The method of exposure assessment used had the advantage that it did not rely on self-reporting, thus eliminating the potential for recall bias, with the disadvantage that it reflected ecological rather than individual exposure to pesticides, and was therefore likely to be associated with substantial exposure misclassification. International Classification of Disease (ICD) codes were not provided.]

Mills & Yang (2005b) reported on a case–control study that was nested in the United Farm Workers of America cohort and followed the same methodology as the study of lympho-haematopoietic cancers described above (Mills et al., 2005a), and included 128 cases of cancer of the breast in women. The association between estimated exposure to diazinon (low/medium/high versus no exposure) was presented separately for cases diagnosed in 1988–1994 (n = 48) and in 1995–2001 (n = 80); some increased risks were observed but they were not statistically significant (see Table 2.1).

2.2.3 Agricultural Health Study

The Agricultural Health Study (AHS) is a prospective cohort of licensed pesticide applicators enrolled in 1993–1997 in Iowa and North Carolina, USA (Alavanja et al., 1996; see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study).

Alavanja et al. (2004) reported on pesticide use and incidence of cancer of the lung in the AHS; 240 incident cases of cancer of the lung were identified. For 22 of the 50 pesticides evaluated (including diazinon, malathion, and parathion), the exposure index "lifetime exposure days" (LEDs) was based on the take-home questionnaire and computed as application days per year × total years of exposure. Unconditional multivariate logistic regression was used to compare cases of cancer of the lung with non-cases for the 50 specific pesticides, adjusting for smoking, age, sex, and total days of any pesticide application. For 7 out of 50 pesticides (including diazinon), LEDs showed some evidence of an exposure–response relationship and were reported. Compared with participants with no exposure to diazinon, odds ratios were 0.93 (95% CI, 0.5–1.8) for < 20 LEDs; 1.4 (95% CI, 0.7–2.7) for 20–108.5 LEDs, and 2.7 (95% CI, 1.2–6.1) for > 108.5 LEDs (P for trend, 0.008) (see Table 2.1). This statistically significant trend remained when the low-exposure category of < 20 LEDs was used as the reference group

(*P* for trend, 0.04). The odds ratios for cancer of the lung did not vary by more than 10% after additional adjustment for non-farm occupational exposures, regular recreational physical activity, alcohol consumption, fruit and vegetable intake, body mass index, medical conditions, medical conditions in a first-degree relative including a history of cancer of the lung, race, state of residence, license type, and education.

Beane Freeman et al. (2005) explored the associations between exposure to diazinon and cancer at multiple sites in the AHS. Results were reported for the following cancers: colorectum, lung, prostate, melanoma, lympho-haematopoietic system, NHL, and leukaemia. Analyses included only male pesticide applicators who had completed the take-home questionnaire that included questions on duration and frequency of diazinon use. Of the 23 106 applicators included in the study, 4961 had reported using diazinon (21%). During the follow-up period ending in December 2002 (approximately 7 years of follow-up), 1269 incident cases of cancer were diagnosed. Poisson regression was used to calculate rate ratios for LEDs and IW-LEDs. For LEDs (categories: none; < 20; 20.0–38.8; > 38.8), increased risks for the highest tertile of exposure (> 38.8 LEDs) and significant trend tests were observed for all neoplasms [OR, 1.39 (95% CI, 1.09–1.78)]; for cancer of the lung [already reported by Alavanja et al. (2004) based on 1 year shorter follow-up]; and for leukaemia [OR, 3.36 (95% CI, 1.08–10.49)] (see Table 2.1). Additional adjustment for use of pesticides most highly correlated with diazinon (ethylene dibromide, aluminium phosphide, metalaxyl, chlordane, and dieldrin), pesticides for which the AHS had reported an increased risk of lympho-haematopoietic cancers and leukaemia (alachlor) (Lee et al., 2004), or cancer of the lung (chlorpyrifos, metolachlor, pendimethalin, and carbofuran) (Alavanja et al., 2004; Bonner et al., 2005), did not markedly alter the results. The exposure–response relationship for IW-LEDs was not as strong as for the reported LEDs. [The intensity index used gave particular weight to dermal exposure and not to the potentially more relevant respiratory exposure, and therefore may have introduced more random error.] No other reported cancer site (including colorectum, prostate, melanoma, and NHL) showed an association with diazinon for the highest tertile of exposure (see Table 2.1).

Engel et al. (2005) examined the association between use of pesticides and incidence of cancer of the breast among farmers' wives in the AHS. Participants were 30 454 women with no history of cancer of the breast before enrolment and excluded licensed pesticide users. Until 2000 (average follow-up, 4.8 years), 309 incident cases of cancer of the breast were identified. Analyses were repeated for two groups: all farmers' wives (n = 30 454), and farmers' wives who had never used pesticides (n = 13 449). For all farmers' wives, exposure was based on a spouse take-home questionnaire, including a question on never versus ever use of diazinon (potential direct exposure). For farmers' wives who had never used pesticides, exposure was based on the husband's enrolment questionnaire, including a question on never versus ever diazinon use (potential indirect exposure). Rate ratios were calculated for individual pesticides using Poisson regression. The relative risk for potential direct exposure to diazinon within the group of all farmers' wives (exposure prevalence, 10%) was 1.0 (95% CI, 0.7–1.5). Potential indirect (husband's) exposure to diazinon within the group of farmers' wives who had never used pesticides (exposure prevalence, 24%) was associated with an odds ratio of 1.4 (95% CI, 0.9–2.0). There was no apparent trend in relation to the husbands' cumulative use of diazinon and risk of cancer of the breast (relative risks not reported). Relative risks were also presented by state and by menopausal status (see Table 2.1), and none reached statistical significance. [The Working Group noted that an increased risk was only observed for indirect (husband's) exposure to diazinon, and not for women's personal (direct) use of diazinon, although the latter was

based on smaller numbers. The strengths of this study included the large sample size, comprehensive exposure assessment, control for potential confounders, and exploration of potential interactions such as family history.]

Lee et al. (2007) studied the risk of cancer of the colorectum associated with exposure to specific pesticides among 56 813 pesticide applicators (women, 2.7%) within the AHS, who were followed up until 31 December 2002, and included 212 incident cases of cancer of the colon and 93 incident cases of cancer of the rectum. Odds ratios for ever use of diazinon were 0.7 (95% CI, 0.5–1.0) for cancer of the colon, and 1.3 (95% CI, 0.8–2.2) for cancer of the rectum. [The Working Group noted that because the follow-up period for this report was the same as that for Beane Freeman et al. (2005), and Beane Freeman et al. had already reported on cancer of the colorectum specifically in relation to exposure to diazinon in the AHS, including detailed dose–response analyses, the results from Lee et al. were not included in Table 2.1. It should be noted, however, that Beane Freeman et al. (2005) reported only on pesticide applicators who completed the take-home questionnaire and for whom LEDs for diazinon could be calculated, while Lee et al. (2007) reported on ever exposure to diazinon based on double the number of study participants. Also, Lee et al. (2007) reported relative risks for cancer of the colon and rectum separately, while Beane Freeman et al. (2005) did not.]

Koutros et al. (2013) studied the risk of cancer of the prostate associated with exposure to specific pesticides among 54 412 male pesticide applicators within the AHS, who were followed up from 1993 to 2007 (approximately 12 years). A total of 1962 incident cases were identified, including 919 aggressive cancers of the prostate. Rate ratios were calculated by Poisson regression to evaluate lifetime use of 48 pesticides for which there were 15 or more exposed cases (incuding diazinon) and cancer of the prostate. Exposure assessment (quartiles of IW-LEDs based on the distribution of exposed cases) included exposure data from data collection phases 1 (1993–1997) and phase 2 (1999–2003 for private applicants in spouses, and 2003–2005 for commercial applicators) of the study. Relative risks were presented for diazinon, but did not show a dose–response association (see Table 2.1). [The Working Group noted that Beane Freeman et al. (2005) had already reported on the association between exposure to diazinon and cancer of the prostate in the AHS, but the study by Koutros et al. (2013) presented analyses that included an additional 5 years of follow-up and relative risk estimates for all cancers of the prostate, as well as aggressive prostate cancers specifically. Because this constituted additional information, the results are reported here and included in the tables.]

Alavanja et al. (2014a, b) reported on an update of the AHS to 31 December 2010 in North Carolina, and 31 December 2011 in Iowa (approximately 15–16 years of follow-up), with a focus on NHL and its subtypes. Analyses included 54 306 male pesticide applicators, among whom there were 523 incident cases of NHL classified into six subtypes using the Surveillance Epidemiology and End Results (SEER) coding scheme (i.e. 148 small B-cell lymphocytic lymphomas (SLL)/chronic B-cell lymphocytic lymphomas (CLL)/mantle cell lymphomas (MCL); 117 diffuse large B-cell lymphomas; 67 follicular lymphomas; 53 other B-cell lymphomas; 97 multiple myelomas; and 19 T-cell NHL and 22 undefined cell types, which were not analysed due to small numbers). Assessment of exposure to diazinon was based on the enrolment questionnaire (never versus ever), take-home applicator questionnaire (LEDs), and the phase 2 follow-up questionnaire. For participants who did not complete the phase 2 questionnaire, use of specific pesticides in phase 2 was imputed. Information on pesticide use from phase 1, phase 2, and imputation for phase 2 was used to construct three cumulative exposure metrics: (i) LEDs (i.e. the product of years of use

of a specific pesticide and the number of days used per year); (ii) IW-LEDs (i.e. the product of lifetime days of use and a measure of exposure intensity); and (iii) data on ever versus never use for each pesticide. Intensity was derived from an exposure algorithm (Coble et al., 2011). [The Working Group noted that these exposure-intensity estimates are not the same as those used in the AHS publications on cancer of the lung (Alavanja et al., 2004; Beane Freeman et al., 2005), the limitations of which were reported in Section 2.2.3.] Poisson models were fitted to estimate rate ratios for tertiles of exposure indices based on the distribution of all exposed cases of NHL, and compared with unexposed cases, for all NHLs, and for the five NHL subtypes. Only the pesticides for which there were 15 or more exposed cases of total NHL were evaluated (26 out of 50 pesticides, including diazinon). Of all cases of NHL, 28% were ever exposed to diazinon, with a rate ratio of 1.0 (95% CI, 0.8–1.3). Rate ratios for ever exposure to diazinon by NHL subtype were also reported, and showed no statistically significant associations (see Table 2.1). LEDs for diazinon were not associated with all NHL (see Table 2.1), but an exposure–response relationship was observed for follicular lymphoma (*P* for trend, 0.02) and suggestive for SLL/CLL/MCL (*P* for trend, 0.06). An exposure–response association was not observed for diffuse large B-cell lymphoma (*P* for trend, 0.72). Polytomous logit models indicated some heterogeneity across subtypes for diazinon, although this did not reach statistical significance ($P = 0.09$). The pattern of increased risk of follicular lymphoma with diazinon use remained after adjusting for tertiles of LEDs of lindane (which was the only other pesticide showing an exposure–response relationship for follicular lymphoma; $P = 0.04$), although the trend was not statistically significant (none: rate ratio, 1.0 (ref.); low: rate ratio, 4.1 (95% CI, 1.5–11.1); high: rate ratio, 2.5 (95% CI, 0.9–7.2); *P* for trend, 0.09).

Jones et al. (2015) reported on the association between exposure to diazinon and seven solid cancers, based on 15–16 years of follow-up of the AHS cohort [an additional 8–9 years of follow-up after the Beane Freeman et al. (2005) report on diazinon]. Included were 22 830 male pesticide applicators who completed the take-home questionnaire and for whom there was complete information for LEDs of diazinon based on exposure data from both data collection phases 2 (1999–2003 for private applicants in spouses, and 2003–2005 for commercial applicators) and phase 3 (2005–2010) of the study. For 28% of the cohort, exposure data from phase 2 were not available and were therefore imputed. Rate ratios were calculated through Poisson regression for tertiles of LED and IW-LED, for cancers of the lung, bladder, kidney, prostate, colon, rectum, and for melanoma. [This was the first report from the AHS on associations between exposure to diazinon and cancers of the bladder, kidney, and lung subtypes.] For cancers of the bladder, prostate, colon, rectum and melanoma, there was no evidence of a dose–response relationship (see Table 2.1). The positive dose–response relationship for cancer of the lung was consistent with previous AHS reports (see Table 2.1), and analyses by subtype suggested an association for adenocarcinoma (rate ratio, LED < median = 1.21, 95% CI, 0.57–2.57; rate ratio, LED ≥ median = 1.37, 95% CI, 0.75–2.51), but not for squamous cell carcinoma (see Table 2.1). For aggressive cancer of the prostate, the highest rate ratios were observed for the highest exposure tertile, without reaching statistical significance (see Table 2.1). For cancer of the kidney, the highest tertile of LEDs for diazinon was associated with a borderline increased risk (rate ratio, 1.77; 95% CI, 0.90–3.51). There was no substantive evidence that dieldrin or five additional most strongly correlated pesticide exposures (from among those with available usage information) were confounders in the reported key analyses for diazinon.

2.3 Case–control studies on lympho-haematopoietic cancers

Two large multicentre case–control studies were identified that reported on the association between specific pesticides, including diazinon, and lympho-haematopoietic cancers: the combined case–control studies in the midwest USA (Section 2.2.1), and the Cross-Canada Case–control Study (Section 2.2.2; see the *Monograph* on Malathion, Section 2.2, for a detailed description of these studies). The case–control studies in the Midwest USA were conducted in the 1980s, initially as three autonomous case–control studies in Iowa and Minnesota (Cantor et al., 1992), Kansas (Hoar et al., 1986), and Nebraska (Hoar Zahm et al., 1990). The study in Iowa and Minnesota included leukaemia and NHL, the study in Nebraska included NHL, Hodgkin lymphoma, multiple myeloma, and CLL, and the study in Kansas included NHL, soft tissue sarcoma, and Hodgkin lymphoma. The data on NHL from these studies were subsequently pooled, which increased the power enabling analyses for specific pesticides.

The Cross-Canada Case-control Study was conducted in the early 1990s, and included NHL, Hodgkin lymphoma, and multiple myeloma (and soft tissue sarcoma, which is covered in the next section) (see Table 2.2).

2.3.1 Studies in the midwest USA

(a) Leukaemia

Brown et al. (1990) reported on the leukaemia component of the case–control study in Iowa and Minnesota. [The analysis included CLL, now a recognized subtype of NHL.] During 1981–1984, all newly diagnosed cases of leukaemia among white men aged ≥ 30 years were ascertained from tumour registry or hospital records. Controls were a population-based stratified sample of white men without lymphatic or haematopoietic cancer, frequency-matched to the leukaemia and NHL cases by 5-year age group, vital status at time of interview, and state of residence. In-person interviews were conducted with the subjects or with close relatives if the subjects were deceased or unable to be interviewed. The questions regarding farming covered farm locations and the number and type of animals raised and crops cultivated. Information concerning the use of 24 animal insecticides, 34 crop insecticides, 38 herbicides, and 16 fungicides on the farm was also obtained, including the first and last year used, and whether the subject personally mixed or applied the pesticide. The number of days per year that each pesticide was used was not collected in the initial interview, but in a supplemental interview in 1987 (only Iowa) for 86 cases (23 living, 63 deceased) and 203 controls (146 living, 57 deceased). The total study population consisted of 578 cases (340 living, 238 deceased; 293 from Iowa, 285 from Minnesota) and 1245 controls (820 living, 425 deceased). The odds ratio comparing farmers who had mixed, handled, or applied diazinon as a crop insecticide to non-farmers (243 cases, 547 controls), was 1.2 (95% CI, 0.6–2.1). Odds ratios according to the number of days per year diazinon was handled were 2.1 (95% CI, 0.8–5.6) for 1–4 days, and 0.5 (95% CI, 0.1–2.4) for 5–9 days; there were no cases exposed for ≥ 10 days (see Table 2.2).

(b) NHL

Cantor et al. (1992) reported relative risks for NHL specifically for diazinon based on case–control studies in the midwest USA, including only the Iowa and Minnesota component (Brown et al., 1990). Between 1980 and 1983, a total of 622 newly diagnosed cases of NHL (white men aged ≥ 30 years) and 1245 population controls (frequency-matched by 5-year age group, vital status, state) were interviewed in-person (the questionnaire was completed by a proxy for 30% of cases and 34% of controls). Exposure to diazinon was defined as having ever personally handled, mixed, or applied diazinon on crops. The odds

Table 2.2 Case–control studies on lympho-haematopoietic cancers and exposure to diazinon

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	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%); white men, newly diagnosed, age ≥ 30 yr Controls: 1245 (response rate, 77–79%); white men, population-based; frequency matched on 5 year age group, vital status, state of residence Exposure assessment method: questionnaire; in-person interview with subject or proxy; farming and pesticide use history for subjects who worked on farm, listing 23 animal insecticides, 34 crop insecticides, 38 herbicides, 16 fungicides. Exposure defined as ever personally handled, mixed or applied; ORs for diazinon refer to use on crops	Leukaemia (including myelo-dysplasia)	Ever vs never use on crops Use (days/yr) 1–4 days/yr 5–9 days/yr ≥ 10 days/yr	17 8 2 0	1.2 (0.6–2.1) 2.1 (0.8–5.6) 0.5 (0.1–2.4) –	Vital status, age, state, tobacco use, family history of lympho-haematopoietic cancer, high-risk occupations, high-risk exposures	Studies in midwest USA Overlaps with Cantor et al. (1992) [Strengths: large study in farming area with high exposure prevalence; detailed questionnaire using list of specific pesticides and quantification of exposure; information on other potential risk factors collected. Limitations: for 41% of cases and 34% of controls the questionnaire was completed by a proxy, for whom recall of specific pesticide use will be problematic and subject to recall bias which may be different for cases and controls; multiple comparisons; self-reported pesticide use]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Cantor et al. (1992) Iowa and Minnesota, USA 1980–1983	Cases: 622 (response rate, 89%); white men newly diagnosed, age ≥ 30 yr Controls: 1245 (response rate, 77–79%); white men; population-based; frequency matched on: 5-year age group, vital status, state of residence Exposure assessment method: questionnaire; in-person interview with subject or proxy; farming and pesticide use history for subjects who worked on farm, listing 23 animal insecticides, 34 crop insecticides, 38 herbicides, 16 fungicides; exposure defined as ever personally handled, mixed or applied; ORs for diazinon refer to use on crops	NHL	Ever vs never As crop insecticide No personal protective equipment Before 1965 Before 1965, Iowa Before 1965, Minnesota	 27 17 14 10 4	 1.5 (0.9–2.5) 1.7 (0.9–3.2) 2.6 (1.2–5.9) 2.4 (0.9–6.2) 3.8 (0.7–22)	Vital status, age, state, smoking status, family history lymphopoietic cancer, high risk occupations, high risk exposures other than farming	Studies in midwest USA Overlaps with Brown et al. (1990) [Strengths: large study; in rural population; questionnaire using list of specific pesticides. Limitations: white men only; for 30% of cases and 34% of controls, the questionnaire was completed by a proxy, for whom recall of specific pesticide use would be problematic and subject to recall bias, which may be different for cases and controls]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Hoar Zahm et al. (1993) Nebraska, USA 1983–1986	Cases: 184 (response rate, 89%); Histologically confirmed cases of NHL diagnosed age ≥ 21 yr and identified through the Nebraska Lymphoma Study Group and area hospitals Controls: 707 (response rate, 86%); residents of the same area 3 : 1 frequency-matched by race, sex, vital status, age (5 yr) (matched to the four cancer sites included in the study i.e. NHL, HD, MM, CCL). For controls aged ≤ 65 yr: random-digit dialling. For living controls age ≥65 yr: Health Care Financing Administration (Medicare) records. Controls for deceased cases: Nebraska state mortality files matched on year of death (excluding causes of death: NHL, HD, MM, leukaemia, malignancy of unknown site, aplastic anaemia, suicide, homicide, legal intervention) Exposure assessment method: questionnaire	NHL	Exposed to diazinon Personally handled diazinon	7 2	1.9 4.1 (0.4–43.2)	Age	Studies in midwest USA [Strengths: the study included women exposed to pesticides. Limitations: relatively small size; number of proxy interviews not stated]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Waddell et al. (2001) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 748 (response rate, NR); white men, newly diagnosed age ≥ 21 yr (Iowa & Minnesota: 462; Kansas: 150; Nebraska: 136) Controls: 2236 (response rate, NR); white men, population-based, frequency matched on: 5-yr age group, vital status, state of residence (Iowa & Minnesota: 927; Kansas: 823; Nebraska: 486) Exposure assessment method: questionnaire; Iowa & Minnesota: see Cantor et al. (1992); Kansas: telephone interview, days/yr of pesticide use and years of use were asked about herbicides and insecticides overall, not by specific pesticide; subjects were asked to volunteer the pesticides they used; Nebraska: telephone interview days per year of use and years of use were asked for each pesticide used; asked about a predetermined list of about 90 pesticides	NHL	Ever use (incl. proxies)	60	1.7 (1.2–2.5)	Age, state of residence, respondent type (proxy/direct), except where otherwise stated	Studies in midwest USA (pooled) Iowa & Minnesota cases and controls overlap those in Cantor et al. (1992). Smaller numbers because of exclusions of those with missing data and those who did not know whether pesticides used [Strengths: large pooled study population; focus of pesticide exposure assessment; risk estimates excluding all proxy respondents are presented; analysis of subtype; cases were pathologically confirmed. Limitations: white men only. Pooled analyses of studies using different questionnaires (days/yr for each active ingredient only available in Iowa & Minnesota and Kansas); no list of pesticides in Kansas); proxies for 33% of cases and 41% of controls (however, risk estimates were presented excluding all proxies)]
			Ever use (excl. proxies)	44	1.3 (0.8–2.0)	Age, state of residence	
			Ever use, Iowa (excl. proxies)	22	1.1 (0.6–2.1)	Age	
			Ever use, Minnesota (excl. proxies)	5	1.3 (0.4–4.0)		
			Ever use, Kansas (excl. proxies)	1	13.0 (0.7–230.0)		
			Ever use, Nebraska (excl. proxies)	16	1.4 (0.7–2.9)		
			First use (excl. proxies): < 20 yr ago	20	1.1 (0.6–2.0)	Age, state of residence	
			First use (excl. proxies): ≥ 20 yr ago	16	1.4 (0.4–2.7)		
			Duration of use (excl. proxies): < 10 yr	20	0.9 (0.5–1.7)	Age, state of residence	
			Duration of use (excl. proxies): 10–19 yr	10	1.8 (0.7–4.4)		

