Diazinon / Diethoxy-(6-methyl-2-propan-2-ylpyrimidin-4-yl)oxy-sulfanyliden-λ5-phosphan

MAK Value Documentation

A. Hartwig[1,*], MAK Commission[2,*]

DOI: 10.1002/3527600418.mb33341e5917

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated diazinon [333-41-5] considering all toxicological endpoints. Available publications and unpublished study reports are described in detail. The critical effect of diazinon is inhibition of the acetylcholinesterase (AChE) at the cholinergic synapses which is measured as AChE activity in erythrocytes and brain. A reduction to 70 and 80% of their respective activities prior to exposure is regarded as adverse. The corresponding NOAELs in subchronic or chronic feeding studies in rats, dogs and monkeys are 0.3, 0.75 and 0.17 mg/kg body weight and day, which are scaled to concentrations of 0.6, 4.5 and 0.7 mg/m^3, respectively. Taking into account a possible enhancement of the effects over time, the maximum concentration at the workplace (MAK value) of 0.1 mg/m^3 for the inhalable fraction is confirmed. The MAK value is also supported by a 3-week inhalation study in rats with a NOAEC of 0.46 mg/m^3.

Since a systemic effect is critical, Peak Limitation Category II is retained. The renal elimination half-life of the dialkyl phosphate metabolites of diazinon in humans is about 2 hours, so that the corresponding excursion factor of 2 is confirmed.

The NOAELs for developmental toxicity are 1.9 mg/kg body weight and day for rats and 100 mg/kg body weight and day for rabbits. As developmental neurotoxicity is induced only at higher doses, the offspring are less sensitive to the inhibition of AChE than the dams, and the margins between the calculated concentrations without effects and the MAK value are sufficiently large, the classification of diazinon in Pregnancy Risk Group C is retained.

Diazinon is not considered to be genotoxic, and therefore not regarded as a germ cell mutagen. In studies not reaching the maximum tolerated dose, diazinon is not carcinogenic in rats and mice. Skin contact is expected to contribute significantly to systemic toxicity, and diazinon remains designated with an "H". Limited data in animals do not show a skin sensitizing potential.

Keywords

diazinon; phosphorothioic acid O,O-diethyl O-[6-methyl-2-(1-methylethyl)-4-pyrimidinyl] ester; acetylcholinesterase inhibitor; mechanism of action; toxicokinetics; metabolism; (sub)acute toxicity; (sub)chronic toxicity; irritation; allergenic effects; reproductive toxicity; fertility; developmental toxicity; genotoxicity; carcinogenicity; peak limitation; prenatal toxicity; germ cell mutagenicity; absorption through the skin; sensitization; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

Author Information

[1] Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Department of Food Chemistry and Toxicology, Institute for Applied Biosciences, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Geb. 50.41, 76131 Karlsruhe, Germany

[2] Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany

* Email: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)

Diazinon

[333-41-5]

supplement 2015

MAK value (1995)	**0.1 mg/m³ I (inhalable fraction)**
Peak limitation (2002)	**Category II, excursion factor 2**
Absorption through the skin (1975)	**H**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (1985)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value (1985)	**reduction of the acetylcholinesterase activity in erythrocytes to 70% of the reference value**

To extrapolate an oral dose from an animal experiment to a concentration in workplace air, since 2010 the Commission has used a species-specific toxicokinetic procedure (DFG 2010). This procedure is used here to verify whether the MAK value of 0.1 mg/m³ I and the classification of diazinon in Pregnancy Risk Group C are still justified.

Diazinon is a thiophosphoric acid ester used as insecticide, acaricide and nematicide (US EPA 2006).

For diazinon, there is documentation from 1975 (documentation "Diazinon" 1998) and supplements from 1992, included in the general chapter on pregnancy (supplement "Sammelkapitel MAK-Werte und Schwangerschaft" 1992, available in German only), 1995 (all end points, incorporated in the English translation of the documentation from 1975: documentation "Diazinon" 1998) and 2002 (regarding the peak limitation category, supplement "Diazinon" 2002).

1 Toxic Effects and Mode of Action

The critical effect of diazinon is the inhibition of acetylcholinesterase (AChE) activity at the cholinergic synapses. The secondary target organs of diazinon are the liver, pancreas and kidneys.

In rabbits, diazinon is not irritating to the skin and, at most, slightly irritating to the eyes.

No clinical findings regarding sensitization or positive patch test results with diazinon are available. The results of animal studies of skin sensitization are not sufficient to derive a skin-sensitizing potential.

In female rats, 3-week inhalation exposure to diazinon (as aerosol) led to the inhibition of AChE activity in the brain to levels of 80% and lower at concentrations of 1.57 mg/m^3 and above.

At doses of about 5 mg/kg body weight and day and above, the AChE activity in the brain was reduced to levels of 80% and lower in rats after 99 weeks and in dogs after 13 and 52 weeks. In a 106-week gavage study in male monkeys, a reduction in the AChE activity in the erythrocytes to levels of 70% and lower was demonstrated at 0.5 mg/kg body weight and day and above.

In male CD-1 mice, histological and functional changes in the reproductive organs were observed at doses of 4.1 mg/kg body weight and day and above after 4-week gavage administration.

In humans, an inverse correlation between the diazinon concentration in the umbilical plasma and the weight at birth and body size was found in newborn babies. In pregnant rats, visceral abnormalities occurred in the foetuses at doses of 3.8 mg/kg body weight and day and above. These are a secondary effect of pronounced maternal toxicity. In a developmental neurotoxicity study in Sprague Dawley rats, delayed body weight gains, delayed sexual maturity, and impaired motor activity, learning and memory were found at 24.2 mg/kg body weight and day. At doses of as little as 2.36 mg/kg body weight and above, a reduction in the AChE activity in erythrocytes and the brain occurred in the dams. In pregnant rabbits, diazinon was toxic to the dams (cholinergic symptoms, mortality) after gavage doses of 30 mg/kg body weight and day and above. No effects were found in the foetuses, however, up to the high dose of 100 mg/kg body weight and day.

Diazinon is not mutagenic in bacteria and mammalian cells. A positive result in the micronucleus test with human fibroblasts and lymphocytes indicates possible clastogenic potential. This could, however, not be confirmed in vivo. In vitro, diazinon and its metabolite diazoxon reduced sperm DNA integrity. In vivo, however, neither SCE (sister chromatid exchange) nor dominant lethal mutations were induced in germ cells.

Carcinogenicity studies with F344 rats and B6C3F1 mice at dose levels below the maximum tolerated dose (MTD) did not produce any evidence of a carcinogenic potential of diazinon.

2 Mechanism of Action

Like other phosphoric acid ester insecticides, diazinon causes AChE inhibition after metabolic oxidation to diazoxon (documentation "Diazinon" 1998). The inhibition of structure-bound AChE at the cholinergic synapses is the only factor responsible for the symptoms following intoxication with AChE inhibitors. The acute inhibition of AChE leads to the accumulation of the transmitter in the tissue ("endogenous acetylcholine poisoning"). AChE is present not only at the cholinergic synapses in the CNS or the motor end plates, but also in erythrocytes. The inhibition of AChE activity in the erythrocytes is a surrogate for inhibition of the peripheral AChE activity. Cholinesterases (ChE; pseudocholinesterases), in contrast, form a group of isoenzymes which are non-specific and are ubiquitous in the organism (Greim and Lehnert 1995).

The inhibition of the erythrocyte AChE to 70% and less of its activity prior to exposure is regarded as adverse (WHO 1986). For AChE activity in the brain, inhibition to levels of 80% and below is considered to be adverse, as the inhibition of the AChE activity in the brain must, toxicologically, be regarded more critically, so that only a lower level of inhibition can be tolerated.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Within 168 hours after a radioactively labelled oral dose of diazinon (dose not specified), between 70% and 80% of the radioactivity was recovered in the urine of rats and between 16% and 24% in the faeces (no other details; ACGIH 2003). Female beagle dogs absorbed at least 85% of a single oral dose of 4.0 mg [^{14}C]-diazinon/kg body weight (ATSDR 1996).

The plasma concentrations of diazinon in male Wistar rats were described most accurately using a two-compartment model after intravenous administration of 10 mg/kg body weight and a one-compartment model after gavage administration of 80 mg/kg body weight. The elimination half-times of diazinon after intravenous and oral administration were 4.70 and 2.86 hours, respectively. The oral bioavailability of the intact diazinon was 35.5%; the low oral bioavailability is explained by a first-pass effect in the liver. The plasma concentration of diazinon was 0.4 to 30 mg/l, of which about 89% was bound to proteins (Wu et al. 1996).

Male Wistar rats were given single gavage doses of diazinon of 23 mg/kg body weight. Four hours after exposure, the maximum concentration in blood of 80.20 ± 1.14 µg/g was reached. In muscle tissue (9.89 ± 0.13 µg/g) and in the liver (3.97 ± 0.057 µg/g) the concentrations were at their highest after 8 hours. The terminal half-life in the blood was about 5 days, in muscle tissue and the liver about 15 and 10 days, respectively. A three-compartment model provided the most adequate description of the plasma concentrations (García-Repetto et al. 1996).

About 68% of an oral dose of radioactively labelled diazinon of 1 mg/kg body weight was found in the urine of mice 60 minutes after administration (no other details; ACGIH 2003).

In rats, the elimination half-time for the heterocyclic part of the molecule was calculated to be 12 hours, and 7 hours for the phosphate/thiophosphate. The substance or its metabolites pass through enterohepatic circulation, which is in agreement with the unusually high amount in some cases (up to > 20%) eliminated with the faeces (IFA 2013).

After single subcutaneous injections of diazinon of 165 μmol/kg body weight (not radioactively labelled), the peak concentration of diazinon in the plasma and brain of male albino mice was found 15 minutes after administration. The half-life in each case was 2.5 hours. Within 24 hours, the urine was free of the metabolites diethyl phosphate and diethyl thiophosphate, the only metabolites determined (de Blaquière et al. 2000).

After dermal application (no other details), between 70% and 80% of the dose was eliminated by the kidneys in rats (WHO 1993). In humans, on the other hand, absorption through the skin after 24-hour open dermal application of diazinon in acetone (2 μg/cm^2) or in lanolin (1.47 μg/cm^2) was merely 2.9% to 3.9%, determined on the basis of the renal elimination over a period of 7 days. As, by the end of the exposure, only between 0.4% and 1.4% of the dose could still be detected on the skin, the authors concluded that the majority of the dose had either been absorbed by clothing or dissipated by volatilization (Wester et al. 1993). The same authors carried out additional in vitro studies with human skin. After 0.25 μg diazinon/cm^2 (dissolved in acetone), 14.1% of the dose was recovered in the receptor fluid within 24 hours. The calculated amount absorbed was the same (0.035 μg/cm^2 and 24 hours (equivalent to a flux of 0.0015 μg/cm^2 and hour)) for both in vitro and in vivo absorption through human skin.

Five volunteers (4 men and 1 woman aged 30 to 50 years) received single oral doses of diazinon of 11 μg/kg body weight (36 nmol/kg body weight) or occlusive dermal doses of 100 mg/person (dissolved in ethanol, concentration 250 g/l, 8 hours, 1.25 mg/cm^2) on the skin of the forearm (80 cm^2). The absorption and elimination of diazinon were studied by monitoring the concentrations of the diazinon metabolites diethyl phosphate and diethyl thiophosphate in their urine samples. Following oral and dermal exposure, the maximum urinary diethyl phosphate concentrations occurred after 2 and 12 hours, respectively. The urinary elimination half-times were approximately 2 and 9 hours, respectively. About 60% of the oral dose and 1% of the dermal dose was eliminated as urinary diethyl phosphate metabolites; 90% of the dermal dose was recovered from the skin surface. In a period of up to 70 hours after dermal application, about 0.47% of the dose was eliminated with the urine in the form of metabolites. Taking into account also amounts of diazinon not renally eliminated (an additional 33% eliminated with the faeces), the authors calculated a dermal flux of 3.7 nmol/cm^2 and hour for an 8-hour exposure, which corresponds to 1.13 μg/cm^2 and hour (Garfitt et al. 2002).

A physiologically-based pharmacokinetic/pharmacodynamic model was developed to describe the toxicokinetics of diazinon, diazoxon and 2-isopropyl-6-methyl-4 (1 H)-pyrimidinone as well as the cholinesterase inhibition in rats and humans after oral and dermal exposure. The dermal permeability coefficient used for humans was 1.31×10^{-4} cm/h (Poet et al. 2004).

3.2 Metabolism

After absorption in the mammalian organism, diazinon is oxidatively metabolized to the actual cholinesterase-inhibiting substance diazoxon via the cleavage of sulfur (IFA 2013). The activation of diazinon is catalyzed by CYP450 enzymes, as is detoxification to 2-isopropyl-6-methyl-4 (1 H)-pyrimidinone and diethyl thiophosphate. Diazoxon can be detoxified also by hepatic and extrahepatic A-esterases (paraoxonase/arylesterase 1 = PON1 hydrolyze organophosphates, although they are not inhibited; Poet et al. 2003) and B-esterases (AChE, butyryl cholinesterase, carboxylesterases, which can be inhibited; Poet et al. 2003) to form 2-isopropyl-6-methyl-4 (1 H)-pyrimidinone and diethyl phosphate (see Figure 1; Poet et al. 2004).

The extent of desulfuration (activation) and dearylation (detoxification) of diazinon was investigated in human liver microsomes. Recombinant human cytochrome P450 isoenzymes were used to determine specific kinetic parameters. The activation of diazinon was mediated primarily by CYP1A1 (K_m = 3.05 µM; V_{max} = 2.35 nmol/min/nmol P450), CYP2C19 (K_m = 7.74 µM; V_{max} = 4.14 nmol/min/nmol P450) and CYP2B6 (K_m = 14.83 µM; V_{max} = 5.44 nmol/min/nmol P450). The isoenzym mainly involved in detoxification was CYP2C19 (K_m = 5.04 µM; V_{max} = 5.58 nmol/min/nmol P450) (Ellison et al. 2012).

In the liver microsomes of 15 subjects (organ donors, no other details), the activity for the formation of diazoxon at diazinon concentrations of 50 and 500 µM was between 11 and 648 or between 164 and 978 pmol/min and mg protein, respectively. CYP2C19 is the main enzyme involved in the activation of diazinon, while other enzymes including CYP1A2 play a more minor role (Kappers et al. 2001).

In an in vitro study with microsomes from the liver and from the enterocytes of male Sprague Dawley rats, the following results were obtained: for the cytochrome 450-mediated metabolism the K_m values for the formation of the oxon and 2-isopropyl-6-methyl-4 (1 H)-pyrimidinone were 498 and 92.9 µM in the liver and 235 and 72.4 µM in the enterocytes, respectively. The values for V_{max} for the formation of the oxon and 2-isopropyl-6-methyl-4 (1 H)-pyrimidinone were 0.357 and 0.03 µM in the liver and 2.79 and 0.5 µM in the enterocytes, respectively. Although the affinity of PON1 for diazoxon was comparable in hepatic and enterocytic microsomes (K_m: 268 and 347 µM, respectively), the V_{max} in the liver was about 48 times higher than in the enterocytes. The intestinal metabolism, especially following low-dose oral exposure, has a considerable impact on the total metabolism (Poet et al. 2003).

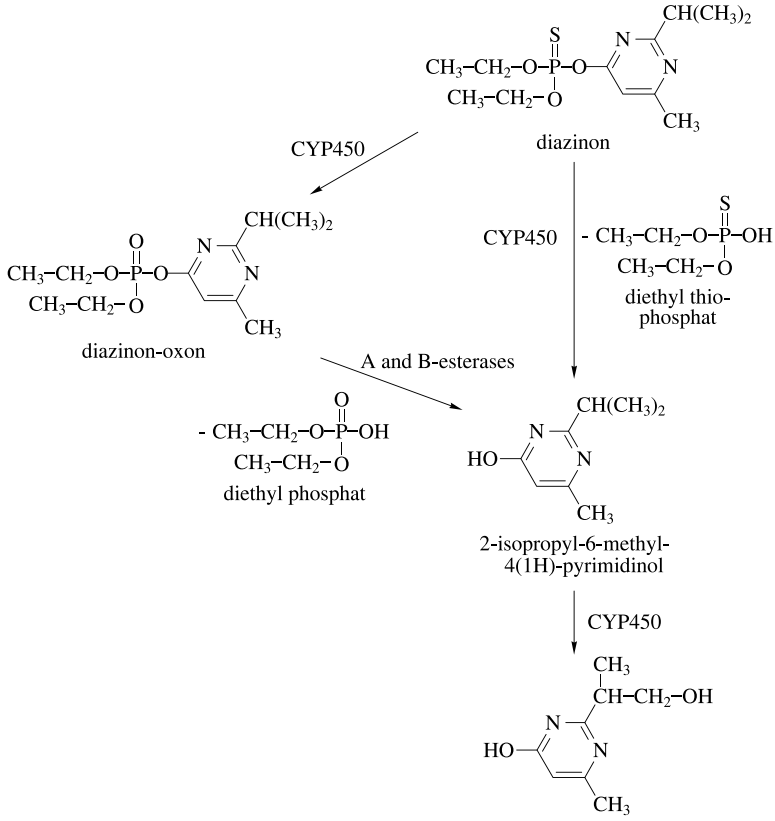

Figure 1 Biotransformation of diazinon in humans (from Poet et al. 2004)

4 Effects in Humans

4.1 Single exposures

In the supplement from 1995 (documentation "Diazinon" 1998) several cases of poisoning after inhalation or oral exposure to diazinon, with fatal results in some cases, were reported. Depending on the severity of the AChE inhibition, signs of parasympathetic stimulation extending to vegetative, motor and central nervous cholinergic crisis were observed. The first clinically manifest symptoms occur only after the AChE activity has decreased to a level markedly below 50% (Greim and Lehnert 1995).