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Waddell et al. (2001) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986 (cont.)			Duration of use (excl. proxies): ≥ 20 yr	1	1.9 (0.1–31.6)		
			Days/yr used (excl. proxies): < 5 days	6	1.3 (0.5–3.9)	Age	
			Days/yr used (excl. proxies): ≥ 5 days	6	2.4 (0.7–8.0)		
			Protective gear used (excl. proxies): yes	12	0.9 (0.4–1.9)	Age, state of residence	
			Protective gear used (excl. proxies): no	17	1.4 (0.7–2.8)		
		Follicular NHL	Ever use (excl. proxies)	17	1.3 (0.7–2.3)	Age, state of residence	
		Diffuse NHL	Ever use (excl. proxies)	13	1.2 (0.6–2.4)	Age, state of residence	
		SLL	Ever use (excl. proxies)	9	2.8 (1.1–7.3)	Age, state of residence	
		Other type NHL	Ever use (excl. proxies)	5	0.7 (0.3–2.0)	Age, state of residence	

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
De Roos et al. (2003) Iowa, Minnesota, Kansas, Nebraska, USA 1979–1986	Cases: 650 (response rate, 74.7%); cancer registries and hospital records; white men Controls: 1933 (response rate, 75.2%); random-digit dialling, Medicare, state mortality files; white men Exposure assessment method: questionnaire and interview (direct or next-of-kin); analyses focused on 47 pesticides to which ≥ 20 persons were exposed; any subject with a missing or "don't know" response for any of the 47 pesticides was excluded from all analyses	NHL	Ever use Logistic regression Hierarchical regression Joint effects of diazinon & atrazine Neither (ref.) Diazinon only Atrazine only Both diazinon and atrazine	 40 40 551 9 59 31	 1.9 (1.1–3.6) 1.7 (1.0–2.8) 1 1.2 (0.5–3.1) 1.5 (1.0–2.3) 3.9 (1.7–8.8)	Age, study site, all the other 46 pesticides to which 20 or more persons were exposed	Studies in midwest USA (pooled). Included participants from Cantor et al. (1992), Hoar Zahm et al. (1990), Hoar et al. (1986), and Brown et al. (1990) Included the same study population of Waddell et al. (2001). Analyses presented are different, with focus on exposure to multiple pesticides and whether there is a more than additive effect. Smaller numbers due to further exclusions (see exposure assessment notes) [Strengths: in addition to the strengths of Waddell et al. (2001), the strength of this analysis was the focus on exposure to multiple pesticides (realistic exposure scenarios), and adjustment of risk estimates for other pesticides. Limitations: In addition to the limitations of Waddell et al. (2001), a limitation of this analysis was that results excluding proxy respondents were not presented]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
McDuffie et al. (2001) Six provinces in Canada (Alberta, Saskatchewan, Manitoba, Quebec, Ontario, British Columbia) 1991–1994	Cases: 517 (response rate, 67.1%), from cancer registries and hospitals; males newly diagnosed, age ≥ 19 yr Controls: 1506 (response rate, 48%); random sample from health insurance and voting records; males age ≥ 19 yr, frequency-matched on province and ± 2 yr to the age distribution of entire case group (which also included soft tissue sarcoma, Hodgkin lymphoma, multiple myeloma) Exposure assessment method: questionnaire; self-administered postal questionnaire, followed by telephone interview for subjects with ≥ 10 hours per year of pesticide exposure and 15% random sample of the remainder; a list of chemical and brand names was mailed to these participants before the telephone interview; exposure defined as used it at work, in home garden or as hobby	NHL	Ever use	18	1.69 (0.88–3.24)	Age, province of residence, medical variables	Cross-Canada Case–control Study [Strengths: large number of cases; population-based; diversity in the occupational exposures; pathological material reviewed; collected information on the number of pesticides used; analysis of use of multiple pesticides; non-occupational use of pesticides considered. Limitations: potential recall bias; low response rate; multiple comparisons; no quantitative exposure data]

Table 2.2 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pahwa et al. (2012) Six provinces in Canada (Alberta, British Columbia, Manitoba, Ontario, Quebec, Saskatchewan) 1991–1994	Cases: 342 (response rate, 58%); men newly diagnosed (age ≥ 19 yr) Controls: 1506 (response rate, 48%); males (age ≥ 19 yr), frequency-matched to province and ± 2 yr to the age distribution of entire case group (which also included soft tissue sarcoma, Hodgkin lymphoma, NHL) Exposure assessment method: questionnaire, postal and telephone interview	Multiple myeloma (ICD-O M 9732/3)	Ever use	9	1.33 (0.59–3.01)	Age, province of residence, medical variables	Cross-Canada Case–control Study [Strengths: large study, detailed pesticide exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders; cases confirmed by pathology review. Limitations: men only; most exposed men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides; no quantitative exposure data]
Karunanayake et al. (2012) Six provinces in Canada (Alberta, Saskatchewan, Manitoba, Quebec, Ontario, British Columbia) 1991–1994	Cases: 316 (response rate, 68.4%); men, newly diagnosed, age ≥ 19 yr Controls: 1506 (response rate, 48%); males (age ≥ 19 yr), frequency-matched to province and ± 2 yr to the age distribution of entire case group (which also included soft tissue sarcoma, multiple myeloma, NHL) Exposure assessment method: questionnaire, postal and telephone interview	Hodgkin lymphoma	Ever use	10	2.08 (0.91–4.77)	Age, province of residence, medical variables	Cross-Canada Case–control Study [Strengths: large study, detailed pesticide exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders; cases confirmed by pathology review. Limitations: men only; most exposed men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides; no quantitative exposure data]

CLL, chronic B-cell lymphocytic lymphoma; IW-LED, intensity-weighted lifetime exposure days; LED, lifetime exposure days; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; SLL; small B-cell lymphocytic NHL; yr, year

ratios for ever use of diazinon was 1.5 (95% CI, 0.9–2.5), and 2.6 (95% CI, 1.2–5.9) for diazinon use before 1965 (see Table 2.2). Adjustment for pesticides from other families of agents did not alter the results. [Odds ratios by days per year of diazinon handling were not presented.]

Hoar Zahm et al. (1993) reported on the female component of the case–control study on NHL in Nebraska, which included 184 women diagnosed with NHL (1983–1986) and 707 controls (from multiple sources; see Table 2.2). For those reporting exposure to diazinon (7 cases, 16 controls) the odds ratio of 1.9 was not statistically significant [95% CI, not reported.] Only 2 cases and 2 controls reported personally handling diazinon (OR, 4.1; 95% CI, 0.4–43.2). [The Working Group noted that this was the only case–control study identified that reported relative risk estimates for cancer in women exposed to diazinon.]

Waddell et al. (2001) reported on the association between exposure to diazinon and NHL based on the pooled database of case–control studies in the midwest USA, including Iowa and Minnesota, Kansas, and Nebraska (see the *Monograph* on Malathion, Section 2.2, for a detailed description of these studies). The odds ratio for ever use of diazinon was 1.7 (95% CI, 1.2–2.5). After excluding all proxy interviews, the odds ratio was 1.3 (95% CI, 0.8–2.0). All subsequent analyses were conducted excluding proxy interviews. As indicated in the table, odds ratios were greater for higher number of years of use, higher number of days of use per year, and for use of diazinon without protective equipment, but none reached statistical significance. Results for ever use of diazinon were also presented by major subtype of NHL (follicular, diffuse, small lymphocytic, other), with SLL associated with an odds ratio of 2.8 (95% CI, 1.1–7.3). After adjusting for fonofos, the odds ratio was 2.5 (95% CI, 0.8–7.6), and after adjusting for malathion, the odds ratio was 2.7 (95% CI, 0.7–10.7). [The Working Group noted that pesticide-specific relative risks have been reported for the Iowa and Minnesota component of the study population (Cantor et al., 1992). Odds ratios were reported by Waddell et al. (2001) by study centre, and were also elevated for the centres not included in the publication by Cantor et al. (1992). The elevated odds ratios reported by Waddell et al. (2001) were thus not entirely attributable to the Iowa and Minnesota component of the study.]

De Roos et al. (2003) also reported on risk estimates for NHL and exposure to diazinon in the pooled case–control studies from the midwest USA, but the focus of analysis was on exposure to multiple pesticides. The odds ratio for ever exposure to diazinon, fully adjusted for exposure to 46 other pesticides, was 1.9 (95% CI, 1.1–3.6). [The Working Group noted that an odds ratio for ever use of diazinon in this study population had already been reported in Waddell et al. (2001). The odds ratio reported in the article by De Roos et al. (2003) suggested that it was not likely to be attributable to confounding by other pesticides, considering the detailed adjustment made for other pesticides. A limitation of this analysis was that results excluding proxy respondents were not presented, although it can be assumed that this analysis probably eliminated many of the proxy interviews because it excluded individuals with missing and "don't know" responses.] Of 48 pesticide combinations, joint effects were more than additive for carbofuran and atrazine; alachlor and atrazine; and diazinon and atrazine. With those never having used diazinon or atrazine as the reference group, the odds ratio for using diazinon and not atrazine was 1.2 (95% CI, 0.5–3.1; 9 exposed cases), the odds ratio for using atrazine was 1.5 (95% CI, 1.0–2.3; 59 exposed cases), and the odds ratio for using both diazinon and atrazine was 3.9 (95% CI, 1.7–8.8; 31 exposed case), indicative of a more than additive effect.

2.3.2 Cross-Canada Case–control Study of Pesticides and Health

(a) NHL

McDuffie et al. (2001) reported the results for NHL (517 incident cases, 1506 population controls) in the Cross-Canada Case–control Study (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Exposure, defined as use of diazinon at work, in the home garden or as a hobby, was associated with an odds ratio of 1.69 (95% CI, 0.88–3.24).

(b) Multiple myeloma

Pahwa et al. (2012) reported the results for multiple myeloma (342 cases, 1506 controls) in the Cross-Canada Case–control Study (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Ever use of diazinon was associated with an odds ratio of 1.33 (95% CI, 0.59–3.01).

(c) Hodgkin lymphoma

Karunanayake et al. (2012) reported the results for Hodgkin lymphoma (315 cases, 1506 controls) in the Cross-Canada Case–control Study (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study). Ever use of diazinon was associated with an odds ratio of 2.08 (95% CI, 0.91–4.77).

2.4 Case–control studies on other cancers

Estimates of risk associated with exposure to diazinon based on a case–control study have been reported for cancers other than lympho-haematopoietic cancers, including soft tissue sarcoma, cancer of the prostate, and cancer of the brain in childhood and in adults (see Table 2.3).

2.4.1 Soft tissue sarcoma

Pahwa et al. (2011) reported the results for soft tissue sarcoma in the Cross-Canada Case–control Study (357 cases, 1506 population controls). Exposure, defined as used diazinon at work, in the home garden or as a hobby, was associated with an odds ratio of 3.31 (95% CI, 1.78–6.23). Aldrin was the only other agent for which a statistically significant association with soft tissue sarcoma was observed and the odds ratio for diazinon did not change substantially after adjustment for use of aldrin (OR, 3.19; 95% CI, 1.69–6.01).

2.4.2 Cancer of the prostate

Band et al. (2011) reported the results of a case–control study that included 1516 patients with cancer of the prostate and 4994 age-matched controls comprising patients with cancer at any other site except lung and cancers of unknown primary site (1153 cases and 3999 controls were included in the final analysis). A total of 47 cases (3.1%) and 109 controls (2.2%) was assessed as being exposed to diazinon (OR, 1.43; 95% CI, 0.99–2.07). By exposure index, the association reached statistical significance for the group with highest exposure (low exposure: OR, 0.91; 95% CI, 0.50–1.68; high exposure: 1.93; 95% CI, 1.21–3.08; *P* for trend, 0.02) compared with never exposed. Similar dose–response relationships were observed for 6 out of 15 fungicides, 3 out of 6 herbicides, and 6 other insecticides out of the total of 19 insecticides. [The Working Group noted that this paper reported high correlation between specific pesticides as assessed through a job-exposure matrix. This, together with the large number of pesticides showing dose–response relationships similar to diazinon, suggested that associations for specific pesticides may have been due to intercorrelation with other pesticides.]

Table 2.3 Case-control studies of other cancers and exposure to diazinon

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Pahwa et al. (2011) Six provinces in Canada (Alberta, Saskatchewan, Manitoba, Quebec, Ontario, British Columbia) 1991–1994	Cases: 357 (response rate, 60.8%); men newly diagnosed, age ≥ 19 yr Controls: 1506 (response rate, 48.0%); men age ≥ 19 yr, frequency matched to province and ± 2 yr to the age distribution of entire case group (which also included NHL, Hodgkin lymphoma, multiple myeloma) Exposure assessment method: self-administered postal questionnaire and telephone interview for subjects with ≥ 10 hours/yr of pesticide exposure and 15% random sample of the remainder; a list of chemical and brand names was mailed to these participants before the telephone interview; exposure defined as use at work, in home garden, or as hobby	Soft tissue sarcoma	Ever use Ever use	20 20	3.31 (1.78–6.23) 3.19 (1.69–6.01)	Age, province of residence, medical variables Age, province of residence, whooping cough, first-degree relative with cancer, aldrin user	Cross-Canada Case-control Study Results presented by soft tissue sarcoma subtype [Strengths: large study, detailed pesticide exposure assessment through telephone interview; deceased were ineligible, reducing the number of surrogate responders. Limitations: men only; most exposed men were exposed to multiple pesticides and multiple classes of pesticides, but risk estimates were not adjusted for other pesticides]

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Band et al. (2011) British Columbia, Canada 1983–1990	Cases: 1153 (response rate, NR); 941 (response rate, 82%) direct respondents; British Columbia cancer registry; with histological confirmation Controls: 3999 (response rate, NR); male cancer patients from the same registry with cancers other than prostate, excluding lung cancer and cancer of unknown primary site Exposure assessment method: JEM; lifetime occupational history was obtained through a self-administered questionnaire and used in conjunction with a JEM to estimate the participants' lifetime cumulative exposure to approximately 180 active compounds in pesticides	Prostate	Ever use	47	1.43 (0.99–2.07)	Age, alcohol consumption, cigarette years, respondent (direct/proxy), education	[Strengths: large number of cases and controls; histologically confirmed incident cancer cases; use of cancer controls which may have limited differential recall; use of JEM limiting differential exposure misclassification; study was conducted before the period of early detection of prostate cancer. Limitations: use of cancer controls; included cancers that may be associated with pesticide exposure; lack of information on family history; potential exposure misclassification; multiple comparisons; use of JEM to assess pesticide exposure resulting in high correlations between specific pesticides; associations for specific pesticides may be due to intercorrelations with other pesticides]
			By exposure index				
			Never use (ref.)	1106	1		
			Low	15	0.91 (0.5–1.68)		
			High	32	1.93 (1.21–3.08)		
			Trend-test *P* value: 0.02				

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Davis et al. (1993) Missouri, USA 1985–1989	Cases: 45 (response rate, NR); cases aged 0–10 yr identified through the population-based Missouri cancer registry Controls: 85 friend controls, 108 cancer controls (response rate, NR) Exposure assessment method: questionnaire; during telephone interviews the biological mothers of cases and controls were asked about the number of times that pesticides had been used for nuisance pests in the home, garden or on pets, during pregnancy, during the interval from birth to age 6 mo, and since age 7 mo, and age of diagnosis; respondents were also asked whether several specific pesticide products had been used at any time from pregnancy to diagnosis	Brain, childhood	In garden or orchard (friend controls) In garden or orchard (cancer controls)	7 7	4.6 (1.2–17.9) 1.4 (0.4–4.7)	Age, environmental tobacco smoke, family income, father's education, mother's education, family member in construction industry, time between diagnosis and interview	[Strengths: study focused on home use of pesticides; during the relevant exposure period, diazinon was widely used as a garden and in-house insecticide; use of individual pesticides, including diazinon, in home and garden was assessed; use of both friend controls and cancer controls. Limitations: very small size; high risk estimates using friend controls (when compared with cancer controls) were likely due to differential recall of parents' use of pesticides between those with sick and healthy children]

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Carreón et al. (2005) Iowa, Michigan, Minnesota, Wisconsin, USA 1 January 1995 – 31 January 1997	Cases: 341 (response rate, 90%); female patients with a histologically confirmed primary intracranial glioma Controls: 527 (response rate, 72%); women with no diagnosis of glioma randomly selected within 10-yr age group strata frequency matching within the state; selection from the state driver's licence/non-drivers identification records (for those aged 18–64 yr) and from Medicare (aged 65–80 yr) Exposure assessment method: questionnaire; postal questionnaire with a list of pesticides – including diazinon – and collecting lifetime pesticide use in farming and non-farming jobs, in the house and the garden. Followed by an interview collecting additional information (first year of use, number of years of use, days per year of use, use on animals and crops, use on buildings or lots)	Brain, intracranial glioma (ICD-O 938–948)	Ever use (incl. proxies) Ever use (excl. proxies)	18 13	1.3 (0.7–2.5) 1.9 (0.9–4.1)	Age, 10-yr age group, education, other pesticides	Upper Midwest Health Study [Strengths: large size for a brain cancer study; first study to look at the association between farm pesticide exposure and glioma in adult women; extensive questionnaire on farm and rural risk factors and pesticide use; cases histologically confirmed and limited to glioma. Limitations: self-reported ever use of specific pesticides; controls older than cases; large proportion of proxy respondents (43% of cases, 2% of controls)]

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Yiin et al. (2012) Iowa, Michigan, Minnesota, Wisconsin, USA 1995–1997	Cases: 798 (response rate, 93%); patients with a histologically confirmed primary intracranial glioma identified through participating medical facilities and offices of neurosurgeon Controls: 1175 (response rate, 70%); selected from the state driver's license/nondriver identification records and centres for Medicare services Exposure assessment method: questionnaire; based on self-report	Brain, intracranial glioma (ICD-O 938–948)	Ever use				
			In non-farm job (incl. proxies)	10	0.61 (0.29–1.29)	Age, 10-yr age group, education, sex, farm pesticide exposure yes/no	Upper Midwest Health Study [Strengths: large number of cases; extensive questionnaire on farm and rural risk factors and pesticide use; population-based design; cases histologically confirmed and limited to glioma. Limitations: controls older than cases; large proportion of proxy respondents (45% of cases)]
			In non-farm job (excl. proxies)	8	0.81 (0.35–1.87)		
			In house and garden (incl. proxies)	57	0.66 (0.47–0.92)		
			In house and garden (excl. proxies)	36	0.75 (0.50–1.12)		

Table 2.3 (continued)

Reference, location follow-up/ enrolment period, study-design	Population size, description, exposure assessment method	Organ site (ICD code)	Exposure category or level	Exposed cases/ deaths	Risk estimate (95% CI)	Covariates controlled	Comments
Lee et al. (2004) Nebraska, USA 1988–1993	Cases: 170 stomach and 137 oesophageal (response rates, 79%, 88%); identified from Nebraska cancer registry and discharge diagnosis and pathology records at 14 hospitals, incident cases age ≥ 21 yr Controls: 502 (response rate, 72%); population controls from a previous case–control study in Nebraska (see Hoar Zahm et al., 1990), from random-digit dialling, and from Medicare files Exposure assessment method: questionnaire; used a list of 16 major insecticides and 14 herbicides used on Nebraska crops over the previous 40 yr including diazinon	Stomach Oesophagus	Ever use Ever use	6 10	0.5 (0.2–1.2) 0.8 (0.4–1.8)	Age, sex	[Strengths: high response rate; use of set list of pesticides in interview. Set in rural area and therefore reasonable exposure prevalence. Limitations: high percentage next-of-kin interviews for whom recall of specific pesticides used will be problematic; self-reported pesticide use; possible misclassification of exposures]

excl., excluding; incl., including; IW-LED, intensity-weighted lifetime exposure days; LED, lifetime exposure days; mo, month; NHL, non-Hodgkin lymphoma; yr, year

2.4.3 Cancer of the brain in childhood

Davis et al. (1993) reported the results of a case–control study that included 45 cases of childhood cancer of the brain (age, 0–10 years), 85 friend controls and 108 cancer controls (predominantly acute lymphoblastic leukaemia), diagnosed in 1985–1989, and interviews were conducted in 1989–1990. During telephone interviews, the biological mothers of cases and controls were asked about the number of times that pesticides had been used for nuisance pests in the home, garden, or on pets, during pregnancy, during the interval from birth to age 6 months, and since age 7 months, and age of diagnosis. Respondents were also asked whether several specific pesticide products had been used at any time between pregnancy and diagnosis. Of the 45 mothers of cases, 7 reported the use of diazinon in the garden or orchard at any time between pregnancy and diagnosis. When compared with friend controls, this yielded an odds ratio of 4.6 (95% CI, 1.2–17.9), and an odds ratio of 1.4 (95% CI, 0.4–4.7) when compared with cancer controls. [The Working Group noted that this was a very small study, but was conducted at a time when diazinon was still widely used in and around the home. The high risk estimate using friend controls as compared with cancer controls suggested differential recall of parents' use of pesticides for sick or healthy children.]