Five volunteers (4 men and 1 woman aged 30 to 50 years and weighing 76 to 90 kg) were given either single oral diazinon doses of 11 µg/kg body weight (about 0.088 mg/person) or occlusive dermal doses of 100 mg/person (about

1.25 mg/kg body weight). There was no statistically significant change in the mean plasma and erythrocyte cholinesterase activities compared with their activities prior to exposure. None of the volunteers reported symptoms or cholinergic effects (Garfitt et al. 2002).

4.2 Repeated exposure

A case study of 99 individuals who were occupationally exposed to diazinon granules 8 hours a day for 39 days during an insecticide application program reported only slight neurological functional deficits (symbol-digit speed and pattern memory accuracy) as a result of exposure. A no observed adverse effect level (NOAEL) of 0.02 mg/kg body weight and day was estimated on the basis of measured diazinon concentrations in passive dermal badges, hand rinses, and full-shift breathing-zone air samples. In the opinion of the ATSDR, multiple exposure routes were involved, so that it was considered difficult to verify the dose calculated by the authors. In addition, the lack of accurate details for the exposure time and recovery from the neurological functional deficits was criticized (ACGIH 2003; ATSDR 1996). For these reasons, this study is not used in the evaluation of diazinon.

Several studies describe the inhibition of cholinesterase activity only under specific working conditions: pest control employees working on average for 8 hours a day and exposed to diazinon, chlorpyrifos or dichlorvos concentrations of 6, 7.1 or 31 μg/m³, had lower levels of cholinesterase activity in the plasma compared with the control group. The activity of acetylcholinesterase in the erythrocytes was unchanged (no other details; ACGIH 2003). As detailed information is lacking, this statement cannot be used in the evaluation of diazinon.

In 18 male Japanese insecticide sprayers working in indoor areas, 5 of whom used mainly diazinon, increased urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) were determined compared with those in 18 control persons not exposed to insecticides. The duration of insecticide application was between half a year and 25 years (average: 5.6 ± 5.8 years). In the summer there was a positive correlation between the 8-OHdG concentration and the concentration of urinary organophosphate metabolites (dimethyl phosphate and total dialkyl phosphates) (Lee et al. 2007). Because of the low number of volunteers examined and the lack of analytical data for the concentrations in the air the study is not included in the evaluation of diazinon.

After moving into a house in Israel (no other details), in which diazinon had been used, the 4 members of the family (mother, father, two daughters) complained of the following symptoms: fatigue, sleeping problems, irritability, headaches, vomiting and heaviness of the chest. Four months later, the family members had increased concentrations of the diazinon metabolite diethyl phosphate in the urine (between 0.45 and 1.70 mg/l) and slightly reduced serum cholinesterase activity (between 78.4% and 93.6% of the normal values). The surface concentration on the walls was between 16 (living room) and 1.051 μg/m² (kitchen) and the concentrations in the air were 5 (guest room) and 27 μg/m³ (entrance to bathroom); also a

number of clothing items were contaminated (between 0.5 and 0.7 μg/g) (Richter et al. 1992). The number of investigated persons is too low to draw any conclusions as to the effect of diazinon at the workplace.

4.3 Local effects on skin and mucous membranes

In patch tests with 294 patients, a 1% diazinon preparation in petrolatum had no irritating effects after 48 and 72 hours (Lisi et al. 1987).

4.4 Allergenic effects

Patch tests with 1% diazinon in petrolatum with 294 patients from a total collective of 652 patients (of whom 274 had contact dermatitis particularly on the hands) did not cause allergic reactions in any of the cases. Of those tested, 125 worked in agriculture at the time or had done so previously (Lisi et al. 1987).

In an incompletely documented publication, 202 cases of contact dermatitis presumably caused by contact with organophosphorous insecticides were reported in Japan between 1972 and 1979. These included two cases of contact dermatitis caused by diazinon alone. According to the authors, also other organophosphorous insecticides in addition to diazinon were responsible for the skin changes in 16 other cases. There was no detailed description of the skin changes and no description of how the patch tests were carried out (Matsushita et al. 1985).

In 56 voluntary test persons, induction and challenge treatment were carried out with technical-grade diazinon (1% emulsified in water with Tween 80) or with a diazinon formulation diluted with water. The induction treatment consisted of 9 applications at intervals of 2 to 3 days. For this purpose, 0.5 ml of the test substance was applied occlusively to the forearm of each person for 24 hours. The challenge treatment took place 14 days after the final induction. Sensitization was found in 6 of the 56 volunteers. In a 2nd and 3rd subsequent test, the volunteers likewise produced a reaction (US EPA 1999).

4.5 Reproductive and developmental toxicity

In a biological monitoring study in Poland, male agricultural workers exposed to pesticides (about 30 pesticides, including diazinon, were listed in the questionnaire), were compared with men not exposed living in the same area (49 exposed and 50 not exposed). In a binary logistic regression analysis, in which the number of malformations and miscarriages after exposure to pesticides, smoking, alcohol and age were investigated, only the exposure to pesticides was directly related to the number of miscarriages (Pastor et al. 2001). As exposure was to a mixture of substances, no conclusions can be drawn about the toxic potential of diazinon on reproduction.

In four American cities, the sperm quality of the partners of 493 pregnant women was investigated between 1999 and 2001. While the differences in sperm morphology and the sperm volumes in men of different cities were low, the sperm concentration and motility in Columbia (Missouri) were significantly reduced compared with in New York (New York State), Minneapolis (Minnesota) and Los Angeles (California). Using multivariate models to monitor abstinence time (reported sexual abstinence between 2 and 5 days), age, race, smoking, sexually transmittable diseases and fever, the significance remained the same (all p-values < 0.01). In a nested case–control study, the metabolites of 8 non-persistent pesticides were measured in the urine (2-isopropyl-6-methyl-4-pyrimidinol as a metabolite of diazinon) of 25 men of the cohort from Columbia with reduced and with normal sperm parameters. In the men with the reduced sperm parameters, the metabolite concentrations of alachlor, atrazine and diazinon were increased compared with the values in control persons (p-value, Wilcoxon rank test = 0.0004 for diazinon). In men with a higher metabolite concentration the probability of a reduction in sperm parameters was higher than in those with lower metabolite concentrations (odds ratio = 16.7 for diazinon) (Swan 2006). As exposure was to a mixture of substances, the study is not included in this evaluation.

From a total of 571 pregnant women in New York City (Columbia Center for Children's Environmental Health, 63% of whom were Afro-American women and 37% from the Dominican Republic), 85% reported that they had been exposed to insecticides in their households during pregnancy. The voluntary participants were recruited between January 1998 and January 2004. Active smokers and mothers, whose cotinin concentration in the plasma or in the umbilical blood at birth was above 15 ng/ml, were excluded from the study. Of the 10 measured insecticides present, chlorpyrifos, diazinon and propoxur were the most frequent. These three compounds were found in between 99.7% and 100% of the 48-hour personal air samples examined during the pregnancy (number of air samples: n = 394) and in between 39% and 70% of the blood samples from the mothers (n = 326) or the infants (n = 341) at birth. There was a high level of correlation between the maternal and the neonatal blood concentrations (r = 0.69 for diazinon, p = 0.001). After consultation between the EPA (Environmental Protection Agency) and the manufacturer, the latter started to phase out the sale of insecticides containing diazinon for use in indoor areas in the USA in January 2001 (completed in December 2002). From this time onwards, the insecticide concentrations in the air and in umbilical blood decreased significantly. In addition, the inverse correlation between chlorpyrifos and diazinon concentrations in the umbilical plasma and the weight at birth and body size was no longer detectable (Whyatt et al. 2005). Corresponding results were also published earlier (Whyatt et al. 2004). No regression analysis was carried out for diazinon alone.