Leiss & Savitz (1995) reported on a case–control study on home pesticide use and childhood cancer. Results specifically for diazinon were not presented, and an association between treatment of the yard (lawn/garden) and cancer of the brain was not observed in this study.

Pogoda & Preston-Martin (1997) reported on a population-based case–control study of childhood tumours of the brain in Los Angeles County, California, USA, that involved 224 cases (diagnosed 1984–1991) and 218 controls; however, the exposure prevalence of diazinon as a garden insecticide was low, and risk estimates for diazinon were not reported.

2.4.4 Cancer of the brain in adults

The association between exposure to farm pesticides and risk of intracranial glioma in adults was studied in the Upper Midwest Health Study (UMHS) (see the *Monograph* on Malathion, Section 2.2, for a detailed description of this study).

Ruder et al. (2004) reported on the UMHS and included 457 male incident cases of intracranial glioma and 648 population controls aged 18–80 years. Proxy interviews were completed for 47% of the cases. Diazinon was among the 14 individual farm pesticides to which the most participants were exposed. Statistically significant associations were not observed for any of these pesticides, either with or without proxy respondents, and the pesticide-specific results were not reported.

Carreón et al. (2005) reported on the UMHS and included 341 female incident cases of intracranial glioma and 528 controls. Reported agricultural use of diazinon was associated with an odds ratio of 1.3 (95% CI, 0.7–2.5), and 1.9 (95% CI, 0.9–4.1) if all proxy interviews (43% of cases and 2% of controls) were excluded from analyses, adjusting for age, education, and any other pesticide exposure.

Yiin et al. (2012) reported on the UMHS and included men and women (798 cases and 1175 controls), aiming to improve on the pesticide exposure assessment to yield a quantitative estimated lifetime cumulative exposure (gram-years), and also investigating non-farm use of pesticides. Positive associations between risk of glioma and estimated quantitive exposure to any of the individual pesticides were not observed and odds ratios were not reported. Ever non-farm occupational use of diazinon was not associated with an increase in risk of glioma (see Table 2.3), nor was house and garden use of diazinon (see Table 2.3).

Table 3.1 Studies of carcinogenicity with diazinon in mice

Species, strain (sex) Duration Reference	Dosing regimen Animal/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M, F) 105–106 wk NTP (1979)	Diet containing diazinon at concentrations of 0 (vehicle control), 100, or 200 ppm, ad libitum, for 103 wk 50 M and 50 F/treated group, and 25 M and 25 F/matched-control group (age, 6 wk)	*Males* Hepatocellular adenoma: 1/21 (5%), 0/46, 3/48 (6%) Hepatocellular carcinoma: 4/21 (19%), 20/46 (43%)*, 10/48 (21%) Hepatocellular adenoma or carcinoma (combined): 5/21 (24%), 20/46 (43%), 13/48 (27%) *Females* No exposure-related increase in tumour incidence	*Males* *P = 0.046 (Fisher exact test) *Females* NS	Purity, 98% No significant increase in mortality in treated mice. The occurrence of hepatocellular carcinoma could not clearly be related to the administration of diazinon Incidence of hepatocellular carcinoma in historical controls, males: 498/2334 (21.3%); range, 8–36% (Haseman et al., 1984)

F, female; M, male; NS, not significant; wk, week

2.4.5 Cancer of the stomach and oesophagus

Lee et al. (2004) reported on a case–control study of incident cases of cancer of the stomach (n = 170) and oesophagus (n = 137) from Nebraska (1988–93) and 502 population controls. Compared with non-farmers, self-reported ever use of diazinon was associated with an odds ratio of 0.5 (95% CI, 0.2–1.2; 6 exposed cases) for cancer of the stomach, and 0.8 (95% CI, 0.4–1.8; 10 exposed cases) for cancer of the oesophagus.

2.5. Meta-analysis

Schinasi & Leon (2014) conducted a systematic review and meta-analysis of NHL and occupational exposure to agricultural pesticides, including diazinon. The meta-analysis for diazinon included three studies (McDuffie et al., 2001; Waddell et al., 2001; Mills et al., 2005a), and yielded a meta risk-ratio of 1.6 (95% CI, 1.2–2.2) with an I^2 value of 0% [indicating no inconsistency between studies].

3. Cancer in Experimental Animals

3.1 Mouse

See Table 3.1

Groups of 50 male and 50 female B6C3F$_1$ mice (age, 6 weeks) were given diets containing diazinon (purity, 98%; dissolved in acetone) at a concentration of 100 or 200 ppm, ad libitum, for 103 weeks, and then held for an additional 2–3 weeks for observation; a group of 25 male and 25 female B6C3F$_1$ mice served as matched controls (NTP, 1979). Survival was 98% (49/50), 90% (45/50), and 84% (21/25) among the males, and 98% (49/50), 100% (50/50), and 96% (24/25) among the females in the groups at the higher and lower dose, and control group, respectively, at week 78. Mean body weights of the treated male and female mice were essentially the same as those of the corresponding controls except for the last 20 weeks of the bioassay, when the mean body weights of the treated females were lower than those of the controls. In males, there was an increase in the incidence of hepatocellular carcinoma, with the incidence at the lower dose (20/46; 43%) being significantly increased (P = 0.046, Fisher exact test) compared with the controls

Table 3.2 Studies of carcinogenicity with diazinon in rats

Species, strain (sex) Duration Reference	Dosing regimen Animal/group at start	Incidence of tumours	Significance	Comments
Rat, F344 (M, F) 104–105 wk NTP (1979)	Diet containing diazinon at concentrations of 0 (vehicle control), 400, or 800 ppm, ad libitum, for 103 wk 50 M and 50 F/treated group, and 25 M and 25 F/matched-control group (age, 7 wk)	*Males* Leukaemia or lymphoma (combined): 5/25 (20% [all leukaemias]), 25/50 (50%)* [leukaemia, 24/50, lymphoma, 1/50], 12/50 (24%) *Females* No exposure-related increase in tumour incidence	*Males* *P = 0.011 (Fisher exact test) *Females* NS	Purity, 98% No significant increase in mortality in treated animals The occurrence of haematopoietic malignancies could not clearly be related to the administration of diazinon Historical control incidence, leukaemia or lymphoma (combined), males: 699/2320 (30.1%); range, 0–46% (Haseman et al., 1984)
Rat, Sprague-Dawley (M, F) 98 wk EPA (1993)	Diet containing diazinon at concentrations of 0 (vehicle control), 0.1, 1.5, 125, or 250 ppm, ad libitum, for 98 wk 20 M and 20 F/group (age, 9 wk)	No exposure-related increase in the incidence of any neoplasm	NS	Purity, 87.7% (impurities not reported) At 97 wk, survival in males was 45%, 30%, 50%, 35%, and 58%, respectively; and survival in females was 58%, 40%, 44%, 68%, and 58%, respectively. Because mortality was higher in the groups at low doses than in the controls, the study was terminated at wk 97

F, female; M, male; NS, not significant; wk, week

(4/21; 19%). [The Working Group concluded that the increase in the incidence of hepatocellular carcinoma could not clearly be related to the administration of diazinon because it was only observed in males at the lower dose, and the incidence was slightly above the upper limit of the range for historical controls in this strain of mice (incidence of hepatocellular carcinoma in historical controls, 498/2334; 21.3%; range, 8–36%; Haseman et al., 1984).] In females, there was no exposure-related increase in tumour incidence.

3.2 Rat

See Table 3.2

Groups of 50 male and 50 female F344 rats (age, 7 weeks) were given diets containing diazinon (purity, 98%; dissolved in acetone) at a concentration of 100 or 200 ppm, ad libitum, for 103 weeks, and then held for an additional 2–3 weeks for observation; a group of 25 male and 25 female F344 rats served as matched controls (NTP, 1979). Survival in male rats was 49/50 (98%) in each treated group, and 24/25 (96%) in the control group at week 78. Survival in female rats was 44/50 (88%) in of each treated group, and 23/25 (92%) in the control group at week 78. Mean body weights of the treated groups of males and females were essentially the same as those of the corresponding controls. In males, there was a significant increase (P = 0.011, Fisher exact test) in the incidence of leukaemia or lymphoma (combined) in rats at the lower dose: 25/50; 50% (leukaemia, 24/50; lymphoma, 1/50) versus 5/25 (all leukaemias) in controls. [The Working Group concluded that the increase in the incidence of

haematopoietic malignancies could not clearly be related to the administration of diazinon because it was observed only in males at the lower dose, and the incidence was slightly above the upper limit of the range for historical controls in this strain of rats (incidence of haematopoietic malignancies in historical controls, 699/2320; 30.1%; range, 0–46%; Haseman et al., 1984).] In females, there was no exposure-related increase in tumour incidence.

The United States Environmental Protection Agency (EPA) provided information on a long-term study in which groups of 20 male and 20 female Sprague-Dawley rats (age, 9 weeks), were given diets containing diazinon (purity, 87.7%; impurities not reported; dissolved in acetone) at a concentration of 0 (control), 0.1, 1.5, 125, or 250 ppm, ad libitum, for 98 weeks (EPA, 1993). There was no adverse effect on body weight in treated rats. At week 97, survival in males was 45% (controls), 30%, 50%, 35%, and 58% in each group, respectively; while survival in females was 58% (controls), 40%, 44%, 68%, and 58%, respectively. Because mortality was higher at the low doses than in the controls, the study was terminated at week 97. There was no exposure-related increase in the incidence of any neoplasm in groups of treated rats compared with controls (EPA, 1993). [The Working Group noted that mortality was higher in rats treated with low doses than in controls, and that the duration of the study was only 97 weeks.]

4. Mechanistic and Other Relevant Data

4.1 Toxicokinetic data

An extensive literature was available on the toxicokinetics of diazinon in humans and in experimental animals.

4.1.1 Absorption

(a) Humans

Dermal exposures resulting from occupational practices and oral exposures from diet are important in humans; there were limited data on exposure to diazinon by inhalation (Knaak et al., 2004; Alavanja et al., 2013). The evidence for absorption of organophosphate pesticides, such as diazinon, has been documented in a large number of biomonitoring studies (Cocker et al., 2002). To cite one example, a cohort of pregnant women belonging to urban minorities in New York City, USA, was evaluated for diazinon exposure by measuring the diazinon levels in personal air samples, and in maternal and umbilical cord sera (Whyatt et al., 2005). Diazinon was detected in 100% of the personal air samples, and in 45% and 44% of the maternal blood and cord blood samples, respectively, with average (± standard deviation) concentrations of 1.3 ± 1.8 pg/g and 1.2 ± 1.4 pg/g, respectively, as assessed by gas chromatography-mass spectrometry (GC-MS) analysis. [The Working Group noted that these data indicated that absorption of diazinon and subsequent internal exposures can occur in humans, and that the developing fetus might also be exposed.]

Diazinon can be absorbed from the gastrointestinal tract by mammals, including humans, via passive diffusion (Poet et al., 2004). Rapid absorption of diazinon was observed after an oral dose of 0.011 mg/kg bw in five volunteers, as shown by the excretion of approximately 60% of the administered dose as dialkylphosphate metabolites in the urine. Most of the administered dose was recovered within 14 hours after dosing (Garfitt et al., 2002). In a woman who intentionally consumed a lethal amount of diazinon (estimated dose, 293 mg/kg bw), diazinon was detected in several tissues (Poklis et al., 1980).

Diazinon was not absorbed very efficiently into the body after dermal exposure; only ~4% of the administered dose of [^{14}C]-labelled diazinon

(vehicle, acetone) was absorbed through the skin of the ventral forearm of volunteers during a 24-hour exposure period (Wester et al., 1993). One possible reason for the poor rate of dermal absorption was that the experimentally determined dermal permeability coefficient for diazinon in human skin ($K_p \approx 1 \times 10^{-9}$ cm/s) was similar to the desquamation rate of skin (Sugino et al., 2014), thus reducing the rate of penetration by diazinon.

The number of studies of dermal absorption in vitro with diazinon was limited. One study in vitro indicated that the absorption of diazinon though human skin was 20% of the applied dermal dose (Moody & Nadeau, 1994).

Other studies evaluated biomarkers of exposure and indicators of absorption, including plasma cholinesterase activity (and decrements thereof) (Poet et al., 2004) and urinary organophosphate metabolites. After oral (11 µg/kg bw) and dermal (100 mg, occluded dermal dose) exposures of human volunteers to diazinon, peak urinary concentrations of diethylphosphate occurred at 2 hours and 12 hours, respectively (Garfitt et al., 2002). Under acidic conditions (pH 1), similar to those in the stomach, diazinon steadily decreased in concentration due to acid-catalysed hydrolysis, exhibiting a half-life of ~90 minutes (Garfitt et al., 2002). [The Working Group noted that this suggested that some degradation of diazinon would occur in the stomach after oral exposures, and that a fraction of the diethylphosphate and IMPY generated in the body might be formed in the stomach.] These metabolites can be readily absorbed from the gastrointestinal tract in rats (Timchalk et al., 2007).

Using the human Caco-2 cell line, a widely used cell model to study intestinal absorption and transport, the levels of P-glycoprotein, which is a xenobiotic transporter that is expressed on the cell surface, were found to be upregulated by diazinon at low concentrations (Lecoeur et al., 2006). [The Working Group noted this suggested that intestinal absorption of diazinon might be reduced after long-term oral exposure to diazinon as a result of enhanced efflux from enterocytes, thus limiting systemic exposure.]

(b) Experimental systems

In male Sprague-Dawley rats exposed orally, diazinon (100 mg/kg bw) was well absorbed from the gastrointestinal tract, as shown by the marked reduction (< 20% of the control values) in plasma cholinesterase activity at 6 hours after dosing (Poet et al., 2004). When male and female Wistar rats were given [^{14}C]-labelled diazinon either as a single oral dose of 4 mg/kg bw or as daily doses of 0.5 mg/kg bw for 10 consecutive days, the rapid absorption of diazinon was shown by the excretion of a large amount of radiolabel in the urine (Mücke et al., 1970). Similar results were obtained in female beagle dogs given a single oral dose of [^{14}C]-labelled diazinon at 4.0 mg/kg bw; absorption was ~85% of the administered radiolabelled dose (Iverson et al., 1975). Toxicokinetic studies in rats (Sprague-Dawley or Wistar strains) and mice (ddy strain) indicated that maximum concentrations of diazinon in blood are reached 1–2 hours after oral and intraperitoneal dosing (Tomokuni et al., 1985; Poet et al., 2004). The oral bioavailability of diazinon in the rat was relatively low (~36%), which was determined by comparing the area under the curve from time-course levels of diazinon in blood after oral and intravenous dosing (Wu et al., 1996).

Rates of dermal absorption of [^{14}C]-labelled diazinon in rats and hairless guinea-pigs in vivo were 56% and 28% of the applied radiolabelled dose, respectively (Moody & Nadeau, 1994); these values are noticeably higher than those for humans (Wester et al., 1993).

4.1.2 Distribution

(a) Humans

Poklis et al. (1980) detected diazinon in tissues (blood, bile, adipose, liver, brain, and kidney) after intentional oral ingestion of diazinon. No other data on tissue distribution of diazinon in humans were available to the Working Group.

(b) Experimental systems

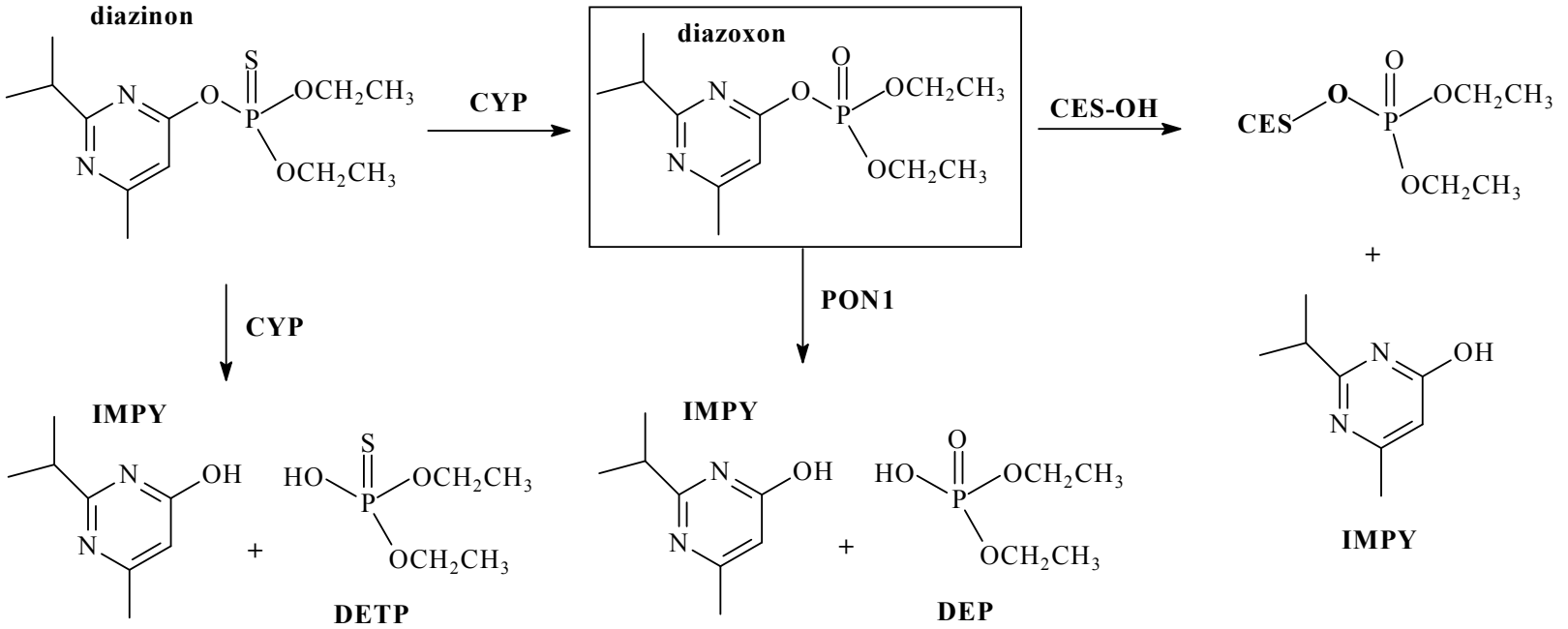
In experimental animals, diazinon is widely distributed to tissues after absorption. The elimination half-life of diazinon in the blood of male Wistar rats given intraperitoneal doses of 20 mg/kg bw or 100 mg/kg bw was estimated to be 4 hours and 6 hours, respectively (Tomokuni et al., 1985). Similarly, immediately after administration of intravenous (10 mg/kg bw) and oral (80 mg/kg bw) doses in rats, plasma concentrations of diazinon indicated half-lives of 4.7 and 2.9 hours, respectively (Wu et al., 1996). Most diazinon in the plasma (89%) is bound non-covalently to albumin and other plasma proteins (Wu et al., 1996; Poet et al., 2004). By 8 hours after intravenous administration (20 mg/kg bw) to rats, the concentration of diazinon was significantly higher in the kidney than in the liver, or brain (Tomokuni et al., 1985). After intravenous dosing (1 or 10 mg/kg bw), diazinon was distributed and eliminated rapidly in male Sprague-Dawley rats, and concentrations of diazinon in saliva were comparable to plasma concentrations of non-protein-bound diazinon (Lu et al., 2003).