4.6 Genotoxicity

Cytogenetic studies in workers in diazinon production were already described in the supplement of 1995. These were not considered valid, as no information was provided on the exposure levels and duration, and exposure was to a mixture of pesticides Király et al. (1979) in documentation "Diazinon" 1998.

In the above-mentioned biological monitoring study with men exposed to pesticides, the number of micronuclei in the lymphocytes and buccal epithelium was not affected (see Section 4.5; Pastor et al. 2001). As exposure was to a mixture of substances, the study is not included in this evaluation.

Three studies with occupational exposure to insecticides, including diazinon, are also described. In workers exposed to insecticides, increased incidences of chromosomal aberrations and sister chromatid exchange in lymphocytes were observed compared with the levels in populations not exposed. According to the authors, the workers were exposed to as many as 80 different substances at unknown exposure levels and for unknown lengths of time (ATSDR 1996). Whether these effects can be attributed to exposure to diazinon, cannot be substantiated as exposure was to a mixture of substances.

4.7 Carcinogenicity

In the 1995 supplement, several studies were cited in which a possible carcinogenic effect of diazinon in humans was investigated. These data were not considered sufficient to evaluate the carcinogenicity of diazinon, as a causal relationship with diazinon exposure was not regarded as confirmed (documentation "Diazinon" 1998).

In a review, 8 cohort studies and 5 case–control studies were analysed in order to evaluate the hypothesis that agricultural exposure to pesticides is causally associated with a risk of prostate cancer. In 2 cohort studies, no relationship between diazinon exposure and prostate cancer was found. Despite sporadic positive findings, these studies did not show any consistency regarding a causal relationship between pesticide exposure and prostate cancer (Mink et al. 2008).

In one of several case–control studies, a relationship between insecticide application and non-Hodgkin's lymphomas in farmers was suspected, in another study there was a correlation between insecticide application and multiple myelomas (ATSDR 1996). In both cases exposure was to a mixture of substances.

In a population-based case-control study in six Canadian provinces, 357 male persons with soft tissue sarcomas and 1506 male control persons were examined. The study design consisted of two stages: a self-administered postal questionnaire (to determine exposure to different herbicides, insecticides and fungicides) and a telephone interview for those who reported pesticide exposure for 10 hours per year or more and a 15% random sample of the remainder. Among those with soft tissue sarcomas, 131 of 357 (37.9%) were non-smokers and among the controls 536

of 1506 (35.7%). There was a positive correlation between exposure to diazinon and the risk of soft tissue sarcomas (odds ratio 3.31; 95% confidence interval: 1.78–6.23). In addition, there was a positive correlation for the occurrence of cancer in first degree relatives and the risk of soft tissue sarcomas (odds ratio 1.30; 95% confidence interval: 1.00–1.68) (Pahwa et al. 2011). The study participants were exposed to different herbicides, insecticides and fungicides. On the basis of exposure to a mixture of substances it is not possible to deduce any carcinogenic effects for the individual substances.

5 Animal Experiments and in vitro Studies

The unpublished reports listed in the supplement from 1995 (documentation "Diazinon" 1998) that are not available in the original are summarized in an unpublished report (Ciba-Geigy 1994).

5.1 Acute toxicity

5.1.1 Inhalation

For rats, the LC_{50} values were more than 2330 mg diazinon/m^3 following whole-body exposure to diazinon aerosols for 4 hours (Ciba-Geigy 1994; WHO 1993) and more than 5440 mg diazinon/m^3 after nose-only exposure (Ciba-Geigy 1994). Further 4-hour LC_{50} values reported were 3500 mg/m^3 for rats, 1600 mg/m^3 for mice and 55 500 mg/m^3 for guinea pigs (ACGIH 2003).

5.1.2 Oral administration

In the supplement of 1995, attention was already drawn to the fact that the LD_{50} values are higher when diazinon is administered at a higher purity of 96% and above (because of a reduction in the formation of toxic by-products and degradation products). Oral LD_{50} values in rats were about 1000 mg diazinon/kg body weight (Ciba-Geigy 1994; WHO 1993).

Male rats given single gavage doses of diazinon of 0, 100, 200 or 400 mg/kg body weight were evaluated using a FOB ("functional observational battery"). Hypoactivity and reduced intestinal defecation were observed at doses of 100 mg/kg body weight and above. In addition, ataxia and tremor occurred at 200 mg/kg body weight and above. Reduced activity, hypothermia, chewing and lacrimation were additionally reported at the high dose of 400 mg/kg body weight. The symptoms were no longer present after 72 hours (no other details; ACGIH 2003).

In another study, in which male and female Sprague Dawley rats (at least 15 animals per group) were given single gavage doses of diazinon of 0, 2, 132, 264 or 528 mg/kg body weight, the following effects in the FOB were observed at 132 mg/kg body

weight and above: ataxia (in the females), abnormal gait (males and females), suppressed maze activity (females) and reduced AChE activity in serum and the erythrocytes (males and females). Autonomous, muscular and central nervous effects increased with the dose. In general, male and female animals were found to have the same spectrum of effects. No effects were observed at 2 mg/kg body weight (no other details; ACGIH 2003; ATSDR 1996).

In male Wistar rats, single gavage doses of diazinon of 80 mg/kg body weight reduced the AChE activity in the erythrocytes to 64% of the control value and the ChE activity in the plasma to 87% within 30 minutes (Wu et al. 1996).

5.1.3 Dermal absorption

Dermal LD_{50} values for rats and rabbits were above 2000 mg diazinon/kg body weight (Ciba-Geigy 1994; WHO 1993).

Pregnant Sprague Dawley rats were treated once semi-occlusively with diazinon in acetone in doses of 65 mg/kg body weight (5 animals per time interval; controls: acetone only). The animals were sacrificed after 1, 2, 4, 12, 24, 48, 72 and 96 hours. Inhibition of the maternal and foetal AChE activity in the brain was highest (48% and 83% of the control value, respectively) after 24 hours. The maximum inhibition of maternal and foetal butyryl cholinesterase (BuChE) activity occurred after 24 hours (76% and 79% of the control value); the activity after 96 hours was 78% and 100%, respectively, of the control value. The inhibition of the BuChE activity in the liver of the dams reached its highest level after 12 hours (76% of the control value) and regained 96% of the control value after 96 hours. After 24 hours, diazinon led to the inhibition of placental AChE and BuChE to about 80% of the control value; after 96 hours, the activity of both enzymes had regained 90% of the control value (Abu-Qare and Abou-Donia 2001).

5.1.4 Subcutaneous injection

ES1 knockout mice of the C57BL/6 strain (ES1: carboxylesterase in the plasma) were given single subcutaneous injections of diazinon of 50 mg/kg body weight in 7% Cremophor EL/10% ethanol/saline solution. Two hours after administration, the AChE activity in the plasma of wild type mice was inhibited to 12% and in $ES1^{-/-}$ mice to 6% of the control value; the BuChE activity was inhibited in both genotypes to 18% of the control value and the ES1 activity in the wild type was down to 0%. After 2 to 4 hours, all mice were found to have slight symptoms of behavioural toxicity, including mucus covering the eyes, palpebral closure, flattened posture, reduced handling reaction and decreased arousal. These effects were no longer observable after 24 hours. In all animals, after 48 hours the AChE activity had returned to the value determined prior to administration, whereas the BuChE activity was still only 60% of the control value in both genotypes after 11 days (Duysen et al. 2012).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Results from toxicity studies with repeated inhalation exposure are shown in Table 1.

Table 1 Studies of the effects of diazinon after repeated inhalation exposure

Species, strain, number per group	Exposure	Findings	References
rat, RAI, 9 ♂ and 9 ♀	**3 weeks**, 0, 151, 245, 559 mg/m³ (nominal), 6 hours/day, 5 days/week, nose-only, aerosol, purity: 97.1%, 4 ♂ and 4 ♀ of the highest concentration group and of the control group, 25-day recovery period	≥ **151 mg/m³**: exophthalmos, diarrhoea, brain AChE ↓; ≥ **245 mg/m³**: body weight gains ↓; **559 mg/m³**: salivation, piloerection, tonic-clonic convulsions within 2 hours after exposure, food consumption ↓, erythrocyte AChE ↓; no mortality, no abnormal findings in gross pathological and microscopic examinations; during the recovery period no abnormal symptoms and changes in AChE reversible	Ciba-Geigy 1994
rat, Tif:RAIf, 10 ♂ and 10 ♀	**3 weeks**, 0, 0.05; 0.46; 1.57; 11.6 mg/m³, 6 hours/day, 5 days/week, nose-only, aerosol generated from diazinon diluted in ethanol, purity: 88%, particle diameter: 0.7–1.4 µm	**0.05 mg/m³**: ♀: brain AChE ↓ (to 76% of the control value, no concentration dependence); **0.46 mg/m³**: ♀: brain AChE ↓ (to 83%), ♀: **NOAEC** for brain AChE ↓; **1.57 mg/m³**: ♀: brain AChE ↓ (to 80%) and erythrocyte AChE ↓ (to 90%); **11.6 mg/m³**: ♀: brain AChE ↓ (to 63%), ♂, ♀: erythrocyte AChE ↓ (to 64% and less); no unusual findings for body weights and food consumption, in haematological examinations, for organ weights, in gross pathological and microscopic examinations	ATSDR 1996; Ciba-Geigy 1994; WHO 1993
rat, Sprague Dawley, 15 ♂ and 15♀	**3 weeks**, 0.1, 1, 10, 100 mg/m³, 6 hours/day, 7 days/week, whole-body, purity: 87%, no other details	**0.1 mg/m³**: ♀:**NOAEC** for erythrocyte AChE ↓; **1 mg/m³**: ♀: erythrocyte AChE ↓ (to 55%), brain AChE ↓ (♂: to 87%, ♀: to 85%); no other abnormalities	US EPA 1999, 2006

AChE: acetyl cholinesterase; ChE: cholinesterase

In a 3-week inhalation study with male and female rats, the AChE activity in the brain of the females was reduced in a concentration-dependent manner to 80% and below after diazinon concentrations of 1.57 mg/m^3 (as aerosol) and above. No concentration dependence was observed at the two low concentrations. In the male rats, the AChE activity in the brain was unchanged. In both sexes, the AChE activity in the erythrocytes was reduced to 60% at 11.6 mg/m^3. Assuming a no adverse effect level (NAEL) of 80% for the AChE activity in the brain, this value is just reached at 1.57 mg/m^3, and the no observed adverse effect concentration (NOAEC) is therefore 0.46 mg/m (see Table 1; ATSDR 1996; Ciba-Geigy 1994; WHO 1993). In another 3-week inhalation study with whole-body exposure on 7 days a week, instead of 5 days a week as in the preceding study, a NOAEC of 0.1 mg/m^3 was obtained for female rats for reduced AChE activity in the erythrocytes to levels of 70% and less of the control value (lowest observed adverse effect concentration (LOAEC) 1 mg/m^3) (US EPA 1999, 2006). This therefore does not contradict the NOAEC 0.46 mg/m^3.

Cholinergic symptoms such as diarrhoea and exophthalmos occurred at diazinon concentrations of 151 mg/m^3 and above in another 3-week study with rats exposed to 0, 151, 245 or 599 mg/m^3 (Ciba-Geigy 1994).

The studies from the documentation from 1975 are not described again here, as only one concentration was used (151 or 245 mg/m^3) at which AChE inhibition in the brain was observed. A NOAEC could therefore not be given (documentation "Diazinon" 1998).

5.2.2 Oral administration

The studies of the toxicity of diazinon after repeated oral administration are shown in Table 2.

Rats

In a 7-day feeding study in 10 male and 10 female Wistar rats, a reduction in ChE activity in the plasma was found at the only dose tested of 0.2 mg/kg body weight and day; the AChE activity in the erythrocytes was unchanged (Davies and Holub 1980 a).

After the administration of diazinon with the drinking water for 21 days, there were changes in haematological and biochemical parameters in the blood of 7 male Wistar rats as well as reduced body weights and reduced liver weights at the only dose tested of 10 mg/kg body weight and day (Messarah et al. 2013).

In a 4-week feeding study in male rats, the AChE activity in the erythrocytes was reduced at 10 mg/kg body weight and day and above (no other details; Bruce et al. 1955] in documentation "Diazinon" 1998).

In a 30-day feeding study with 10 male and 10 female Wistar rats, the AChE activity in the erythrocytes was reduced in male and female animals and in the brain in female animals at the only tested dose of 2.3 to 2.5 mg/kg body weight and day. The authors concluded that female rats are more sensitive to diazinon than males (Davies and Holub 1980 a).

Table 2 Studies of the toxicity of diazinon after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat			
Wistar, 10 ♂ and 10 ♀	7 days, 0, 2 mg/kg diet (about 0, 0.2 mg/kg body weight and day assuming food consumption amounting to 10% of the body weight/day), purity: 99.2%	**0.2 mg/kg body weight**: ♀: plasma ChE ↓ (to 71% of the control value); no changes: body weight gains, food consumption; erythrocyte AChE	Davies and Holub 1980 a
Wistar, 7 ♂	21 days, 0, 10 mg/kg body weight and day, drinking water, purity: 60% (commercial formulation)	**10 mg/kg body weight**: body weights ↓, absolute and relative liver weights ↓, haemoglobin and haematocrit ↓, triglycerides ↓, cholesterol ↑, AST, ALT, LDH ↑, TBARS in liver and erythrocytes ↑, GSH in liver and erythrocytes ↓; simultaneous administration of vitamin E or curcumin reduced the effects	Messarah et al. 2013
♂, no other details	4 weeks, 0, 100, 1000 mg/kg diet (about 0, 10, 100 mg/kg body weight and day assuming food consumption amounting to 10% of the body weight/day), purity: not specified	**10 mg/kg body weight**: erythrocyte AChE ↓; **100 mg/kg body weight**: body weight gains ↓; brain AChE ↓	Bruce et al. 1955 in documentation "Diazinon" 1998
Wistar, 10 ♂ and 10 ♀	30 days, 0, 25 mg/kg diet (about 0, 2.3–2.5 mg/kg body weight and day assuming food consumption amounting to 10% of the body weight/day), purity: 99.2%	**2.3–2.5 mg/kg body weight**: ♂ and ♀: plasma ChE ↓ (♂: to 48%–56% and ♀ to 24%–33% of the control value; on day 3 and beyond) and erythrocyte AChE ↓ (♂: to 56%–79% and ♀ to 40%–81%; on day 3 and beyond); ♀: brain AChE ↓ (to 97% and 93% on days 15 and 30, respectively); no changes in body weight gains, food consumption	Davies and Holub 1980 a

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
Wistar, 8–10 ♀	35 days, 0, 0.1, 0.5, 1.0, 2.0 mg/kg diet (about 0, 0.01, 0.05, 0.1, 0.2 mg/kg body weight and day assuming food consumption amounting to 10% of the body weight/day), purity: 99.2%	≥ **0.05 mg/kg body weight**: plasma ChE ↓ (to about 58% and less of the control value); **0.2 mg/kg body weight**: **NOAEL** for erythrocyte AChE ↓; no changes in body weight gains, food consumption, erythrocyte AChE	Davies and Holub 1980 b
Sprague Dawley, 10 ♂ and 10 ♀	6 weeks, 0, 0.5, 2, 100, 1000/2000/4000 mg/kg diet (♂: 0, 0.04, 0.2, 8.4, 165; ♀: 0, 0.05, 0.2, 9.4, 198 mg/kg body weight and day), purity: 87.7%	**0.2 mg/kg body weight**: ♀: plasma ChE ↓ (to 65% of the control value, no details of higher doses); **8.4 (♂)/9.4 (♀) mg/kg body weight**: erythrocyte AChE ↓ (♂: to 79%; ♀: to 79%, no details of higher doses); ♀: brain AChE ↓ (to 76%); **165 (♂)/198 (♀) mg/kg body weight**: soft faeces, body weight gains ↓ (♂: 4%–19%; ♀: 6%–9%), food consumption ↓	US EPA 1999
Sprague Dawley, 10 ♂ and 10 ♀	6 weeks, 0, 0.2, 0.5, 2, 20, 100, 300 mg/kg diet (♂: 0, 0.02, 0.04, 0.17, 1.68, 8.60, 25.8; ♀: 0, 0.02, 0.05, 0.19, 1.82, 9.27, 29 mg/kg body weight and day), purity: technical-grade, no other details	**0.02 mg/kg body weight**: ♀: plasma ChE ↓ (to 88% of the control value); **0.04 (♂)/0.05 (♀) mg/kg body weight**: plasma ChE ↓ (♂: to 88%; ♀: to 83%); **0.17 (♂)/0.19 (♀) mg/kg body weight**: plasma ChE ↓ (♂: to 87%; ♀: to 51%; no details of higher doses); **1.68 (♂)/1.82 (♀) mg/kg body weight**: erythrocyte AChE ↓ (♂: to 65%–71%; ♀: to 65%–84%, no details of higher doses); ≥ **8.60 (♂)/9.27 (♀) mg/kg body weight**: ♂: brain AChE ↓; **25.8 (♂)/29 (♀) mg/kg body weight**: ♀: brain AChE ↓; no systemic effects (no other details)	US EPA 1999