4.1.3 Metabolism

(a) Overview of metabolism of diazinon

Organophosphate pesticides are subject to similar metabolic pathways in humans and experimental animals in vivo (Casida & Quistad, 2004); see also Section 4.1.3 of the *Monograph* on Malathion in the present volume. Biotransformation of organophosphates occurs primarily in the liver, and to a lesser extent in the small intestine, after oral exposure (Barr & Angerer, 2006). After absorption by the dermal or oral route, diazinon is rapidly biotransformed by several enzymes – including cytochrome P450 (CYP), paraoxonases, and carboxylesterases (CES) – to water-soluble metabolites that are rapidly eliminated (see Fig. 4.1). Both desulfuration and dearylation of diazinon are mediated by CYP. The bioactive diazoxon metabolite can be detoxified by paraoxonase (PON1)-catalysed reactions (Costa et al., 2013), yielding alcohol and diethylphosphate products. Alternatively, diazoxon can be subject to inhibition of CES function (Crow et al., 2012; Fig. 4.1). The oxon metabolite can escape detoxication by CES or PON1 in the liver and instead covalently modify (and inhibit) various serine hydrolase enzymes, including the B-esterase targets butyrylcholinesterase, acetylcholinesterase, and CES (Casida & Quistad, 2004; see Fig. 4.2). The bioactive oxon metabolite is generated by CYP-catalysed desulfuration (Buratti et al., 2005; Barr & Angerer, 2006). If the oxon is not degraded by hepatic paraoxonase or carboxylesterases, it can escape the liver and instead covalently modify (and inhibit) various serine hydrolase enzymes, including the B-esterase targets butyrylcholinesterase, acetylcholinesterase, and carboxylesterases (Casida & Quistad, 2004; see Fig. 4.2). Generation of the oxon metabolite is a bioactivation reaction, because the oxon is a much more potent inhibitor of B-esterases than the parent compound (Casida & Quistad, 2004). In general, analytical measurement of the oxons in blood is difficult due to the small quantities of metabolite that are formed and its relative instability (Timchalk et al., 2002). Nevertheless, the oxons are potent inhibitors of serine hydrolases, exhibiting bimolecular rate constants of inhibition varying from 10^3 to 10^7 $M^{-1}s^{-1}$, depending on the hydrolase and the specific oxon (Casida & Quistad, 2004; Crow et al., 2012). Most important with respect to the insecticidal and toxicological activity of the oxon is acetylcholinesterase, the

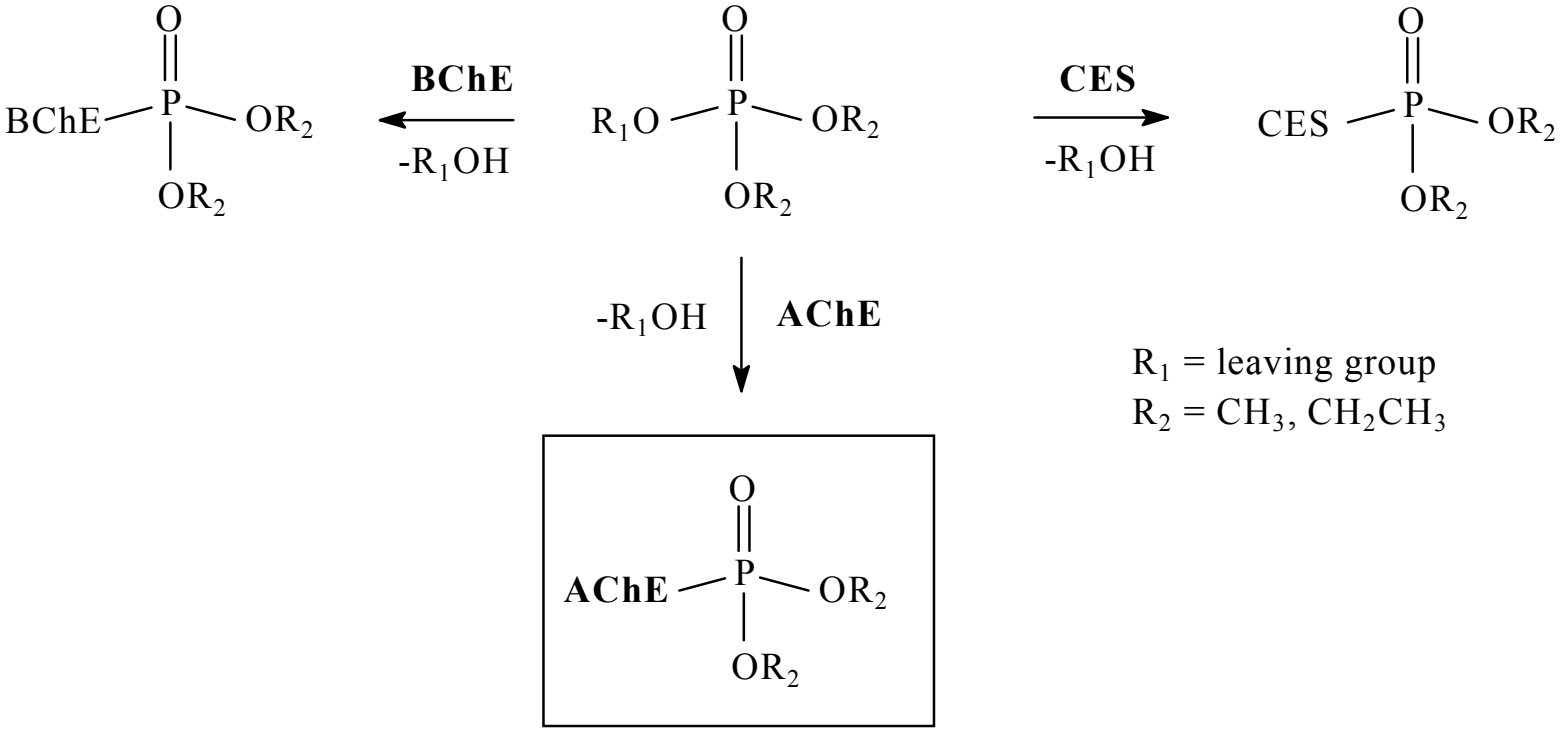
Fig. 4.1 Biotransformation of diazinon



From Poet et al. (2004); copyright (2004), modified with permission from Elsevier

Reactions catalysed by cytochrome P450 (CYP) produce the desulfuration metabolite (oxon) or aryl alcohol and dialkylthiophosphate products. Paraoxonase-1 (PON1) and carboxylesterase (CES) contribute to diazoxon metabolism reactions. The bioactive diazoxon metabolite is indicated by the box. 2-Isopropyl-4-methyl-6-hydroxypyrimidine (IMPY) is the dearylation product and the major metabolite of diazinon. CES-OH indicates carboxylesterase with –OH being the functionality of the active-site serine residue that is covalently modified by the oxon metabolite.

Fig. 4.2 Reactions of a generic oxon metabolite with esterases



Adapted with permission from Casida & Quistad (2004); copyright (2004) American Chemical Society
The reaction of the oxon metabolite common to several organophosphate pesticides (in this case, diazinon, diazoxon) with the canonical target leads to inhibition of CES, AChE, and BChE activity. The neurotoxicity displayed by organophosphate pesticides is attributed to the product (shown in the box) of reaction between the oxon metabolite and AChE.
AChE, acetylcholinesterase; BChE, butyrylcholinesterase; CES, carboxylesterase

esterase responsible for terminating the signalling action of the neurotransmitter acetylcholine in the central and peripheral nervous systems (Casida & Quistad, 2004; Crow et al., 2012).

(b) Humans

Multiple human CYPs are implicated in diazinon metabolism. The major human CYP isoforms involved in the metabolism of diazinon to diazoxon are CYP1A1, CYP2C19, and CYP2B6, while CYP2C19 is also responsible for the dearylation (detoxification) of diazinon (Ellison et al., 2012). One study showed that recombinant CYPs 2D6, 2C19, 3A4, and 3A5 were also efficient at producing diazoxon or IMPY and diethylthiophosphate from diazinon (Mutch & Williams, 2006). Most of these biotransformation reactions take place in the liver where CYPs are most abundant. Using heterologously expressed CYP proteins, human CYP2C19 was identified to be the major isoform responsible for diazinon metabolism in liver, while other enzymes including CYP1A2 had a minor role (Kappers et al., 2001). On the basis of intrinsic clearance rates ($Cl_{int} = V_{max}/K_m$), the dearylation metabolism rate for diazinon was 2.5-fold that of the desulfuration metabolism rate in human liver microsomes (Sams et al., 2004). Desulfuration and dearylation reactions of diazinon were catalysed by individual CYP isoforms at roughly similar rates, in the following rank order: CYP2C 19 > CYP1A2 > CYP2B6 > CYP3A4 (Sams et al., 2004).

[The Working Group noted that CYP-mediated biotransformation of diazinon is an important metabolic pathway. The Working Group also noted the variation in organophosphate substrate specificity and rates of oxidation for individual CYP isoforms.]

PON1 is also an important detoxication enzyme of diazoxon. PON1 catalyses the hydrolytic degradation of diazoxon and possesses polymorphic variants (Costa et al., 2013). Coding region polymorphisms in human *PON1*, specifically the glutamine/arginine substitution at position 192 (192 Q/R) alloforms, can affect the catalytic efficiency of oxon hydrolysis for certain organophosphates (Povey, 2010). For example, when pure recombinant PON1 enzymes were examined, the $PON1_{R192}$ polymorphic isoform hydrolysed chlorpyrifos oxon more efficiently than the $PON1_{Q192}$ isoform, while both alloforms hydrolysed diazoxon with the same catalytic efficiency (Li et al., 2000). It was hypothesized that the PON1 Q192R polymorphism can influence susceptibility to organophosphates (Povey, 2010). In a cross-sectional study, farmers with ill health who had reportedly mixed and applied pesticides were more likely to possess a 192R allele than a 192Q allele when compared with healthy farmers (OR, 1.93; 95% CI, 1.24–3.01) (Cherry et al., 2002). In support of this notion, Davies and co-workers (Davies et al., 1996) showed using plasma samples that individuals who were 192QQ homozygotes were more efficient at hydrolysing diazoxon than 192RR homozygotes (Davies et al., 1996). However, another study showed opposite results: individuals with the RR genotype had the highest serum activity of diazoxonase, while activity was slightly reduced in individuals with the QR genotype, and reduced even further in those with the QQ genotype (O'Leary et al., 2005). The contrast in the results reported by the two studies was attributed to the different reaction conditions employed. High salt conditions (NaCl, 2 M; pH 8.5) were used in the study by Davies et al. (1996), while more physiologically relevant buffer conditions (NaCl, 150 mM; pH 7.4) were used in the study by O'Leary et al. (2005). [The Working Group noted that associations between the different polymorphisms at position 192 and PON1 activity towards diazoxon are unclear.]

It has also been suggested that protection or susceptibility to diazinon-induced toxicity is primarily determined by the expression level of PON1 protein and is not dependent on the Q192R genotype (Costa et al., 2013). Injection of *PON1*$^{-/-}$ mice with either recombinant human $PON1_{R192}$

or recombinant $PON1_{Q192}$ proteins afforded equal measures of protection against diazinon-induced toxicity (Li et al., 2000; Stevens et al., 2008).

When another human genetic polymorphism in PON1 was examined – leucine (L)/methionine (M) at codon 55, 55 L/M alloforms – there were also significant differences in enzyme activity towards diazoxon, with the following rank order: LL > LM > MM genotypes (O'Leary et al., 2005). Thus individuals exhibiting haplotypes combining 192Q and 55M alleles might have a reduced capacity to detoxify diazoxon, which suggests they would have a greater susceptibility to toxicity associated with diazinon (O'Leary et al., 2005).

In insects, glutathione transferases (GSTs) play an important role in resistance to organophosphates, and limited data suggested that GST-mediated *O*-dealkylation might also occur in humans. For example, when glutathione (1 mM) and methyl parathion (300 µM) are incubated together with recombinant GST enzymes, human GSTs hGSTT1-1 and hGSTA1-1 exhibited significant *O*-dealkylation activity: 546 and 65 nmol/min per mg, respectively (Abel et al., 2004). When expression level and enzymatic activity were considered, it was estimated that hGSTA1-1 was responsible for the majority of *O*-dealkylation of methyl parathion in human hepatic cytosol. [The Working Group noted that although no specific GST-mediated metabolism data for diazinon could be identified, it could be speculated that in organs such as brain and skeletal muscle, where hGSTT1-1 is expressed, hGSTT1-1-mediated biotransformation of organophosphate pesticides might be an important extrahepatic detoxication mechanism.] Furthermore, organophosphate pesticides have been shown to induce GSTα (GSTA1) in a human HepG2 cell line, which might aid their own detoxication (Medina-Díaz et al., 2011).

(c) Experimental systems

IMPY (also called pyrimidinol) is the dearylation product of diazinon (see Fig. 4.1) and a major metabolite of diazinon in vivo. CYP2C11, CYP3A2, and CYP2B1/2 are rat P450 isoforms responsible for oxidative dearylation of diazinon, affording IMPY (Fabrizi et al., 1999). Plasma concentrations of IMPY were ~20-fold those of diazinon at 3 hours after a single oral dose of diazinon of 100 mg/kg bw in Sprague-Dawley rats (Poet et al., 2004). These data demonstrate the rapid metabolism of diazinon that occurs in vivo in rats. [The Working Group noted that very few toxicological data concerning IMPY were available in the peer-reviewed and published literature.]

In a metabolomics study using a liquid chromatography–quadrupole–time-of-flight instrument, a novel metabolite (1-hydroxyisopropyl diazinon), was detectable in the plasma of male Sprague-Dawley rats given diazinon by intraperitoneal administration, or when diazinon was incubated with rat liver microsomes supplemented with reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Ibáñez et al., 2006). Absolute concentrations of this novel metabolite were not determined.

An important species difference is that human plasma contains no carboxylesterase 1c (CES1c), in contrast to the robust expression in experimental animals (such as mice, rats, and rabbits) (Li et al., 2005). This could potentially have an impact on the ability of humans to detoxify the bioactive diazoxon metabolite. However, it was demonstrated that *Ces1c*$^{-/-}$ knockout mice (which do not have Ces1c in plasma) were no more sensitive to the toxic effects of diazinon, delivered subcutaneously at 50 mg/kg bw, than were wildtype mice (Duysen et al. 2012). This was because the Ces1c present in the plasma of wildtype mice was insufficient to detoxify the diazoxon produced in vivo.

4.1.4 Excretion

(a) Humans

Because of its rapid metabolism in vivo, unchanged diazinon is not detected in the urine in humans. The metabolites and their glucuronide or sulfate conjugates are mainly excreted in the urine. However, the major metabolite of diazinon, IMPY, can be readily excreted from the body via urine and was detected in 29% of the population of the USA in urine samples collected for the National Health and Nutrition Examination Survey (NHANES, 1990–2000) in 1997, before residential use of diazinon was banned (Barr et al., 2005). In this study, the 95th percentile for IMPY concentration was 3.7 µg/L (3.4 µg/g creatinine). Dialkylphosphate metabolites are also found in human urine; after oral administration of diazinon, 66 ± 12% of an administered dose of 11 µg/kg bw was recovered, in contrast to only 0.5 ± 0.2% of a dermal dose (100 mg for 8 hours) (Garfitt et al., 2002). Unmetabolized diazinon was not detectable in the urine in either exposure scenario, nor was plasma cholinesterase activity reduced, indicating that measurement of urinary dialkylphosphate metabolites is a more sensitive biomarker of exposure than decreased plasma cholinesterase activity for biological monitoring purposes.

(b) Experimental systems

In female rhesus monkeys given [^{14}C]-labelled diazinon by intravenous administration, the cumulative level of ^{14}C residue in the urine after 7 days was 56% of the administered dose, while 23% was eliminated in the faeces (Wester et al., 1993). Similar findings with regard to excretion have been found in toxicokinetic studies in rodents (Poet et al., 2004). Thus experimental animals, like humans, absorb and metabolize diazinon very efficiently, and rapidly excrete the metabolites via the urine, with lesser amounts in the faeces. There was no evidence on the accumulation of diazinon and its metabolites in the body in either humans or experimental animals.

4.2 Mechanisms of carcinogenesis

4.2.1 Genotoxicity and related effects

Diazinon and its metabolites have been studied for genotoxic potential in a variety of assays. Table 4.1, Table 4.2, Table 4.3, Table 4.4, and Table 4.5 summarize the studies carried out in exposed humans, in human cells in vitro, in non-human mammals and non-mammals in vivo, in non-human mammalian cells in vitro, and in non-mammalian systems in vitro, respectively.

(a) Humans

(i) Studies in exposed humans

See Table 4.1

In peripheral blood lymphocytes from 34 workers engaged in the production of diazinon, a significant increase in the frequency of stable chromosomal aberrations was found, compared with a control group (Király et al., 1979). [The Working Group noted that diazinon was not the only chemical to which these individuals may have been exposed.] A significant increase in the frequency of sister-chromatid exchange was observed in peripheral blood lymphocytes of subjects after exposure to a sheep dip containing diazinon, compared with before exposure; however, the formulation also contained other unspecified ingredients (Hatjian et al., 2000).

Other studies showed that long-term occupational exposure to multiple insecticides, including diazinon, is associated with an increase in the frequency of chromosomal aberration and sister-chromatid exchange in peripheral blood lymphocytes, compared with non-exposed populations (De Ferrari et al., 1991).

Table 4.1 Genetic and related effects of diazinon in exposed humans

Tissue	Cell type (if specified)	End-point	Test	Description of exposure and controls	Response[a]/ significance	Comments	Reference
Peripheral blood	Lymphocytes	Chromosomal damage	Chromosomal aberration	34 workers engaged in diazinon production 49 controls, mainly males, Genetic Counselling Clinic of the National Institute of Hygiene	+ [no *P* calculation]	Significant increase in stable chromosomal aberrations in workers vs controls	Király et al. (1979)
Peripheral blood	Lymphocytes	Chromosomal damage	Chromosomal aberration	32 floriculturists exposed diazinon and other pesticides[b] 31 controls living in the same area, and with no history of occupational exposure to pesticides	(+) [see Comments]	Exposure to numerous pesticides, including diazinon Significant increase in structural ($P < 0.01$) and numerical ($P < 0.001$) chromosomal aberrations in exposed group vs controls	De Ferrari et al. (1991)
Peripheral blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	32 floriculturists exposed to diazinon and other pesticides[b] 31 controls living in the same area, and with no history of occupational exposure to pesticides	(+) $P < 0.01$	Exposure to numerous pesticides, including diazinon Significant increase in sister-chromatid exchange in exposed group vs controls	De Ferrari et al. (1991)
Peripheral blood	Lymphocytes	Chromosomal damage	Sister-chromatid exchange	8 volunteer agricultural college students exposed to sheep dip containing approximately 45% diazinon 8 age-and ethnicity-matched controls, non-smoking male university research staff	+ $P < 0.001$	Diazinon formulation contained other unspecified ingredients Significant increase after, compared with before, exposure; no difference between groups before dipping	Hatjian et al. (2000)

[a] +, positive; (+), positive result in a study of limited quality

[b] Other pesticides included 18 nitro-organic herbicides/fungicides, 9 nitro-organic fungicides, 12 organophosphate and organochlorophosphate insecticides, 4 hydrocarbon-derivative herbicides and 5 inorganic fungicides and insecticides

vs, versus

(ii) Humans cells in vitro

See Table 4.2

There was more evidence for diazinon-induced genotoxicity in human cells than in other mammalian cells. Diazinon induced genotoxicity in all studies in human cells in vitro, except in one quite old study. Diazinon induced DNA damage (comet assay) in human mucosal cells from the nose (Tisch et al., 2002), and from the tonsils (Tisch et al., 2007), as well as sister-chromatid exchange in lymphocytes (Sobti et al. 1982; Hatjian et al., 2000). DNA damage was also induced in spermatozoa (Salazar-Arredondo et al. 2008). Micronuclei were formed in blood lymphocytes exposed to diazinon (Colović et al., 2010; Karamian et al., 2013; Shokrzadeh et al., 2014), in skin fibroblasts (Colović et al., 2010), and in breast cancer (MCF-7) cells (Ukpebor et al., 2011).

Diazoxon was more active than diazinon in inducing DNA damage in spermatozoa (Salazar-Arredondo et al., 2008), while diethylthiophosphate (DETP), another diazinon metabolite, induced DNA damage in human hepatic cell lines (Vega et al., 2009). The metabolite IMPY induced formation of micronuclei in blood lymphocytes, skin fibroblasts, and MCF-7 cells (Colović et al., 2010; Ukpebor et al., 2011).

(b) Experimental animals

(i) Non-human mammals in vivo

See Table 4.3

Diazinon caused oxidative DNA damage (shown by increases in apurinic/apyrimidinic or abasic sites) in liver and kidney of rabbits given repeated oral doses over several months (Tsitsimpikou et al., 2013). Micronucleus formation was observed in peripheral blood lymphocytes of rats treated by intraperitoneal doses for 30 days (Shadboorestan et al., 2013; Shokrzadeh et al., 2013), and in bone-marrow cells in mice given repeated doses (Ni et al., 1993). Diazinon also induced micronucleus formation in blood cells of rats given repeated oral doses for 4 weeks (Hariri et al., 2011). Diazinon failed to induce sister-chromatid exchange in bone-marrow cells of mice treated by gavage (EPA, 1992a). A diazinon-based formulation also induced DNA damage in the testicular germinal epithelium and micronucleaus formation in bone marrow of mice given a single intraperitoneal dose (Sarabia et al., 2009a).

(ii) Non-human mammalian cells in vitro

See Table 4.4

Conflicting results were obtained in the mouse lymphoma assay: McGregor et al. (1988) showed that diazinon induced mutation without metabolic activation, while the EPA (1989a) reported that diazinon did not induce mutation with or without metabolic activation. In Chinese hamster lung cells, diazinon caused chromosomal aberration in the presence of metabolic activation (Matsuoka et al., 1979). Diazinon did not cause micronucleus formation in rat hepatocytes (Frölichsthal & Piatti, 1996), or in Chinese hamster ovary cells (Kirpnick et al., 2005). Moreover, diazinon did not induce sister-chromatid exchange in Chinese hamster lung (V79) cells (Chen et al., 1981, 1982; Kuroda et al., 1992), or in Chinese hamster ovary cells (Nishio & Uyeki, 1981). Diazoxon caused sister-chromatid exchange in Chinese hamster ovary cells (Nishio & Uyeki, 1981).

In Chinese hamster ovary cells, there was an increase in the frequency of chromatid aberration after exposure to urine collected during spraying from non-smoking, male orchardists (n = 22) using 16 pesticides including diazinon, when compared with urine from the same individuals before spraying ($P < 0.001$) (See et al., 1990).

(iii) Non-mammalian systems in vivo

See Table 4.3

Diazinon induced sister-chromatid exchange in fish, *Umbra limi* (Vigfusson et al., 1983). DNA

Table 4.2 Genetic and related effects of diazinon, diazoxon, diethylthiophosphate, and IMPY in human cells in vitro

Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Diazinon							
Primary nasal mucosal cells	DNA damage	DNA strand break, comet assay	+	NT	500 µM [152 µg/mL]	Positive for both cell types tested (middle and inferior turbinate)	Tisch et al. (2002)
Mucosal epithelial cells from human tonsil tissue	DNA damage	DNA strand break, comet assay	+	NT	50 µM [15.2 µg/mL]		Tisch et al. (2007)
Spermatozoa	DNA damage	Sperm-chromatin structure assay	+	NT	500 µM [152 µg/mL]		Salazar-Arredondo et al. (2008)
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	20 µg/mL		Hatjian et al. (2000)
Lymphoid cell line (LAZ-007)	Chromosomal damage	Sister-chromatid exchange	–	+	20 µg/mL	Only one concentration tested with metabolic activation [20 µg/mL]	Sobti et al. (1982)
Peripheral blood lymphocytes	Chromosomal damage	Chromosomal aberrations	–	NT	30 µg/mL		Lopez et al. (1986)
Blood lymphocytes	Chromosomal damage	Micronucleus formation	+	NT	750 µM [228 µg/mL]	Only one concentration tested	Shokrzadeh et al. (2014)
Peripheral blood lymphocytes	Chromosomal damage	Micronucleus formation	+	NT	750 µM [228 µg/mL]	Only one concentration tested	Karamian et al. (2013)
Breast adenocarcinoma cell line (MCF-7)	Chromosomal aberration	Micronucleus formation	+	NT	10^{-6} µM [0.3×10^{-6} µg/mL]		Ukpebor et al. (2011)
Blood lymphocytes	Chromosomal damage	Micronucleus formation	+	NT	0.02 µM [6×10^{-3} µg/mL]		Colović et al. (2010)
Skin fibroblasts	Chromosomal damage	Micronucleus formation	+	NT	0.02 µM [6×10^{-3} µg/mL]		Colović et al. (2010)
Peripheral blood lymphocytes	Chromosomal damage	Micronucleus formation	(+)	NT	4 µg/mL		Bianchi-Santamaria et al. (1997)

Table 4.2 (continued)

Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
			Without metabolic activation	With metabolic activation			
Diazoxon							
Spermatozoa	DNA damage	Sperm chromatin structure assay	+	NT	300 µM [86.5 µg/mL]	Diazoxon was more active than diazinon	Salazar-Arredondo et al. (2008)
IMPY							
MCF-7, breast adenocarcinoma cell line	Chromosomal damage	Micronucleus formation	+	NT	10^{-6} µM [0.152 × 10^{-6} µg/mL]		Ukpebor et al. (2011)
Blood lymphocytes	Chromosomal damage	Micronucleus formation	+	NT	0.02 µM [3 × 10^{-3} µg/mL]	IMPY was more active than diazinon	Colović et al. (2010)
Skin fibroblasts	Chromosomal damage	Micronucleus formation	+	NT	0.02 µM [3 × 10^{-3} µg/mL]		Colović et al. (2010)
DETP							
HepG2, hepatocellular carcinoma cell line	DNA damage	DNA strand break Comet assay	+	NT	1 µM [0.17 µg/mL]		Vega et al. (2009)
WRL68, embryonic hepatic non-transformed cell line	DNA damage	DNA strand break Comet assay	+	NT	1 µM [0.17 µg/mL]	Positive effect linked to CYP450 enzymes: addition of sulconazole, a CYP450 inhibitor, inhibited the DNA damage	Vega et al. (2009)
HeLa, cervical adenocarcinoma cell line	DNA damage	DNA strand break, comet assay	–	NT	500 µM [85 µg/mL]		Vega et al. (2009)
Peripheral blood mononucleated cells	DNA damage	DNA strand break, comet assay	–	NT	500 µM [85 µg/mL]		Vega et al. (2009)
Diazinon-based formulation							
Lymphocytes	Chromosomal damage	Sister-chromatid exchange	+	NT	Diazinon, 45% NR		Hatjian et al. (2000)

[a] +, positive; –, negative; (+), weakly positive

DETP, diethyl thiophosphate; HIC, highest ineffective concentration; IMPY, 2-isopropyl-4-methyl-6-hydroxypyrimidine; LEC, lowest effective concentration; NR, not reported; NT, not tested

Table 4.3 Genetic and related effects of diazinon in non-human mammals in vivo

Species, strain, sex	Tissue	End-point	Test	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Diazinon								
Rabbit, New Zealand White, F	Liver	DNA damage	Oxidative DNA damage AP sites	+	2.64 mg/kg bw per day	p.o., 12 mo (every 2 days for 3 mo, 8 mo without treatment, then 1 mo of treatment every 2 days)	Significant increase in apurinic/apyrimidinic or abasic (AP) sites with both doses tested (2.64 and 5.28 mg/kg bw) compared with controls Higher effect in liver than kidney $P < 0.001$	Tsitsimpikou et al. (2013)
Rabbit, New Zealand White, F	Kidney	DNA damage	Oxidative DNA damage AP sites	+	2.64 mg/kg bw per day	Gavage, 12 mo (every 2 days during 3 mo, 8 mo without treatment, then 1 mo of treatment every 2 days)	Significant increase in AP sites with both doses tested (2.64 and 5.28 mg/kg bw) compared with controls Higher effect in liver than kidney $P < 0.001$	Tsitsimpikou et al. (2013)
Rat, Wistar, M	Peripheral blood lymphocytes	Chromosomal damage	Micronucleus formation	+	20 mg/kg bw per day	i.p. × 30 days	Only one dose tested, $P < 0.0001$; L-carnitine had antigenotoxic effect	Shadboorestan et al. (2013)
Rat, Wistar, M	Peripheral blood lymphocytes	Chromosomal damage	Micronucleus formation	+	20 mg/kg bw per day	i.p. × 30 days	Only one dose tested; $P < 0.0001$; selenium had antigenotoxic effect	Shokrzadeh et al. (2013)
Rat	Blood Cells not specified	Chromosomal damage	Micronucleus formation	+	20 mg/kg bw per day	p.o., 1×/day, ×4 wk	One dose tested; $P < 0.001$	Hariri et al. (2011)
Mouse	Bone marrow Polychromatic erythrocytes	Chromosomal damage	Micronucleus formation	+	0.1, 0.2, 0.4, 0.6 and 0.8 × LD_{50}	i.p. 1×/day, ×4 days	LD_{50}, NR; LED, NR	Ni et al. (1993)
Mouse, ICR	Bone marrow	Chromosomal damage	Sister-chromatid exchange	–	100 mg/kg bw	Gavage, × 1		EPA (1992a)

Table 4.3 (continued)

Species, strain, sex	Tissue	End-point	Test	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Insect, *Drosophila melanogaster*		Mutation	Somatic mutation and recombination test (SMART)	+	1 ppm [1 µg/mL] feeding			Çakir & Sarikaya (2005)
Insect, *Drosophila melanogaster*		Chromosomal damage	Complete and partial chromosome losses	–	100 ppb [0.1 µg/mL]			Woodruff et al. (1983)
Diazinon-based formulation								
Mouse, CF-1, M	Germinal epithelium of testis, spermatocytes	DNA damage	DNA strand breaks, comet assay	+	43.33 mg/kg bw	i.p. × 1	Diazinon, 60% Two doses tested corresponding to 1/3 and 2/3 of the LD_{50} (65 mg/kg bw) Significant increase at higher dose (43.33 mg/kg bw); $P < 0.001$; melatonin prevented DNA damage $P < 0.001$	Sarabia et al. (2009a)
Mouse, CF-1, M	Bone marrow	Chromosomal damage	Micronucleus formation	+	21.66 mg/kg bw	i.p. × 1	Diazinon, 60% Significant increase in micronucleus formation with the two doses tested, 21.66 and 43.33 mg/kg bw: $P < 0.01$ pre-treatment with melatonin prevented micronucleus formation	Sarabia et al. (2009a)
Fish, *Umbra limi*		Chromosomal damage	Sister-chromatid exchange	+	5.4×10^{-10} M [0.164 µg/L]		Diazinon, 48.72% The highest concentration tolerated by fish was 5.4×10^{-9} M	Vigfusson et al. (1983)
Freshwater mussel, *Utterbackia imbecilis*	Glochidia	DNA damage	DNA strand break, comet assay	+	0.28 µg/mL		Diazinon, 22.4% 0.28 µg/mL corresponds to 1/4 NOAC, positive response at level below the NOAEC	Conners & Black (2004)

[a] +, positive; –, negative

AP, apurinic, apyrimidinic or abasic sites; F, female; HID, highest ineffective dose; i.p., intraperitoneal; ; LD50, median lethal dose LED, lowest effective dose (units as reported); M, male; mo, month; NOAEC, no-observed-adverse-effect concentration; NT, not tested; p.o., oral

Table 4.4 Genetic and related effects of diazinon in non-human mammalian cells in vitro

Species	Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Urine from exposed humans								
Hamster	Chinese hamster ovary cells (CHO)	Chromosomal damage	Chromatid aberrations	(+)	NT	1–8 mg/mL creatine equivalent	Extracts of urine from 22 non-smoker male orchardists using 16 pesticides including diazinon 21 subjects non-smoking males and females Urine samples collected during spraying period had increased chromatid aberration frequency compared with urine before spraying ($P > 0.001$). (before use of pesticide, urine of orchardists caused same level of chromatid aberrations as urine of control group)	See et al. (1990)
Diazinon								
Mouse	Mouse lymphoma L5178Y cells	Mutation	*Tk*$^{+/-}$	+	NT	60 µg/mL		McGregor et al. (1988)
Mouse	Mouse lymphoma L5178Y	Mutation	*Tk*$^{+/-}$	–	–	108 µg/mL		EPA (1989a)
Rat	Hepatocytes	Chromosomal damage	Micronucleus formation	–	NT	54 µg/mL		Frölichsthal & Piatti (1996)
Hamster	Chinese hamster lung cells	Chromosomal damage	Chromosomal aberration	Toxic	+	100 µg/mL	– S9, 100 µg/mL was cytotoxic	Matsuoka et al. (1979)
Hamster	Chinese hamster ovary cells (CHO)	Chromosomal damage	Micronucleus formation	–	–	94 µg/mL		Kirpnick et al. (2005)
Hamster	Chinese hamster lung cells	Chromosomal damage	Micronucleus formation	–	NT	NR	Only one dose tested: highest dose that induced 50% cell death (NR)	Ni et al. (1993)

Table 4.4 (continued)

Species	Tissue, cell line	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Hamster	Chinese hamster lung fibroblast V79 cells	Chromosomal damage	Sister-chromatid exchange	–	NT	0.4 µg/mL		Kuroda et al. (1992)
Hamster	Chinese hamster lung fibroblast V79 cells	Chromosomal damage	Sister chromatid exchange	–	–	80 µg/mL		Chen et al. (1981, 1982)
Hamster	Chinese hamster ovary cells (CHO)	Chromosomal damage	Sister-chromatid exchange	–	NT	1 mM [304 µg/mL]		Nishio & Uyeki (1981)
Diazoxon								
Hamster	Chinese hamster ovary cells (CHO)	Chromosomal damage	Sister-chromatid exchange	+	NT	1 mM [288 µg/mL]		Nishio & Uyeki (1981)

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

HIC, highest ineffective concentration; LEC, lowest effective concentration; NR, not reported; NT, not tested; S9, 9000 × *g* supernatant

damage was induced in freshwater mussels exposed to diazinon, as shown by the comet assay (Conners & Black, 2004). In *Drosophila melanogaster,* diazinon induced mutation in the somatic mutation and recombination test (Çakir & Sarikaya, 2005), but did not cause complete or partial chromosome losses (Woodruff et al., 1983).

(iv) Non-mammalian systems in vitro

See Table 4.5

Diazinon did not induce chromosomal damage in *Saccharomyces cerevisiae* strain *RS112* (Kirpnick et al., 2005), nor mutation in most studies in *S. typhimurium* (Marshall et al., 1976; Wong et al., 1989; Kubo et al., 2002). Diazinon induced gene mutation in a single Ames assay in *S. typhimurium* in the presence (but not the absence) of metabolic activation (Wong et al., 1989). Moreover, diazinon did not induce DNA damage in the rec assay in *B. subtilis* without metabolic activation (Shirasu et al., 1976). A study in an acellular system with calf thymus DNA showed non-intercalative binding of diazinon with DNA (Kashanian et al., 2008).

4.2.2 Receptor-mediated mechanisms

(a) Neurotoxicity-pathway receptors

Diazinon is bioactivated to diazoxon in insects and mammals (Section 4.1.3; Casida & Quistad, 2004). Diazoxon can covalently modify the catalytic serine residue and inhibit the activity of several B-esterases, including the recognized target acetylcholinesterase, resulting in acute neurotoxicity in insects and mammals. Acetylcholinesterase is responsible for terminating the signalling action of the neurotransmitter acetylcholine in the central and peripheral nervous systems. Blockage results in acetylcholine overload and the overstimulation of nicotinic and muscarinic acetylcholine receptors.

Additional receptor targets of diazinon that can affect neurotoxicity include the cannabinoid receptor and butyrylcholinesterase (Quistad et al., 2002; Costa et al., 2011). The mechanistic relevance of these effects to carcinogenicity is unknown.

(b) Sex-hormone pathway disruption

(i) Humans

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

Diazinon showed weak estrogenic activity in vitro in the E-Calex assay, in ovarian carcinoma cells, BG1, that are stably transfected with an estrogen-responsive luciferase reporter gene plasmid; the concentration that produced 10% of the maximal estradiol activity was 460 µM (Kojima et al., 2005).

Diazinon (10^{-6} to 100 µM) gave negative results for estrogenicity in estrogen-receptor-positive breast cancer cells (MCF-7), and did not cause estrogen-receptor-negative cells (MDA MB 231) to proliferate (Oh et al., 2007).

In androgen-receptor and estrogen-receptor α and β reporter-gene assays in Chinese hamster ovary cells (CHO-K1), diazinon did not show agonist or antagonist activity (Kojima et al., 2004, 2010).

(ii) Non-human mammalian experimental systems

In male mice treated daily by gavage for 4 weeks, diazinon (4.1 or 8.2 mg/kg bw) substantially reduced levels of luteinizing hormone and follicle-stimulating hormone, while a lower dose (2 mg/kg bw) was without effect (ElMazoudy & Attia, 2012). At 4.1 mg/kg bw, plasma testosterone concentration was nearly double that of controls (5.9 versus 3.1 ng/mL), and at 8.2 mg/kg bw it was roughly one third of that of controls (1.1 versus 3.1 ng/mL). For prolactin, a similar pattern was seen of increase in concentration in the group at 4.1 mg/kg bw, and decrease in the group at the highest dose; for estradiol, only the group at 4.1 mg/kg bw showed significant increase in concentration. Jayachandra &

Table 4.5 Genetic and related effects of diazinon in non-mammalian systems in vitro

Phylogenetic class	Test system (species, strain)	End-point	Test	Results[a]		Concentration (LEC or HIC)	Comments	Reference
				Without metabolic activation	With metabolic activation			
Prokaryote (bacteria)	*Salmonella typhimurium*, TA1535, TA1536, TA1537, TA1538	Mutation	Reverse mutation	–	–	1000 µg/plate		Marshall et al. (1976)
	Salmonella typhimurium, TA98, TA100	Mutation	Reverse mutation	–	–	1 mM [304 µg/mL]		Kubo et al. (2002)
	Salmonella typhimurium, TA98	Mutation	Reverse mutation	–	+	NR	Concentration tested was between non-toxic and 50% toxic concentration: 20–80 ppm [20–80 µg/mL]	Wong et al. (1989)
	Salmonella typhimurium, TA102, TA1535, TA1537	Mutation	Reverse mutation	–	–	80 ppm [80 µg/mL]		Wong et al. (1989)
	Bacillus subtilis	DNA damage	Rec-assay, differential toxicity	–	NT	20 µg/disk		Shirasu et al. (1976)
Yeast	*Saccharomyces cerevisiae* strain RS112	Chromosomal damage	Deletion assay Intrachromosomal recombination	–	–	10 000 µg/mL		Kirpnick et al. (2005)
Acellular systems	Calf thymus DNA	DNA damage	DNA binding	+	NT	4.92 µM [1.5 µg/mL]	Formation of stable 1 : 2 complex of DNA–diazinon	Kashanian et al. (2008)

[a] +, positive; –, negative

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

D'Souza (2014) found decreased concentrations of gonadotropins at puberty and in adulthood in male offspring of Sprague-Dawley rats exposed to diazinon during mating, pregnancy, and lactation. At puberty and in adulthood, male offspring of dams exposed to diazinon (30 mg/kg bw) had significantly reduced plasma concentrations of luteinizing hormone, follicle-stimulating hormone, and prolactin; prolactin was also reduced at 15 mg/kg bw. At puberty, offspring also had reduced concentrations of testosterone, compared with control levels. Several abnormalities were found in sperm and other reproductive parameters in adults and pubescent animals at each dose level.