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
Wistar, 8–10 ♀	42 days, 0, 1, 2, 3, 4 mg/kg diet (about 0, 0.1, 0.2, 0.3, 0.4 mg/kg body weight and day assuming food consumption amounting to 10% of the body weight/day), purity: 99.2%	≥ **0.1 mg/kg body weight**: plasma ChE ↓ (to about 40% and less of the control value); ≥ **0.3 mg/kg body weight**: erythrocyte AChE ↓ (to about 80% and less); **0.4 mg/kg body weight**: **NOAEL** for erythrocyte AChE ↓; **0.4 mg/kg body weight**: erythrocyte AChE ↓ (to about 78%); no changes in body weight gains, food consumption, brain AChE	Davies and Holub 1980 b
Wistar, 8 ♂	7 weeks, 0, 10 mg/kg body weight and day, by gavage, in corn oil, purity: 99%	**10 mg/kg body weight**: serum: total protein ↓, albumin ↓, ALP ↑, ALT ↑, AST ↑, total cholesterol ↑, triglycerides ↓, VLDL cholesterol ↓, hepatocytes: swelling of the mitochondria and breaking up of the mitochondrial christae; 200 mg vitamin E twice/week: diazinon toxicity ↓, but not completely	Kalender et al. 2005
Wistar, 8 ♂	1, 4, 7 weeks, 0, 10 mg/kg body weight and day, gavage, in corn oil, purity: 99%	**10 mg/kg body weight**: body weight ↓ (after 4 and 7 weeks), serum after 1–7 weeks: ChE activity ↓, erythrocytes ↓, haemoglobin ↓, haematocrit ↓, leukocytes ↑, MCV ↑, MCHC ↓, thrombocytes ↓; 200 mg vitamin E twice/week: ↓ of the diazinon toxicity, but not completely	Kalender et al. 2006

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
Wistar, 6 ♀	92 days, 0, 5, 10, 15 mg/kg diet (about 0, 0.375, 0.75, 1.13 mg/kg body weight and day assuming food consumption amounting to 7.5% of the body weight/day), purity: 99.2%	≥ **0.375 mg/kg body weight**: plasma ChE ↓ (to about 26% and less of the control value), erythrocyte AChE ↓ (to about 80% and less); ≥ **0.75 mg/kg body weight**: erythrocyte AChE ↓ (to about 68% and less); **1.13 mg/kg body weight**: brain AChE ↓ (to about 98%); no cholinergic signs, no changes in body weight gains, food consumption	Davies and Holub 1980 b
F344, 10 ♂ and 10 ♀	13 weeks, range-finding study, 0, 50, 100, 200, 400, 800, 1600, 3200 mg/kg diet (about 0, 3.75, 7.5, 15, 30, 60, 120, 240 mg/kg body weight and day assuming food consumption amounting to 7.5% of the body weight/day), purity: 98%	≥ **120 mg/kg**: body weight ↓; **240 mg/kg**: mortality ↑ (♂: 7/10, ♀: 6/10); no other examinations	NCI 1976
Crl:VAF/plus, CD (SD)Br, 15 ♂ and 15 ♀	13 weeks, 0, 0.5, 5, 250, 2500 mg/kg diet (0, 0.03, 0.3, 15, 168 mg/kg body weight and day), purity: 87.7%	**0.3 mg/kg body weight**: ♀: **NOAEL** for brain AChE ↓; **0.3 mg/kg body weight**: ♂ and ♀: serum ChE ↓ (♂: to 74% of the control value, ♀: to 22%); **15 mg/kg body weight**: erythrocyte AChE ↓ (♂: to 73%, ♀: to 59%); ♀: brain AChE ↓ (up to 59%), ♀: brain hepatocellular hypertrophy (only this dose); **168 mg/kg body weight**: hyperactivity, hypersensitivity to touch and sound, soft faeces, body weight gains ↓ (♂: 6%, ♀: 13%), ♂: aggressiveness; ♀: leukocytes and reticulocytes ↑, haemoglobin ↓, haematocrit ↓, absolute and relative liver weights ↑; erythrocyte AChE ↓ (♂: 74%), brain AChE ↓ (♂:51%)	Ciba-Geigy 1994

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
♂ and ♀, no other details	15–16 weeks, 0, 1, 5, 25, 125 mg/kg diet (about 0, 0.075, 0.375, 1.875, 9.375 mg/kg body weight and day assuming a body weight of 300 g and food consumption of 25 g/day), purity: not specified	≥ **1.875 mg/kg body weight**: erythrocyte AChE ↓ and plasma ChE ↓	Edson and Noakes 1960 in documentation "Diazinon" 1998
♂ and ♀, no other details	6 months, 0, 1, 2, 4, 8, 16 mg/kg diet (about 0, 0.075, 0.15, 0.3, 0.6, 1.2 mg/kg body weight and day assuming a body weight of 300 g and food consumption of 25 g/day), purity: not specified	≥ **0.3 mg/kg body weight**: erythrocyte AChE ↓	Melis et al. 1959 in documentation "Diazinon" 1998
♂ and ♀, no other details	72 weeks, 0, 10, 100, 1000 mg/kg diet (about 0, 0.5, 5, 50 mg/kg body weight and day assuming a body weight of 400 g and food consumption of 20 g), purity: not specified	≥ **50 mg/kg body weight**: erythrocyte and brain AChE ↓	Bruce et al. 1955 in documentation "Diazinon" 1998
F344, 50 ♂ and 50 ♀, controls: 25 ♂ and 25 ♀	103 weeks, carcinogenicity study, 0, 400, 800 mg/kg diet (about 0, 20, 40 mg/kg body weight and day assuming a body weight of 400 g and food consumption of 20 g), purity: 98%, recovery period 1–2 weeks	≥ **20 mg/kg body weight**: ♂ and ♀: accelerated breathing; ♂: hyperactivity; ♀: distended abdomen, vaginal haemorrhage and discharge; **40 mg/kg body weight**: ♀: hyperactivity, discoloured urine; body weights not changed, MTD not attained	NCI 1976

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
Sprague Dawley, 30–40 ♂ and 30–40 ♀	98–99 weeks, carcinogenicity study, 0, 0.1, 1.5, 125, 250 mg/kg diet (♂: 0, 0.004, 0.06, 5, 10 mg/kg body weight and day; ♀: 0, 0.005, 0.07, 6, 12 mg/kg body weight and day); vehicle control 26.5 mg epoxidized soybean oil/kg diet; controls: untreated animals, purity: 87.7%, interim sacrifice after 52 weeks: 8–10 animals per sex and group; interim sacrifice after 52 weeks and 4 weeks without exposure: 9–10 animals per sex in control groups and highest dose group	**0.06 (♂)/0.07 (♀) mg/kg body weight**: **NOAEL** for brain AChE ↓; **0.06 (♂)/0.07 (♀) mg/kg body weight**: serum ChE ↓ (♂: to 49.3% of the control value; ♀: to 70.4%); **5 (♂)/6 (♀) mg/kg body weight**: erythrocyte AChE ↓ (♂: to 79.3%; ♀: to 74.4%), brain AChE ↓ (♂: to 76%; ♀: to 71.4%); **10 (♂)/12 (♀) mg/kg body weight**: body weight and food consumption ↑ (palatability ↑ due to soy oil), erythrocyte AChE ↓ (♂: to 78.8%; ♀: to 75%), brain AChE ↓ (♂: to 57.6%; ♀: to 51.6%) ↓; no substance-related mortality, no gross pathological or microscopic abnormalities, MTD not attained	Ciba-Geigy 1994
mouse			
Albino, 10, sex not specified	5, 15, 20, 30 days, 0, 50 mg/kg body weight and day, drinking water, purity: not specified	**50 mg/kg body weight**: after 30 days: IL-2, IL-4, IL-10, IL-12, IFN-β ↓ in splenocytes, IL-10 ↑ in $CD4^+$-, $CD8^+$- and B cells, INF-β ↓ in B cells, INF-β mRNA synthesis ↑ in splenocytes, $CD4^+$-, $CD8^+$- and B cells; IL-2 mRNA synthesis ↑ only in $CD4^+$ cells	Alluwaimi and Hussein 2007
Swiss CD, 15 ♂	45 days, 0, 300 mg/kg diet (about 0, 30 mg/kg body weight and day assuming a body weight of 100 g and food consumption of 10 g/day), with or without supplementation of up to 40% protein or 200% corn oil in the diet, subsequent recovery period: 2 weeks, purity: not specified	**30 mg/kg body weight**: necrotic degeneration of the trabeculae (spleen and thymus), hyperplasia of cortex and medulla (thymus and lymph nodes), hyperplasia of the white and red pulp (spleen), occasional haemorrhage (all tissues), blood smears often with crenated/hypochromic red blood cells and vacuolated white blood cells with abnormal nuclei; post-exposure recovery was limited, for the authors an indication of oxidative stress	Handy et al. 2002