Serum testosterone concentrations were significantly reduced in male Sprague-Dawley rats exposed for 8 weeks to diazinon (10, 15 or 30 mg/kg bw per day by gavage; $P < 0.05$) Leong et al. (2013). After 1 week, serum testosterone concentrations were significantly increased by diazinon (15 or 30 mg/kg bw per day). A single high dose of diazinon (75 mg/kg bw) administered orally to Wistar rats for 28 days also increased serum testosterone concentrations (Alahyary et al., 2008).

Marked and dose-dependent decreases in progesterone compared with controls were seen in female Wistar rats treated orally with diazinon (50, 100, or 150 mg/kg bw per day for 14 days) (Johari et al., 2010). There were no significant changes for estrogen, luteinizing hormone, or follicle-stimulating hormone.

In an in-vitro study, diazinon increased the proliferation of the 17-β estradiol-sensitive MtT/Se cell line derived from rat pituitary tumour cells in which estrogen receptor α is dominant (Manabe et al., 2006).

(iii) Non-mammalian experimental systems

In female bluegill fish (*Lepomis macrochirus*), continuous exposure to diazinon (60 µg/L in aquaria water) reduced blood estradiol measurements at all time-points (24, 48, 72, and 96 hours, 1 and 2 weeks), with significant reductions at all time-points except 96 hours. Estradiol was undetectable at 24 hours and 2 weeks. Alterations in estradiol concentration reflected the damage present within the ovarian structure (Maxwell & Dutta, 2005).

(c) Other pathways

(i) Humans

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

In an in-vitro human pregnane X receptor (PXR) reporter-gene assay in a CHO-K1 cell line, diazinon did not exhibit agonist activity (Kojima et al., 2010).

(ii) Non-human mammalian experimental systems

Thyroid hormone status was evaluated in healthy Swiss albino mice, and in mice treated with diazinon alone for 9 and 17 weeks or in combination with a drug, and with and without *Schistosoma masoni* (Hanna et al., 2003). There were non-significant increases in triiodothyronine (T3) (by 16.5% and 22.4% at 9 and 17 weeks, respectively) and thyroxine (by 2.8% and 5.3% at 9 and 17 weeks, respectively) compared with controls.

In livers from mice exposed in utero to a low dose of diazinon (0.18 mg/kg bw to dams during pregnancy), hepatic metabolism of corticosterone was impaired. Plasma concentrations of corticosterone were elevated in resting male and female mice, but normal under stress (Cranmer et al., 1978). High doses (9 mg/kg bw) were without effect.

In in-vitro studies, diazinon was not an agonist for mouse peroxisome proliferator-activated receptors α or γ (PARP α or γ) in reporter-gene assays in CV-1 monkey kidney cells (Takeuchi et al., 2006; Kojima et al., 2010). Diazinon was not an agonist for the aryl hydrocarbon receptor (AhR) in mouse hepatoma Hepa1c1c7 cells stably transfected with a reporter plasmid containing

copies of a dioxin-responsive element (Takeuchi et al., 2008; Kojima et al., 2010).

(iii) Non-mammalian experimental systems

Thyroid-stimulating hormone (TSH) and thyroxine (T4) were substantially reduced at 24, 48, 72, and 96 hours in all dose groups in Caspian roach (*Rutilus rutilus*) fingerling fish from the north-east of the Islamic Republic of Iran exposed in aquaria to a diazon-based formulation (purity, 60%; 0, 1, 2, and 3 mg/L in fresh water for 96 hours) (Katuli et al., 2014). Triiodothyronine (T3) was also reduced except at the highest dose at 24 hours after exposure. Whole-body cortisol levels were increased in diazinon-exposed fish, but decreased to the control levels by 96 hours after fish were transferred to diazinon-free brackish water.

In adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*), the effective dose of diazinon that inhibited by 50% (EC_{50}) the stimulated cortisol secretion in response to adrenocorticotropic hormone (ACTH) was similar to the doses that were lethal to cells ($LC50/EC_{50} = 1.3$) (Bisson & Hontela, 2002).

4.2.3 Oxidative stress, inflammation, and immunosuppression

(a) Oxidative stress

(i) Humans

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

In human erythrocytes, diazinon (0.0033–33 mM; for 60 or 180 minutes) significantly increased malondialdehyde concentrations and the activity of superoxide dismutase and glutathione peroxidase at all dose levels in a concentration- and duration-dependent manner. Catalase activity remained unchanged. In haemolized erythrocytes, superoxide dismutase activity was significantly decreased at 33 mM (both time-points), and glutathione peroxidase activity was significantly increased at 0.3 and 33 mM (both time-points).

Diazinon and its photolysis product IMPY increased lipid peroxidation in human lymphocytes (freshly prepared from one donor) in vitro (Colović et al., 2010). On incubation for 72 hours, there were significant elevations in amounts of thiobarbituric acid-reactive substances with diazinon at concentrations of 2×10^{-5} M or higher, and with IMPY at 2×10^{-6} M or higher. The effect of IMPY was approximately 50–80% stronger (statistically significant) than that of diazinon at the same concentrations.

(ii) Non-human mammalian experimental systems

In vivo

Most of the experimental studies of oxidative stress and diazinon were conducted in rats and examined a range of end-points, exposure durations, doses, administration routes, and tissues. Specifically, it was found that diazinon induces the production of free radicals and oxidative stress in rat tissues through alteration of antioxidant-enzyme activity, depletion of glutathione, and increasing lipid peroxidation. Increases in oxidative-stress biomarkers upon exposure to diazinon in vivo have been observed in blood (Shadnia et al., 2007; Sutcu et al., 2007; Abdou & ElMazoudy, 2010; Messarah et al., 2013; El-Demerdash & Nasr, 2014; Moallem et al., 2014), liver (Teimouri et al., 2006; Amirkabirian et al., 2007; Lari et al., 2013; Lari et al., 2014), myocardium (Akturk et al., 2006; Jafari et al., 2012; Razavi et al., 2014a), testis (Leong et al., 2013; Oksay et al., 2013), kidney (Shah & Iqbal, 2010; Boroushaki et al., 2013), brain (Jafari et al., 2012; Yilmaz et al., 2012), blood vessels (Razavi et al., 2014b), adipose (Pakzad et al., 2013) and spleen (Jafari et al., 2012). Some studies used pre-treatments with various antioxidants and demonstrated that diazinon-related oxidative stress is mitigated by antioxidants (Shadnia et al., 2007; Sutcu et al., 2007; Messarah et al., 2013; El-Demerdash & Nasr, 2014). Jafari et al. (2012) performed a comparative analysis of tissue

susceptibility to diazinon-associated oxidative stress, and observed that induction of oxidative stress in diazinon-treated rats is in the rank order of brain > heart > spleen.

A study in mice given a single intraperitoneal injection of diazinon (22 or 43 mg/kg bw) showed an increase in superoxide dismutase activity in the testis (Sarabia et al., 2009b). Two studies examined oxidative stress end-points in rabbits exposed to diazinon. Tsitsimpikou et al. (2013) reported histopathological lesions and oxidative stress in liver and kidneys after long-term exposure of rabbits to diazinon. Zafiropoulos et al. (2014) observed diazinon-induced oxidative stress in the rabbit myocardium.

In vitro

Four reports presented the effects of diazinon on oxidative stress end-points in rat or mouse cells in vitro. Slotkin et al. (Slotkin et al., 2007; Slotkin & Seidler, 2009) used rat neuronotypic pheochromocytoma PC12 cells to explore whether diazinon affects the lipid peroxidation and transcriptional profiles of oxidative-stress response genes. Diazinon (30 µM) significantly increased levels of thiobarbituric acid-reactive substances in PC12 cells. In addition, the same concentration of diazinon (30 µm) had both positive and negative effects (all less than 1.5-fold) on several glutathione synthesis-related genes, catalase, and superoxide dismutase isoforms (Slotkin & Seidler, 2009). Pizzurro et al. (2014) showed that diazinon and its oxygen metabolite diazoxon cause oxidative stress in cultures of primary rat hippocampal neurons as a mechanism of inhibition of neurite outgrowth. Antioxidants prevented neurite outgrowth inhibition by diazinon. The concentrations of both compounds used in these studies were not cytotoxic, and caused limited inhibition of acetylcholinesterase activity in astrocytes. Finally, Giordano et al. (2007) explored the role of oxidative stress on the neurotoxicity of diazinon and diazoxon in neuronal cells from wildtype mice (*Gclm*$^{+/+}$) and mice lacking the modifier subunit of glutamate cysteine ligase (*Gclm*$^{-/-}$), the first and limiting enzyme in the synthesis of glutathione. Both diazinon and diazoxon increased intracellular levels of reactive oxygen species and lipid peroxidation, and in both cases the effects were greater in neurons from *Gclm* null mice. There was no change in intracellular concentrations of glutathione, but there was a significant increase in levels of oxidized glutathione.

(iii) Non-mammalian experimental systems

Positive associations between exposure to diazinon and oxidative stress were reported in various tissues in fish models in vivo (Oruç & Usta, 2007; Uner et al., 2007; Girón-Pérez et al., 2009; Oruç, 2011; Banaee et al., 2013).

(b) Inflammation and immunomodulation

(i) Humans

Three publications (Hoppin et al., 2007; Valcin et al., 2007; Slager et al., 2010) suggested that exposure to diazinon, among other pesticides, may be associated with an increased incidence of chronic inflammatory and allergic diseases of the respiratory system (bronchitis and rhinitis) in agricultural workers exposed to these agents. They used data from the AHS, a large study of pesticide applicators and their spouses enrolled in Iowa and North Carolina, USA, in 1993–1997. [The Working Group noted that these data should be interpreted with caution since the exposures were to mixtures of pesticides and dust.]

In in-vitro studies using human lymphoblastic T-cell lines (Jurkat), diazinon (> 125 µM) significantly decreased induction of interferon γ (IFNγ) and interleukin 4 (IL4) promoters in the presence of phytohaemagglutinin, or without any stimulus, but had no effect on viability (≥ 1 mM) (Oostingh et al., 2009). Diazinon had similar effects in human peripheral blood mononuclear cells, reducing the secretion of TH1-cytokine IFNγ, and TH2 cytokines IL-4 and IL-13 significantly at concentrations above

10 μM. Shao et al. (2013) demonstrated upregulation of several adaptive immune-response genes by diazinon in the transcriptome of the human Jurkat T-cell line in vitro.

(ii) Non-human mammalian experimental systems

Pro-inflammatory effects of diazinon have been observed in studies in experimental animals. Pakzad et al. (2013) treated rats with diazinon (70 mg/kg bw) by daily gavage for 4 weeks and evaluated molecular changes in the adipose tissue, finding that levels of tumour necrosis factor α (TNFα) doubled after exposure to diazinon. Moallem et al. (2014) evaluated levels of TNFα in rat serum after oral exposure to diazinon at 20 mg/kg bw per day for 4 weeks and also observed a significant induction of more than threefold. Studies in female rabbits given diazinon (5 mg/kg bw per day) orally every other day for up to 12 months reported focal inflammation and fibrosis in the liver and kidneys (Tsitsimpikou et al., 2013).

Pathological effects of diazinon on the immune system have been reported. Jeong et al. (1995) observed a significant decrease in thymus weight at the highest dose (20 mg/kg bw) in $B6C3F_1$ mice given diazinon by intraperitoneal injection for 7 days. Long-term oral exposure to diazinon (300 mg/kg food, by dry weight) for 45 days in CD-1 mice resulted in necrotic degeneration of trabeculae (spleen and thymus), hyperplasia of cortex and medulla (lymph nodes, thymus) and hyperplasia of the white and red pulp of the spleen (Handy et al., 2002). In C57BL/6 female mice given diazinon (0.2, 2, or 25 mg/kg bw; five intraperitoneal injections per week) for 28 days, there was a decrease in the ratio of thymus weight to body weight at doses > 2 mg/kg bw, and gross histopathological changes were observed in the thymus and spleen of mice at 25 mg/kg bw (Neishabouri et al., 2004). In a study in rats given diazinon at a dose of 20 mg/kg bw (administered orally every second day, for 35 days), there was a marked increase in the number of spleen lymphocytes, without a significant gain in relative spleen weight (Baconi et al., 2013). Diazinon also caused an increase in the number of mononuclear cells per spleen weight. However, splenic lymphocyte proliferation stimulated with concanavalin A ex vivo was not affected.

Suppression of the humoral immune response by diazinon has been reported in studies in mice. Suppression of humoral functional responses, such as haemagglutination titration and IgM plaque-forming colonies, was observed in female C57BL/6 mice treated with diazinon at 25 mg/kg bw for 28 days (five intraperitoneal injections per week) (Neishabouri et al., 2004). In mice given diazinon at 50 mg/kg bw for 30 days, there was a gradual significant decrease in the concenetrations of interleukins IL-2, IL-4, IL-10, and IL-12, and IFNγ (both protein and mRNA) in the splenocyte cultures that were stimulated with phytohaemagglutinin (Alluwaimi & Hussein, 2007). In pregnant mice fed diets containing diazinon (9 mg/kg) throughout gestation, there were significant effects on serum concentrations of IgG1 and IgG2a in male and female offspring at age 3 months (Barnett et al., 1980). No effects were observed on levels of IgG2b, IgA, or IgM at any time-point.

Cell-mediated effects of diazinon on the immune system were demonstrated in studies in mice. Suppression of the cellular functional responses, such as delayed-type hypersensitivity to sheep erythrocytes and T-cell subtyping (CD4/CD8) was observed in female C57BL/6 mice treated with diazinon at 25 mg/kg bw for 28 days (five intraperitoneal injections per week) (Neishabouri et al., 2004).

(iii) Non-mammalian experimental systems

Positive associations between exposure to diazinon and immunotoxicity in fish have been observed. There have been several reports on the effects of diazinon on immune system parameters in Nile tilapia (*Oreochromis*

niloticus) (Girón-Pérez et al., 2007, 2008, 2009). Splenocyte proliferation and phagocytic indices were significantly decreased after acute exposure to diazinon (Girón-Pérez et al., 2007). Diazinon (1.96 mg/L) significantly increased respiratory burst and IgM concentration in splenocytes (Girón-Pérez et al., 2009). In an ex-vivo study, acetylcholinesterase activity was lower, and acetylcholine concentration was higher, in spleen from Nile tilapia exposed to diazinon than in non-exposed controls. Pre-exposure to acetylcholine depleted the proliferative function of spleen cells, suggesting that the immunotoxic effects of diazinon in fish may be indirect and could involve the lymphocyte cholinergic system (Girón-Pérez et al., 2008). Also in Nile tilapia, diazinon decreased lymphocyte count and suppressed humoral immune responses in vaccinated fish, as shown by a decrease in primary antibody response and antibody plaque-forming cell number (Khalaf-Allah, 1999). In a study in iridescent shark (*Pangasius hypophthalmus*) exposed to diazinon (0.5 and 1 ppm for 7 days), leukocytosis, lymphopenia, and neutrophilia were observed (Hedayati & Tarkhani, 2014).

4.2.4 Cell proliferation and death

(a) Humans

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

In experiments in vitro, a human teratocarcinoma cell line (NTera2/D1) (NT2) that has properties of neuronal precursor cells was used to explore the role of acetylcholinesterase in the modulation of apoptosis by diazinon (Aluigi et al., 2010). Diazinon (1 µM; a concentration that did not result in significant inhibition of acetylcholinesterase activity) increased the number of viable cells (by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay). At 10 and 100 µM, acetylcholinesterase activity was inhibited, and cell viability was decreased, as dose and duration increased. At 10 µM, various measures of apoptosis were affected by diazinon, including activation of caspases and nuclear fragmentation (measured by a flow-cytometry procedure).

Diazinon (3.9–1000 µM) had no negative effect on cell viability, and in fact showed a significant increase above control levels at any concentration of co-administration in lung epithelial carcinoma (A549) cells transfected with an insert encoding different promoter regions, including that for TNFα, and treated with recombinant human TNFα (rhTNFα) (0, 1, 20, or 300 ng/mL) (Oostingh et al., 2009). Diazinon at the same concentrations had no significant positive or negative effect on cell viability in a human lymphoblastic T-cell line (Jurkat) incubated with phytohaemagglutinin at 0 or 10 µg/mL.

In colonic epithelial cell lines established from primary cultures of surgically resected tissue, diazinon (0.05–50 µM; in dimethyl sulfoxide, DMSO) caused an increase in cell growth as measured by the MTT assay after 1 day (Greenman et al., 1997). After 3 days, cell growth remained elevated at 1 and 50 µM, but did not significantly differ from control levels at 0.5 and 10 µM.

In a colorectal adenocarcinoma cell line (Caco-2 cells), cell growth (as measured by the MTT assay) was not elevated above control levels after exposure to diazinon (15, 45, or 135 µM for 5 days) (Habibollahi et al., 2011). Indeed, cell viability substantially decreased with increasing exposure, but descendants of cells that were treated for 4.5 months with gradually increasing concentrations of diazinon (from 0.02 µM to 20 µM) were more resistant to effects on cell viability than were the parent cells. [Data on cell growth after a shorter period of exposure were not provided.]

In a lymphocyte culture derived from blood drawn from a healthy male (age, 30 years), cell proliferation potential (evaluated by cytokinesis-block proliferation index) was inhibited by diazinon (0.02–20 µM) (Colović et al., 2010). This

was also the case for similarly exposed skin fibroblasts (source not specified).

(b) *Non-human mammalian experimental systems*

(i) *In vivo*

In bioassays in rats and mice carried out by the National Toxicology Program (NTP), diazinon caused an increase in the incidence of proliferative lesions of the uterus (NTP, 1979). In female rats, the incidence of proliferative lesions of the uterus in treated animals was roughly double that in controls (P = 0.05, Cochran-Armitage trend). In female mice, the incidence of uterine hyperplasia was significantly increased (P = 0.05, Cochran-Armitage trend).

Male Wistar rats receiving diazinon at a dose of 15 or 30 mg/kg bw per day in corn oil by gavage for 4 weeks showed no differences in markers of apoptotic effects in brain tissue (Marzieh et al., 2013). Western-blot analyses of caspases 3 and 9 and related active forms, or Bax/Bcl2, did not differ between treated and control rats.

In an experiment on liver foci, male F344 rats were injected intraperitoneally with diethylnitrosamine as an initiator, and then received diets containing diazinon (500 or 100 ppm) for 6 weeks; diazinon had no effect on the number of foci that were positive for glutathione *S*-transferase placental (GSTP) form (Kato et al., 1995).

In adult male Wistar rats receiving daily doses of diazinon (15 mg/kg bw) in corn oil for 4 weeks, liver caspases 3 and 9 were activated and the Bax/Bcl2 ratio was increased (Lari et al., 2013). The antioxidant crocin had a protective effect, as indicated by decreased levels of caspases 3 and 9 activation and Bax/Bcl2 ratio in rats receiving diazinon plus crocin. In a follow-up study in similarly treated rats, proteomic analysis showed that levels of liver proteins involved in apoptosis pathways were perturbed (Lari et al., 2014). For example, levels of glucose-regulated protein GRP78 (a member of the family of heat-shock proteins that functions as an endoplasmic reticulum chaperone with anti-apoptotic properties) and regucalcin (RGN, involved in cellular calcium homeostasis) were reduced.

(i) *In vitro*

In a rat intestinal cell line (IEC-6) incubated with diazinon in DMSO, cell growth (MTT assay) was elevated after 1 day with diazinon at 1, 10 and 50 μM, after 2 days at 1 μM, and after 3 days at 1 or 10 μM (Greenman et al., 1997).

Diazinon (0.01–10 μM) induced cell proliferation in rat pituitary tumour cells (MtT/Se), which are responsive to stimulation by 17β-estradiol (Manabe et al., 2006).

Diazinon was tested in Swiss Webster mice, on cultures of neuronal and mixed cortical cell lines derived from fetal mixed cortical cells, and glial cultures derived from mice aged 1 or 2 days (Rush et al., 2010). Diazinon at a concentration of 30 or 100 μM caused a high percentage of neuronal death, while diazoxon had no measurable effect. The toxicity of diazinon was mitigated by co-exposure to a caspase inhibitor. Diazinon induced chromatin condensation characteristic of apoptosis. Glutamate receptor antagonists, as well as atropine and mecamylamine, were not protective, and addition of acetylcholine and its non-hydrolysable analogue, carbachol, did not increase toxicity as would be expected if inhibition of acetylcholinesterase activity were playing a role.

In a study designed to test the neuroprotective effects of cannabinoids, diazinon (50–200 μM) induced apoptosis in a dose-dependent fashion, as measured by TUNEL (terminal uridine deoxynucleotidyl transferase dUTP nick end labelling) staining, in the rat PC12 neuronal cell line (Sadri et al., 2010). Apoptosis was mitigated when cells were pre-treated with the cannabinoid receptor agonist WIN-55, 212-2.

4.3 Data relevant to comparisons across agents and end-points

4.3.1 General description of the database

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

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

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. [The Working Group noted that the limited activity of the oxon metabolites in in-vitro systems may be attributed to the high reactivity and short half-life of these compounds, hindering interpretation of the results of in-vitro assays.]

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

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

1. *Is electrophilic or can undergo metabolic activation (31 end-points)*: the 31 assay end-points that were mapped to this characteristic measure cytochrome p450 (CYP) inhibition (29 end-points) and aromatase inhibition (2 end-points). All 29 assays for CYP inhibition are cell-free. These assay end-points are not direct measures of electrophilicity or metabolic activation.
2. *Is genotoxic (9 end-points):* the only assay end-points that mapped to this characteristic measure TP53 activity. [The Working Group noted that while these assays are not direct measures of genotoxicity, they are an indicator of DNA damage.]
3. *Alters DNA repair or causes genomic instability (0 end-points)*: no assay end-points were mapped to this characteristic.
4. *Induces epigenetic alterations (11 end-points):* assay end-points mapped to this characteristic

measure targets associated with DNA binding (4 end-points) and histone modification (7 end-points) (e.g. histone deacetylase).

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

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

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

Next, to integrate the data across individual assay end-points into the cumulative score for each "key characteristic," the toxicological prioritization index (ToxPi) approach (Reif et al., 2010) and associated software (Reif et al., 2013) were used. In the Working Group's analyses, the ToxPi score provides a measure of the potential for a chemical to be associated with a "key characteristic" relative to 178 other chemicals that have been previously evaluated by the *IARC Monographs* and that had been screened by ToxCast. Assay end-point data were available in ToxCast for these 178 chemicals, and not for other chemicals previously evaluated by *IARC Monographs*. ToxPi is a dimensionless index score that integrates of multiple different assay results and displays them visually. The overall score for a chemical takes into account score for all other chemicals in the analysis. Different data are translated into ToxPi scores to derive slice-wise scores for all compounds as detailed below,

and in the publications describing the approach and the associated software package (Reif et al., 2013). Within the individual slice, the values are normalized from 0 to 1 based on the range of responses across all chemicals that were included in the analysis by the Working Group.

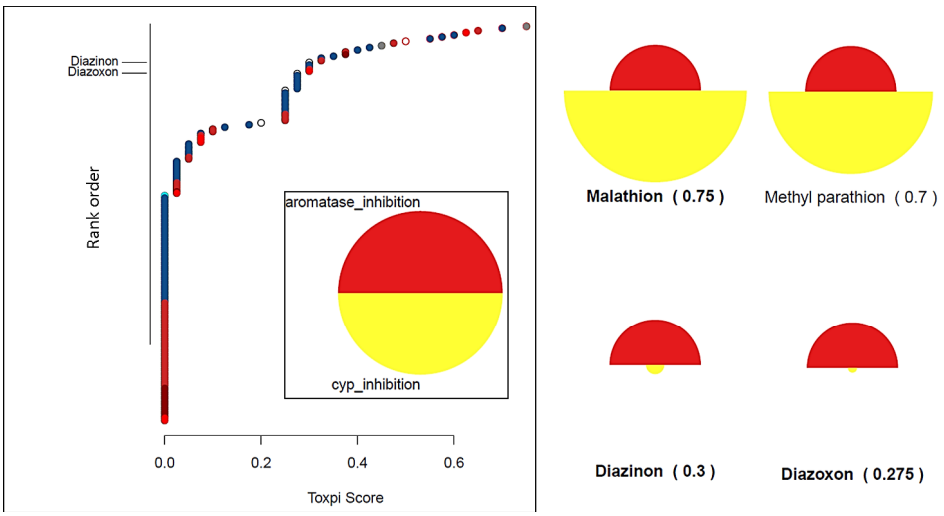
The list of ToxCast/Tox21 assay end-points included in the analysis by the Working Group, description of the target and/or model system for each end-point (e.g. cell type, species, detection technology, etc.), their mapping to 7 of the 10 "key characteristics" of known human carcinogens, and the decision as to whether each chemical was "active" or "inactive" are available as supplemental material to *Monograph* Volume 112 (IARC, 2015). The output files generated for each "key characteristic" are also provided in the supplemental material, and can be opened using ToxPi software that is freely available for download without a licence (Reif et al., 2013).

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

The relative effects of diazinon were compared with those of 178 chemicals selected from the more than 800 chemicals previously evaluated by the *IARC Monographs* and also screened by the ToxCast/Tox21 programmes, and with those of the other three compounds evaluated in the present volume of the *IARC Monographs* (Volume 112) and with three of their metabolites. Of these 178 chemicals previously evaluated by the *IARC Monographs* and screened in the ToxCast/Tox21 programmes, 8 are classified in Group 1 (*carcinogenic to humans*), 16 are in Group 2A (*probably carcinogenic to humans*), 58 are in Group 2B (*possibly carcinogenic to humans)*, 95 are in Group 3 (*not classifiable as to its carcinogenicity to humans*), and 1 is in Group 4 (*probably not carcinogenic to humans).* The results are presented as a rank order of all compounds in the analysis arranged in the order of their relative effect. The relative positions of diazinon and diazoxon in the ranked list is also shown on the *y* axis. The inset in the scatter plot shows the components of the ToxPi chart as subcategories that comprise assay end-points in each characteristic, as well as their respective colour-coding. On the top part of the graph on the right-hand side, the two highest-ranked chemicals in each analysis are shown to represent the maximum ToxPi scores (with the scores in parentheses). At the bottom of the right-hand side, ToxPi images and scores (in parentheses) for diazinon and diazoxon are shown.

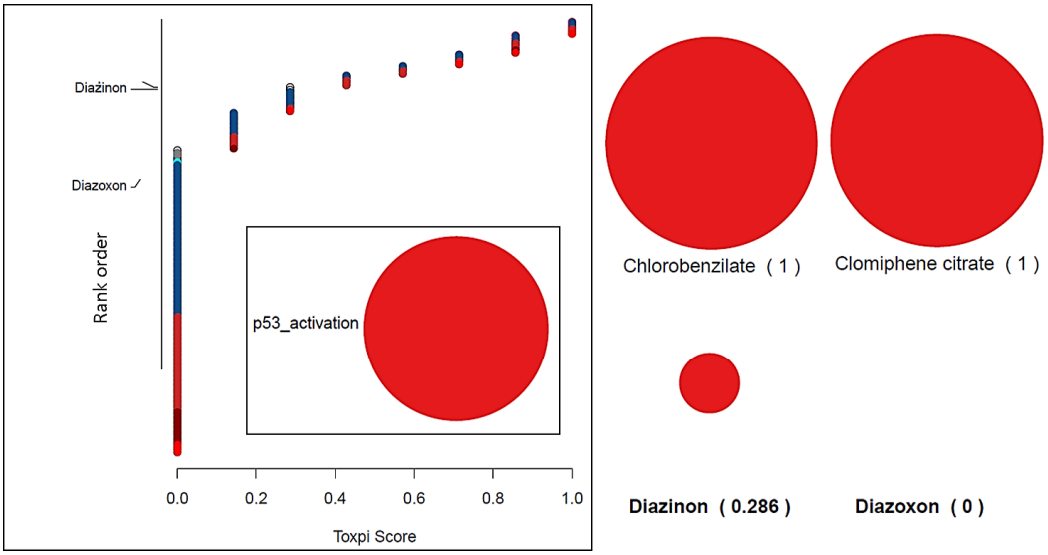
- Characteristic (1) *Is electrophilic or can undergo metabolic activation:* Diazinon and diazoxon were tested for 31 assay end-points and were found to be active for 3 and 2, respectively, of the assay end-points related to CYP inhibition. The highest ranked of the 178 chemicals included in the comparison was malathion, which was active for 20 out of 29 assay end-points. Diazinon and diazoxon were tested for two assays end-points related to aromatase inhibition, and were found to be active for one end-point each (Fig. 4.3).
- Characteristic (2) *Is genotoxic*: Diazinon and diazoxon were tested for nine assay end-points related to TP53 activity. Diazinon was found to be active for two assay end-points. The highest ranked chemicals tested were chlorobenzilate and clomiphene citrate, which were active for seven out of of nine assay end-points. Diazoxon was not active for any of these assay end-points (Fig. 4.4).
- Characteristic (4) *Induces epigenetic alterations:* Diazinon and diazoxon were found to be inactive for all 11 assay end-points for which they were tested (4 end-points related to DNA binding, and 7 end-points related to histone modification) (Fig. 4.5).
- Characteristic (5) *Induces oxidative stress*: Diazinon and diazoxon were tested for 18 assay end-points. Diazinon showed negligible

Fig. 4.3 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to enzyme inhibition



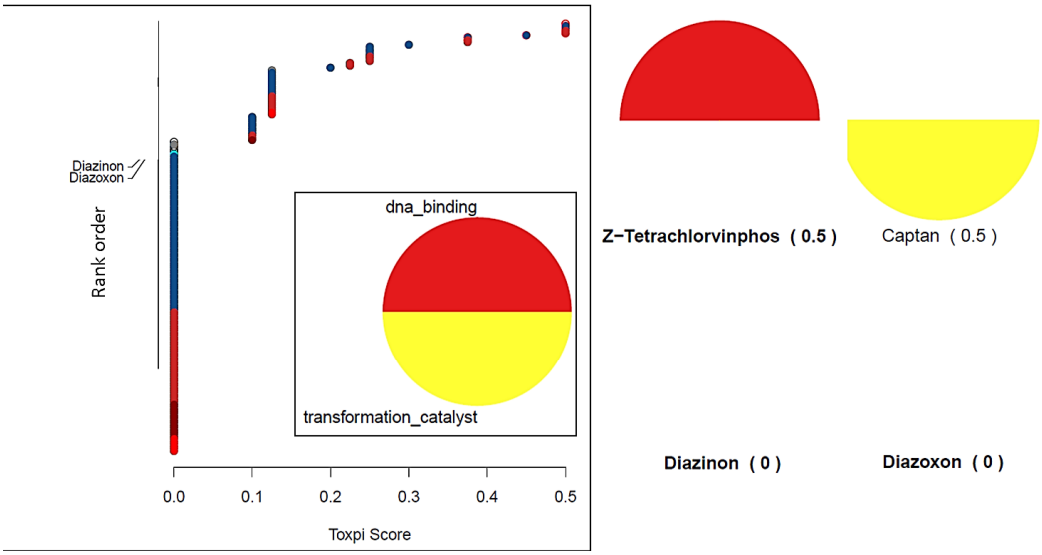
On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, malathion and methyl parathion), and the target chemicals (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.4 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to genotoxicity



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

Fig. 4.5 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to epigenetic alterations



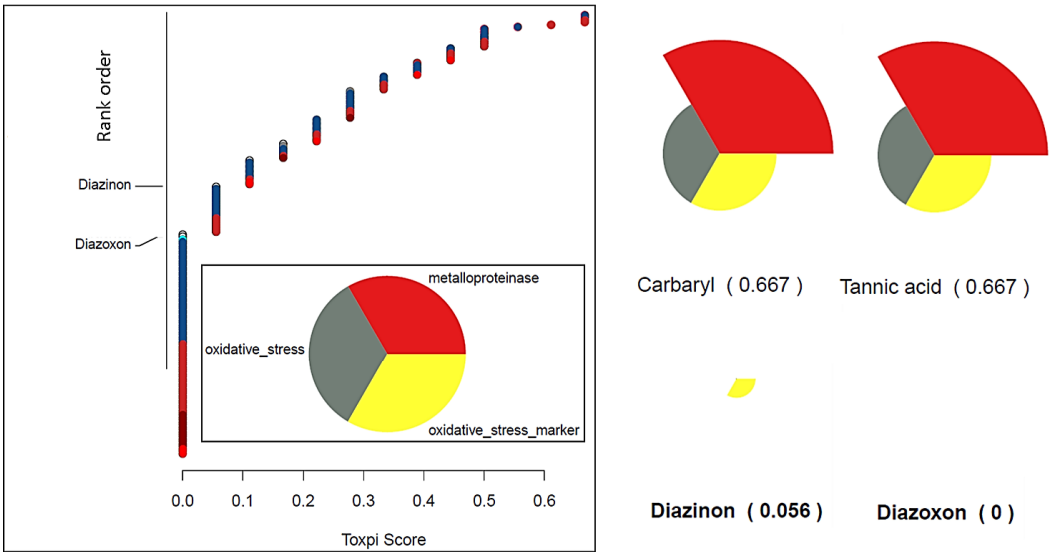
On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the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

activity. Diazoxon showed no activity (Fig. 4.6).

- Characteristic (6) *Induces chronic inflammation*: Diazinon and diazoxon were tested for 45 assay end-points; and no activity was observed for either chemical (Fig. 4.7).
- Characteristic (8) *Modulates receptor-mediated effects:* Diazinon and diazoxon were tested for 81 assay end-points. Diazinon was active for 16 of these end-points, including both end-points relating to AhR, a subset of end-points relating to estrogen receptor (both α and β), and other end-points relating to nuclear receptors. Diazoxon showed no activity for any of these assay end-points (Fig. 4.8).
- Characteristic (10) *Alters cell proliferation, cell death, or nutrient supply:* Diazinon and diazoxon were both tested for 67 of the 68 assay end-points. Diazinon was found to be active for 3 assay end-points relating to cytotoxicity, while diazoxon was active for 1 end-point. In comparison to the highest ranked chemicals, ziram and clomiphene citrate, diazinon and diazoxon showed little cellular toxicity under the conditions of the assay (Fig. 4.9).

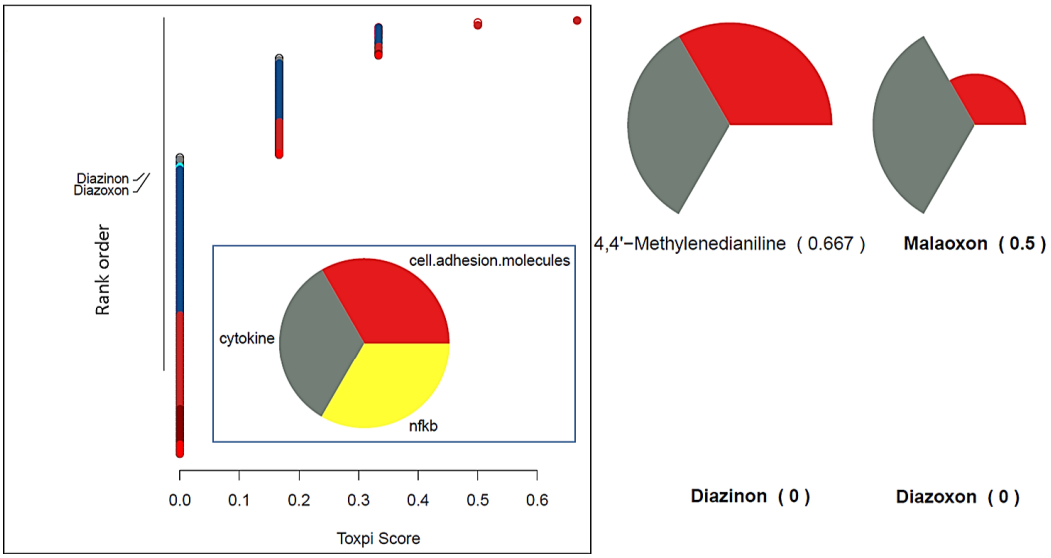
Overall, diazinon demonstrated activity in both AhR assays, and additional effects in a subset of assay end-points relating to estrogen receptor α and β. Diazoxon exhibited little activity across the 263 assay end-points, being found active for only 3 assay end-points. The limited activity of diazoxon may be attributed to the high reactivity and short half-life of this compound, which hinder interpretation of the results of the assay end-points.

Fig. 4.6 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to oxidative stress



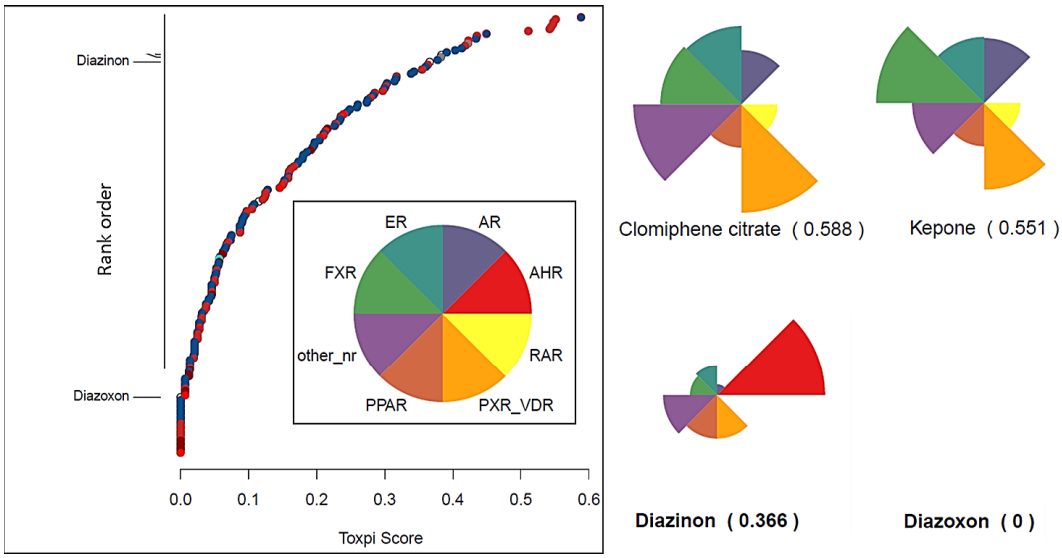
On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, carbaryl and tannic acid) and the target chemicals (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.7 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to chronic inflammation



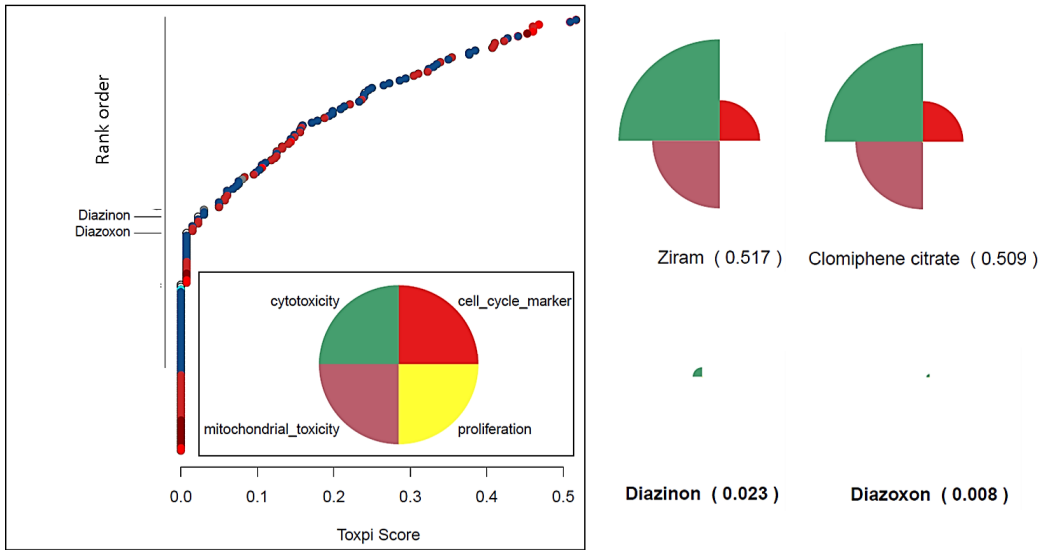
On the left-hand side, the relative ranks of diazinon, and its metabolite diazoxon, are shown (*y* axis) with respect to their toxicological prioritization index (ToxPi) score (*x* axis). The rank is relative to all other chemicals evaluated by the *IARC Monographs* that have also been tested in the ToxCast assays (including other chemicals in the present volume and 178 chemicals previously evaluated by IARC). The inset in the scatter plot shows subcategories of the ToxPi chart, as well as their respective colour coding. On the right-hand side, the ToxPi charts of the two highest-ranked chemicals (in this case, 4,4′methylenedianiline and malaoxon) and the target chemicals (diazinon and diazoxon) are shown with their respective ToxPi score in parentheses.