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
B6C3F1, 10 ♂ and 10 ♀	13 weeks, range-finding study, 0, 50, 100, 200, 400, 800, 1600, 3200 mg/kg diet (about 0, 8.75, 17.5, 35, 70, 140, 280, 560 mg/kg body weight and day assuming a body weight of 20 g and food consumption of 3.5 g/day), purity: 98%	≥ **140 mg/kg body weight**: body weights ↓; ≥ **280 mg/kg body weight**: mortality 100%; no other examinations	NCI 1976
B6C3F1, 50 ♂ and 50 ♀, controls: 25 ♂ and 25 ♀	103 weeks, carcinogenicity study, 0, 100, 200 mg/kg diet (about 0, 12.5, 25 mg/kg body weight and day assuming a body weight of 20 g and food consumption of 2.5 g/day), purity: 98%, recovery period 2–3 weeks	≥ **12.5 mg/kg body weight**: ♂ and ♀: hyperactivity; body weights not changed, MTD not attained	NCI 1976
dog			
beagle, 4 ♂ and 4 ♀	4 weeks, pilot study to 13-week study, 0, 0.5, 2, 20, 500 mg/kg diet (♂: 0, 0.02, 0.073, 0.80, 14.68 mg/kg body weight and day; ♀: 0, 0.023, 0.082, 0.75, 15.99 mg/kg body weight and day), purity: 87.7%	**0.023 (♀) mg/kg body weight**: plasma ChE ↓ (to about 71%); **0.80 (♂)/0.75 (♀) mg/kg body weight**: **NOAEL** for erythrocyte and brain AChE ↓; **14.68 (♂)/15.99 (♀) mg/kg body weight**: body weight gains and food consumption ↓, vomiting, erythrocyte AChE ↓ (to 61%–74%) and brain AChE ↓ (♂: to 56%, ♀: to 50%);	US EPA 1999
beagle, number of animals not specified	12 weeks, 0, 0.25, 0.75, 75 mg/kg diet (0, 0.01, 0.02, 2.0 mg/kg body weight and day), purity: not specified	≥ **0.02 mg/kg body weight**: plasma ChE ↓; **2.0 mg/kg body weight**: erythrocyte AChE ↓ (also after a further 6 weeks without administration); no unusual symptoms	ACGIH 2003; Williams et al. 1959 in documentation "Diazinon" 1998

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
beagle, 4 ♂ and 4 ♀	13 weeks, 0, 0.1, 0.5, 150, 300 mg/kg diet (0, 0.003, 0.02, 5.6, 10.9 mg/kg body weight and day), purity: 87.7%	**0.02 mg/kg body weight**: **NOAEL** for erythrocytes (only ♀) and brain AChE ↓ (♂ and ♀); **5.6 mg/kg body weight**: vomiting, ♀: body weight gains ↓, plasma ChE ↓ (to about 20%), erythrocyte AChE ↓ (♂: to about 75%, ♀: to about 69%), brain AChE ↓ (♂: to about 69%, ♀: to about 70%); **10.9 mg/kg body weight**: ♂: moderate atrophy of the pancreatic acini (1/4)	Ciba-Geigy 1994; US EPA 1999
beagle, 4 ♂ and 4 ♀	52 weeks, 0, 0.1, 0.5, 150, 300 mg/kg diet (♂: 0, 0.0032, 0.015, 4.7, 7.7 mg/kg body weight and day; ♀: 0, 0.0037, 0.02, 4.5, 9.1 mg/kg body weight and day), after 14 weeks in highest dose group: dose reduction to 225 mg/kg diet (6.3 mg/kg body weight and day), purity: 87.7%	**0.015 (♂)/0.02 (♀) mg/kg body weight**: **NOAEL** for erythrocyte AChE ↓; **0.02 mg/kg body weight**: ♀: **NOAEL** for brain AChE ↓; **0.015 (♂)/0.02 (♀) mg/kg body weight**: plasma ChE ↓ (♂: to 75%–95% of the reference values prior to exposure, ♀: to 60%–82%); **4.7 mg/kg body weight**: ♂: **NOAEL** for brain AChE ↓; **4.7 (♂)/4.5 (♀) mg/kg body weight**: body weight gains ↓ (to 64%) and food consumption ↓ (to 27%), erythrocyte AChE ↓ (♂: to 66%–75%, ♀: to 67%–74%) and brain AChE ↓ (♂: to about 85%, ♀: to about 74%); serum amylase ↑ (24%–59%); **7.7 (♂)/9.1 (♀) mg/kg body weight**: brain AChE ↓ (♂: to about 75%, ♀: to about 65%); haematology, urinalysis, organ weights, gross pathological and microscopic examinations without unusual findings	Ciba-Geigy 1994; US EPA 1999

Table 2 (continued)

Species, strain, number per group	Exposure	Findings	References
monkey			
Rhesus, 3 ♂ and 3 ♀	106 weeks, gavage; 0, 0.1, 1.0, 10 mg/kg body weight and day (related to diazinon), from week 3 onwards: marked AChE inhibition in high dose group, therefore from week 5 onwards: 0, 0.05, 0.5, 5 mg/kg body weight and day; purity: 48.6%, no other details	**0.05 mg/kg body weight**: ♂: **NOAEL** for erythrocyte AChE ↓; **0.05 mg/kg body weight**: body weight gains ↓ (only mild, no other details), plasma ChE ↓ (♂: to 64%–90% of the reference values prior to exposure); **0.5 mg/kg body weight**: ♀: **NOAEL** for erythrocyte AChE ↓; **0.5 mg/kg body weight**: soft faeces, plasma ChE ↓ (♂ and ♀: to 10%–82%) and erythrocyte AChE ↓ (♂: to 56%–82%, ♀: to at least 80%); **5 mg/kg body weight**: tremor, brain AChE in one animal ↓; 4 animals (one animal per dose group) died from infection, necropsy without abnormal findings	Ciba-Geigy 1994; US EPA 1999

$p < 0.05$; AChE: acetyl cholinesterase; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ChE: cholinesterase; GSH: glutathione; IL: interleukin; INF-β: interferon-β; LDH: lactate dehydrogenase; MCHC: mean corpuscular haemoglobin concentration; MCV: mean cell volume of the erythrocyte; TBARS: thiobarbituric acid reactive substances; VLDL: very low density lipoprotein

In two 6-week studies with 10 male and 10 female Sprague Dawley rats, various effects, such as a reduction in AChE activity in the erythrocytes, were described (US EPA 1999). However, the documentation is inadequate, and the studies are therefore not included in this evaluation.

Gavage administration for 1, 4 or 7 weeks of the only dose tested of 10 mg/kg body weight and day led in male Wistar rats to increased liver enzyme activities and histological changes in the liver (Kalender et al. 2005, 2006). A NOAEL cannot be derived, as only one dose was used.

In several feeding studies in female Wistar rats with different doses and exposure durations, the NOAEL for a reduction in AChE activity to 70% of the control value and below in the erythrocytes was 0.375 mg/kg body weight and day after exposure for 35 or 42 days. The authors emphasized the lower sensitivity of AChE in the brain compared with AChE in the erythrocytes after administration with the diet. Determination of the erythrocyte AChE activity after doses of 0, 0.375, 0.75 and 1.13 mg/kg body weight and day revealed an initial increase in enzyme inhibition over time at all doses, which, however, reached a plateau after about 30 days in each case (Davies and Holub 1980 b).