Fig. 4.8 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to receptor-mediated effects



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

Fig. 4.9 ToxPi ranking for diazinon and its metabolite diazoxon using ToxCast assay end-points mapped to cytotoxicity and cell proliferation



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

4.4 Susceptibility

Indirect evidence for an association between risk of cancer and exposure to diazinon was reported from two studies in the same population. Searles Nielsen et al. (2005) explored the relationship between exposure to common residential insecticides (with chlorpyrifos and diazinon presumed to be most likely exposures, albeit not measured in this study), two common *PON1* polymorphisms, C-108T and Q192R, and occurrence of brain tumours in childhood. This population-based study with 66 cases and 236 controls found an inverse association between PON1 levels and occurrence of brain tumours in childhood; the risk of childhood brain tumour was non-significantly increased in relation to the inefficient *PON1* promoter allele [per $PON1_{-108T}$ allele, relative to $PON1_{-108CC}$: OR, 1.4; 95% CI, 1.0–2.2; *P* for trend, 0.07]. Notably, the association for childhood brain tumours was statistically significant among children whose mothers reported chemical treatment of the home for pests during pregnancy or childhood (per $PON1_{-108T}$ allele: among exposed, OR, 2.6; 95% CI, 1.2–5.5; among unexposed, OR, 0.9; 95% CI, 0.5–1.6) and for primitive neuroectodermal tumours (per $PON1_{-108T}$ allele: OR, 2.4; 95% CI, 1.1–5.4). The Q192R polymorphism was not associated with risk of childhood brain tumour, nor was the $PON1_{C-108T/Q192R}$ haplotype.

In a follow-up study, Searles Nielsen et al. (2010) examined the same single nucleotide polymorphisms (SNPs) for PON1 and six additional genetic polymorphisms that affect insecticide metabolism, using the same number of cases and controls born in Washington State, USA (66 cases, 236 controls) expanded with 26 cases and 50 controls from San Francisco, and 110 cases and 99 controls from Los Angeles. Of the six additional genetic polymorphisms studied, the $BCHE_{539T}$ allele, associated with reduced in-vivo activity of the butyrylcholinesterase enzyme, was associated with increased risk of childhood brain tumours only among insecticide-exposed individuals, but this association was not statistically significant.

4.5 Other adverse effects

4.5.1 Humans

In a nested case–control study, men with diazinon metabolites in urine samples were more likely to exhibit lower sperm concentration and motility (Swan, 2006). Sperm DNA damage was observed after incubation of spermatozoa from healthy volunteers with several organophosphate compounds and their oxons, including diazinon (concentration, 50–750 μM) (Salazar-Arredondo et al., 2008; see Section 4.2.1(a)(ii) in this *Monograph*).

4.5.2 Experimental systems

Diazinon was tested in thirteen regulatory toxicity submissions included in the Toxicity Reference Database (ToxRefDB) (EPA, 2015c). Specifically, study design, treatment group, and treatment-related effect information were captured for five long-term studies of toxicity or carcinogenicity, two short-term studies of toxicity, two studies of developmental toxicity, two multigenerational studies of reproductive toxicity, and two studies of developmental neurotoxicity. Diazinon was also tested in bioassays in both rats and mice by the United States National Cancer Institute (NTP, 1979). [The Working Group noted that although long-term studies with diazinon were available, the ability to determine a full range of adverse effect potential may be limited by sensitivity to the cholinergic effects of diazinon, which limits the available dosing range.]

Cholinergic effects were observed in numerous studies in which cholinesterase inhibition was evaluated, and included inhibition of plasma, erythrocyte, and brain cholinesterase

activity at doses as low as 0.1 mg/kg bw per day (NTP, 1979; EPA, 1988, 1991). Corresponding clinical signs were also observed at doses as low as 50 mg/kg bw per day, and included increased salivation, abnormal gait, tremors, and reduced activity. Mild hyperactivity was also noted in rats and mice in bioassays carried out by the National Cancer Institute (NTP, 1979).

Liver hypertrophy and increases in liver weight were observed in female rats at the highest dietary dose tested (212 mg/kg bw per day) (EPA, 1988).

Although not specifically attributed to the stomach, gastrointestinal-tract issues were observed in rabbits given diazinon at the highest dose (100 mg/kg bw per day) in a study of developmental toxicity. Congestion, erosion, and haemorrhage were observed in the gastrointestinal tract of rabbits that died (EPA, 1981).

Under various exposure conditions, including in utero and during lactation, diazinon has been shown to decrease testicular weight, decrease sperm count and quality, and alter levels of various endocrine hormones (Jayachandra & D'Souza, 2013, 2014; ElMazoudy & Attia, 2012).

In a long-term study in dogs, lung weights were decreased in females fed diets containing diazinon at all doses (range, 0.0037–9.1 mg/kg bw per day) (EPA, 1991). Weights of the mandibular salivary gland were decreased in female dogs exposed to diazinon at the two higher doses tested (4.5 and 9.1 mg/kg bw per day) (EPA, 1991). Reduced body weight was observed in males at the intermediate dose, and in males and females at the highest dose (EPA, 1991).

In rats given diazinon at a dose of 15 mg/kg bw per day by gavage for 4 weeks, mitochondrial-mediated apoptosis occurred in heart tissue, as measured by levels of apoptotic proteins (Bax, Bcl2, and caspase 3), and the effects were ameliorated by co-exposure to the antioxidant crocin at 50/kg bw per day (Razavi et al., 2013). Evidence for cardiotoxicity has also been demonstrated in the form of dose-dependent degeneration of cardiac and skeletal muscle fibres in female rats exposed to diazinon (Abdou & ElMazoudy, 2010). In female mice, uterine cystic hyperplasia was observed in 22 out of 46 mice receiving diazinon at the highest dose tested (200 ppm), compared with zero in the matched controls (NTP, 1979).

In a two-generation study of reproductive toxicity, reduced mating, litter size, and viability index were observed in rats at the highest dose of 35.15/41.43 mg/kg bw per day (males/females). Fertility and gestational interval were reduced in females at the highest dose (EPA, 1989b).

In a study of developmental toxicity in rats, diazinon (100 mg/kg bw per day) increased rudimentary T-14 ribs and decreased fetal weights (EPA, 1985). In a study of developmental neurotoxicity in rats, diazinon (24.2 mg/kg bw per day) decreased pup weight in males and females and delayed vaginal opening in females, and preputial separation in males (EPA, 2003).

In a study of developmental neurotoxicity in rats, diazinon (24.2 mg/kg bw per day) increased the number of errors and latent period in males assessed for learning and memory in a maze (EPA, 2003).

In a dose range-finding study for the study by EPA (2003), diazinon (38.06 mg/kg bw per day) decreased pup weight in males and females, and decreased surface righting reflex in females (EPA, 2002).

5. Summary of Data Reported

5.1 Exposure data

Diazinon is an organophosphate insecticide that was developed in the 1950s and acts on a wide range of insects on crops, gardens, livestock, and pets. Production volumes have been relatively low (about 5000 tonnes in the USA in 1990) and have decreased further since use of diazinon was restricted in the USA in 2004, and in the European Union in 2006. In the USA,

outdoor residential use accounted for most of the diazinon used. Exposures in agricultural workers vary considerably, with higher exposure related to higher volume of diazinon used, inappropriate application methods, inadequate worker protection, and poor hygienic practices. Diazinon has been found in soil and dust. Levels in water and food are reported to be low.

5.2 Human carcinogenicity data

In its evaluation of the epidemiological studies reporting on cancer risks associated with exposure to diazinon, the Working Group identified 9 reports from 3 cohort studies, and 14 reports on 6 case–control studies, that reported on associations between cancer and exposure to diazinon specifically. Several large studies each provided multiple reports, notably the Agricultural Health Study cohort, case–control studies in the midwest USA, and the Cross-Canada Case–control Study of Pesticides and Health, which were considered to be key studies for the evaluation because of relatively large study size and because individual information was provided on specific pesticide exposures. Reports from more than two independent studies were available for non-Hodgkin lymphoma (NHL) and leukaemia. For cancers of the lung, breast, and prostate, results from two independent studies were available. For cancers of the colorectum, melanoma, bladder, kidney, multiple myeloma, Hodgkin lymphoma, soft tissue sarcoma, brain in childhood or in adults, stomach, and oesophagus, results from a single study for each cancer site were available for evaluation.

5.2.1 NHL

Two large case–control studies on NHL reported a positive association for diazinon: a pooled analysis from the USA (OR, 1.7; 95% CI, 1.2–2.5; including proxy respondents; OR, 1.3; 95% CI, 0.8–2.0; excluding proxy respondents), and a study from Canada (OR, 1.7; 95% CI, 0.9–3.2; including proxy respondents). The pooled analysis from the USA showed a positive exposure–response relationship with years of diazinon use when proxy respondents were excluded, and adjustment for other pesticides did not alter the results (OR, 1.9; 95% CI, 1.1–3.6; including proxy respondents). Subtype-specific analyses indicated a positive association for small lymphocytic lymphoma. The positive association for all NHL was not replicated in the Agricultural Health Study (OR, 1.0; 95% CI, 0.8–1.3), but analyses by subtype indicated an increased risk and positive exposure–response relationship with lifetime exposure days for follicular lymphoma (*P* for trend, 0.02) and suggestive evidence for a similar association for small B-cell lymphocytic lymphoma/chronic B-cell lymphocytic lymphoma/mantle cell lymphoma (*P* for trend, 0.06), as well as for all lympho-haematopoietic cancers combined (*P* for trend, 0.09). An association was absent for diffuse large B-cell lymphoma, the largest subtype within NHL, and there was some evidence of heterogeneity among subtypes. There was no evidence for major confounding by other pesticides.

The Working Group noted that: (i) positive associations for NHL or its subtypes were reported for both case–control studies and a large cohort study; (ii) both case–control studies and the cohort study suggest a positive exposure–response relationship; (iii) both case–control studies and the cohort study assessed exposure to multiple pesticides through self-reporting, which in the case of the cohort study was before diagnosis, thus excluding differential exposure misclassification as a likely explanation for the observed association in the cohort study; and (iv) there was no evidence that confounding by other pesticides could explain the observed associations.

5.2.2 Leukaemia

One case–control study on leukaemia in the USA (OR, 1.2; 95% CI, 0.6–2.1), and one case–control study nested in a cohort of farmworkers in California (OR, 1.32; 95% CI, 0.65–2.65) reported risk estimates for diazinon, neither reporting a consistently increased risk, although in one study elevated risks were reported for both chronic lymphocytic leukaemia (OR, 1.4; 95% CI, 0.5–4.4) and granulocytic leukaemia (OR, 1.9; 95% CI, 0.7–5.7). [The Working Group noted that in current classifications, chronic lymphocytic leukaemia would now be classified as NHL.] In the large Agricultural Health Study cohort, an exposure–response association (*P* for trend = 0.03) was observed for leukaemia with a rate ratio of > 3 for the highest exposure tertile. Adjustment for a list of other pesticides that were associated with increased risks within the Agricultural Health Study did not markedly alter the results.

The Working Group noted that: (i) the large Agricultural Health Study cohort provided evidence of a positive association between use of diazinon and leukaemia, which was strengthened by the presence of a monotonic increase in risk by cumulative exposure, and adjustment for other pesticides without changing the results; (ii) there was a suggestion of an increased risk for both lymphocytic and granulocytic leukaemia in a case–control study nested within a cohort from California (United Farm Workers of America).

5.2.3 Cancer of the lung

Within the large Agricultural Health Study cohort, risk estimates for cancer of the lung were reported multiple times for different updates for this prospective cohort, in 2004, 2005, and 2015. Results for cancer of the lung were very consistent over these three updates, consistently showing a positive exposure–response relationship (*P* for trend, 0.02). These risk estimates were fully adjusted for smoking; adjustment for other pesticides and other agricultural exposures did not markedly change the results. No case–control studies on cancer of the lung were identified that reported specifically on exposure to diazinon. However, one study nested in a cohort of pest-control workers from Florida, showed an increased risk of cancer of the lung associated with diazinon exposure that was not statistically significant (OR, 2.0; 95% CI, 0.7–5.5; compared with deceased controls; and OR, 1.3; 95% CI, 0.6–3.1; compared with living controls); limitations in the exposure assessment of this study were noted.

The Working Group noted that: (i) the cumulative exposure-dependent increased risk for cancer of the lung is a consistent and robust finding within the large Agricultural Health Study cohort, arguing against chance as an explanation; (ii) there was no evidence that confounding by other pesticides, smoking, or other established risk factors for cancer of the lung could explain the observed association. However, the Working Group also noted that no other cohort studies or good-quality case–control studies of cancer of the lung were identified that also reported on diazinon, thus meaning that this finding was not replicated in other study populations.

5.2.4 Cancer of the breast

Two studies were identified that reported on diazinon and cancer of the breast in women: a study nested in the United Farm Workers of America cohort and the Agricultural Health Study; neither provided consistent evidence of an increased risk.

5.2.5 Cancer of the prostate

One case–control study on cancer of the prostate was identified that reported on exposure to diazinon as assessed through a job-exposure matrix as one of 180 pesticides evaluated,

reporting an exposure–response relationship for diazinon. Limitations in the exposure assessment were noted, in particular the high correlation among pesticides assessed through the job-exposure matrix, and lack of adjustment for other pesticides. Within the large Agricultural Health Study cohort, three updates reported on cancer of the prostate in 2005, 2013, and 2015. Although based on large numbers, there was no evidence that risk of cancer of the prostate was elevated for those exposed to diazinon, and risk did not increase by cumulative exposure.

The Working Group noted that the increased risk of cancer of the prostate observed for the case–control study was not replicated in the Agricultural Health Study cohort.

5.2.6 Other cancer sites

For cancers of the bladder, colorectum, kidney, stomach, oesophagus, and tumours of the brain in childhood or in adults, and for melanoma, multiple myeloma, Hodgkin lymphoma, and soft tissue sarcoma, results from a single study for each site were available for evaluation.

For cancer of the kidney, there was some suggestion of an increased risk for the highest category of diazinon exposure (based on one report from the Agricultural Health Study).

For multiple myeloma and Hodgkin lymphoma, there was some suggestion of an elevated risk (based on the Cross-Canada Case–control Study). In the same study, an increased risk of soft tissue sarcoma was also observed, and the threefold increased risk observed did not change after adjusting for aldrin, which was the only other pesticide also associated with soft tissue sarcoma besides diazinon.

An increased risk of childhood tumours of the brain and garden use of diazinon was observed (based on a very small study), but other studies could not evaluate this association because of small numbers.

No increased risk was observed for cancers of the colorectum (based on the Agricultural Health Study), stomach and oesophagus (based on a case–control study), bladder (based on the Agricultural Health Study), melanoma (based on the Agricultural Health Study), or adult glioma (based on a case–control study).

The risk for all cancers combined was evaluated in the large Agricultural Health Study cohort, which showed an increased risk with an exposure–response relationship (P for trend = 0.009).

In conclusion, positive associations and exposure–response trends were noted for NHL, leukaemia, and cancer of the lung. The Working Group noted that the number of studies available was relatively small and confounding by other pesticides as an explanation for the increased risks could not be fully excluded.

5.3 Animal carcinogenicity data

Diazinon was tested for carcinogenicity in one 2-year feeding study in male and female mice, and two 2-year feeding studies in male and female rats.

Diazinon induced a significant increase in the incidence of hepatocellular carcinoma in male mice at the lowest dose. This increase could not be clearly related to the administration of diazinon because it was only observed in male mice at the lowest dose, at an incidence slightly above the upper limit of the range for historical controls for this tumour in this strain of mouse. There were no significant findings in males at the highest dose, or in female mice at any dose.

In the first study in rats, diazinon induced a significant increase in the incidence of leukaemia or lymphoma (combined) in male rats at the lowest dose. This could not clearly be related to the administration of diazinon because it was observed only in males at the lowest dose, at an incidence slightly above the upper limit of the range for historical controls for these tumours in this strain of rat. There were no significant

findings in males at the highest dose, or in female rats at any dose. There were no significant increases in tumour incidence in the second study.

5.4 Mechanistic and other relevant data

The majority of orally administered diazinon is absorbed, in humans, dogs, and rodents. Studies in human volunteers indicate that dermal absorption of diazinon is considerably slower than oral absorption. Few data on systemic tissue distribution in humans were available to the Working Group. Studies in experimental animals indicate that diazinon is widely distributed via blood. Overall, metabolism of diazinon involves cytochrome P450 (CYP450), paraoxonase 1 (PON1) and carboxylesterases. It is well established that diazinon metabolism is similar in humans and experimental species. Diazinon is rapidly metabolized to short-lived diazoxon or 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMPY) by several cytochrome P450s. PON1 can metabolize diazoxon to IMPY and diethylphosphate (DEP). Carboxylesterase can degrade diazoxon to liberate IMPY. In humans and experimental animals, diazinon is excreted as IMPY, DEP, or other metabolites (e.g. diethylthiophosphate).

The evidence for the genotoxicity of diazinon is *strong* and appears to operate in humans. No studies in humans in vivo exposed to diazinon only were available. Studies in experimental animals in vivo showed either DNA damage (oxidative DNA damage, DNA strand breaks) or chromosomal damage (micronuclei). In vitro, human cell lines also showed DNA damage (DNA strand breaks) or chromosomal damage (micronucleus formation, sister-chromatid exchange). The results of studies in humans exposed to multiple compounds including diazinon are consistent with these findings. In studies in non-human species in vitro, results were mixed.

The evidence that diazinon can induce oxidative stress is *strong*. Diazinon induced oxidative stress in human and mammalian cells in vitro, and in a variety of tissues in numerous studies in rodents in vivo. Studies employing pre-exposures to various antioxidants mitigated the effects. Diazinon induces oxidative stress through alteration of antioxidant enzyme activity, depletion of glutathione, and increasing lipid peroxidation. Several studies in fish also report similar findings. Pro-inflammatory effects are also observed in vivo in studies in rodents.

The evidence for receptor-mediated mechanisms in the potential carcinogenicity of diazinon is *weak*. In vivo, diazinon modulated gonadotropin levels in several studies in rats. The diazinon metabolite diazoxon binds to acetylcholinesterase and other serine esterases such as butyrylcholinesterase. It is unclear what role, if any, the sequelae can play in carcinogenesis.

Overall, the effects on proliferation are *weak*, with a few studies showing apoptotic effects in some diazinon-exposed human and rodent cell lines, and in a few other studies showing no cell proliferation or apoptotic effect. Diazinon induced uterine cystic hyperplasia in mice.

Because of the limited available data, the evidence for immunosuppression as a mechanism of carcinogenicity for diazinon is *weak*. In human cell lines, diazinon decreased the induction of regulators of immune system function, while pathological effects on the immune system, suppression of humoral immune response, and cellular functional responses have been observed in rodents in vivo. Immunotoxicity was seen in model fish species.

There were few data on the other key characteristics of carcinogens.

In studies in humans and experimental animals, diazinon exhibited effects of sperm quality, count, and motility, with corresponding testicular pathology in animals. In addition to

cholinergic effects, non-neoplastic pathology was also observed in lung, stomach, heart, and liver tissues in studies in experimental animals.

Overall, the mechanistic data provide strong support for carcinogenicity findings of diazinon. This includes strong evidence for genotoxicity and oxidative stress. There is evidence that these effects can operate in humans.

6. Evaluation

6.1 Cancer in humans

There is *limited evidence* in humans for the carcinogenicity of diazinon. A positive association has been observed for non-Hodgkin lymphoma, leukaemia, and cancer of the lung.

6.2 Cancer in experimental animals

There is *limited evidence* in experimental animals for the carcinogenicity of diazinon.

6.3 Overall evaluation

Diazinon is *probably carcinogenic to humans (Group 2A).*

6.4 Rationale

There is strong evidence that diazinon can operate through two key characteristics of known human carcinogens and that these can be operative in humans. Specifically:

- There is strong evidence that exposure to diazinon is genotoxic, from studies in experimental animals in vivo, and in studies in animal cell lines. In addition, studies in human cell lines in vitro show effects on chromosomal damage; this demonstrates that this mechanism can operate in humans. Additional support for human relevance is provided by positive results in a study of a small number of volunteers exposed to diazinon.
- There is also strong evidence that diazinon can act to induce oxidative stress. This evidence is from studies in experimental animals in vivo, and studies in human and animal cell lines in vitro. This mechanism has been challenged experimentally by administering antioxidants, treatment that abrogated the effects of diazinon on oxidative stress.

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