In a 13-week range-finding study with 10 male and 10 female F344 rats, body weights were reduced at 120 mg/kg body weight and day and above. No other examinations were carried out (NCI 1976). The study cannot therefore be included in this evaluation.

Administration with the diet for 13 weeks at 15 mg/kg body weight and day and above reduced the AChE activity in the brain of female rats to 59% of the control value; the NOAEL was 0.3 mg/kg body weight and day (Ciba-Geigy 1994).

In three studies with rats lasting several weeks, already cited in the documentation from 1975, reduced AChE activities were found after doses of 1.875 mg/kg body weight and day and above (Edson and Noakes 1960 in documentation "Diazinon" 1998), 0.3 mg/kg body weight and day (Melis et al. 1959 in documentation "Diazinon" 1998) or 50 mg/kg body weight and day (Bruce et al. 1955 in documentation "Diazinon" 1998).

In a carcinogenicity study with groups of 50 male and 50 female F344 rats, cholinergic symptoms (accelerated breathing, hyperactivity) were observed at 20 mg/kg body weight and day and above (NCI 1976). AChE activities were not determined.

In another carcinogenicity study with groups of 30 to 40 male and female Sprague Dawley rats, the AChE activity in the brain was reduced to 80% and below at 5 mg/kg body weight and day and above in the males and 6 mg/kg body weight and day in the females. The NOAEL was 0.06 (♂) and 0.07 (♀) mg/kg body weight and day (Ciba-Geigy 1994).

A study with rats cited in the 1975 documentation is not described here, as only the highest dose produced cholinesterase inhibition in the plasma (Hazleton Laboratories 1956 in documentation "Diazinon" 1998), nor is a study in the 1995 supplement described, in which only one dose was administered twice a week (Anthony et al. 1986 in documentation "Diazinon" 1998).

Mice

In a 30-day drinking water study in albino mice, effects on various immunological parameters were determined at the only dose tested of 50 mg/kg body weight and day (Alluwaimi and Hussein 2007). It is not possible to give a NOAEL. From a 45-day feeding study in mice with only one dose of about 30 mg/kg body weight and day, in which effects on the spleen and thymus were found (Handy et al. 2002), a NOAEL likewise cannot be derived. In addition, effects on these organs were not reported in any other study.

In a 13-week range-finding study in B6C3F1 mice, there was a reduction in body weights at 140 mg/kg body weight and day and above (NCI 1976). As no further investigations were carried out, the study is not included in this evaluation.

In a 103-week carcinogenicity study in B6C3F1 mice, hyperactivity was observed at 12.5 mg/kg body weight and day and above (see Section 5.7.2; NCI 1976). As AChE activity was not determined, the study is not included in this evaluation.

The carcinogenicity study carried out by Ciba-Geigy (1994) in CD-1 mice is not included in this evaluation because of the lack of data and contradictions (see Section 5.7.2).

Dogs

In a 4-week pilot study with 4 male and 4 female beagle dogs per group, body weight gains, food consumption and the AChE activity in erythrocytes and the brain were decreased at dose levels of 14.68 (♂) and 15.99 (♀) mg/kg body weight and day and above. The NOAEL was 0.80 (♂) and 0.75 (♀) mg/kg body weight and day (US EPA 1999).

In a 12-week study in beagle dogs, the AChE activity in the erythrocytes was decreased at 2.0 mg/kg body weight and day (ACGIH 2003; Williams et al. 1959 in documentation "Diazinon" 1998). However, no details of the number of animals or the percentage change in AChE activity were given.

In addition, in a 13-week study with groups of 4 male and 4 female beagle dogs, the AChE activity in the erythrocytes was decreased at doses of 5.6 mg/kg body weight and day and above. The NOAEL was 0.02 mg/kg body weight and day (Ciba-Geigy 1994; US EPA 1999). Because of the large difference between the NOAEL and the lowest observed adverse effect level (LOAEL), the true NAEL may be higher.

In a 52-week study in male and female beagle dogs, the following effects were found at 4.7 (♂) and 4.5 (♀) mg/kg body weight and day and above: reduced body weight gains and food consumption, and reduced AChE activity in the erythrocytes (between 66% and 75% of the control value in the ♂, between 67% and 74% in the ♀) and in the brain (to about 74% in the ♀ only). The NOAEL was 0.015 (♂) and 0.02 (♀) mg/kg body weight (Ciba-Geigy 1994; US EPA 1999). Because of the large difference between the NOAEL and the LOAEL, the true NAEL may be higher.

Two studies in dogs mentioned in the documentation from 1975 are not cited again here as either cholinesterase activities were not determined (Earl et al. 1971 in documentation "Diazinon" 1998) or the highest dose produced merely cholinesterase inhibition in the plasma (Hazleton 1956 in documentation "Diazinon" 1998).

Monkeys

In a 106-week gavage study, groups of 3 male and 3 female Rhesus monkeys were initially given 0, 0.1, 1.0 or 10 mg/kg body weight and day and then 0, 0.05, 0.5 or 5 mg/kg body weight and day from week 5 onwards as a result of marked AChE inhibition in the high dose group. At doses of 0.5 mg/kg body weight and day and above, AChE activity in the erythrocytes was reduced to between 56% and 82% of the baseline value before exposure in the males and to at least 80% in the females. The NOAEL for reduced AChE activity in the erythrocytes was 0.05 mg/kg body weight and day in the males and 0.5 mg/kg body weight and day in the females (Ciba-Geigy 1994; US EPA 1999).

Conclusions

In rats, administration with the diet of diazinon doses of 15 mg/kg body weight and day and above for 13 weeks decreased the AChE activity in the brain (to 59% of the control value) in female rats; the NOAEL was 0.3 mg/kg body weight and day (Ciba-Geigy 1994). In the 99-week carcinogenicity study, NOAELs of 0.06 (♂) and 0.07 (♀) mg/kg body weight and day were obtained for this effect. The LOAELs were considerably higher at 5 mg/kg body weight and day (♂) and 6 mg/kg body weight and day (♂) (Ciba-Geigy 1994).

For dogs, LOAELs for reduced AChE activity in the brain of 4.7 (♂) and 4.5 (♀) mg/kg body weight and day were derived from a 52-week feeding study; the NOAELs were 0.015 (♂) and 0.02 (♀) mg/kg body weight (Ciba-Geigy 1994; US EPA 1999).

Because of the large difference between the NOAELs and LOAELs, the true NAELs for rats and dogs could well be higher.

In male monkeys, a NOAEL of 0.05 mg/kg body weight and day was obtained from a 106-week gavage study for the reduction of AChE activity in the erythrocytes to 70% and less; the LOAEL was 0.5 mg/kg body weight and day (Ciba-Geigy 1994; US EPA 1999).

5.2.3 Dermal application

Groups of 5 male and 5 female albino rabbits were exposed semi-occlusively to diazinon in doses of 0, 1, 5 or 100 mg/kg body weight and day (purity: 97.1%) for 3 weeks. After doses of 5 mg/kg body weight and day and above, the cholinesterase activity in serum, erythrocytes and the brain was decreased. The NOAEL was 1 mg/kg body weight and day (Ciba-Geigy 1994).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

The skin of 3 male and 3 female New Zealand White rabbits was exposed semi-occlusively for 4 hours to 0.5 ml undiluted diazinon. As the mean irritation scores

calculated from those obtained after 24, 48 and 72 hours were below the critical score of 2 laid down by the European Union for irritating effects according to Directive 93/21 (EU 2000) diazinon was not considered to be irritating to the skin (Ciba-Geigy 1994).

The skin of 6 New Zealand White rabbits was shaved, but not abraded. Then 0.5 ml diazinon (purity: 87.7%) was applied occlusively for 4 hours. A maximum primary irritation score of 2.8 was calculated (no details of maximum score). The substance was regarded as slightly irritating (US EPA 1999). According to OECD Test Guideline 405, the test substance should be applied semi-occlusively.

5.3.2 Eyes

A volume of 0.1 ml undiluted diazinon was introduced into the conjunctival sac of 3 male and 6 female New Zealand White rabbits. The eyes of 3 females were rinsed with deionized water 30 seconds later. In these animals, the mean irritation score was slightly below that of the animals whose eyes had not been rinsed. As the mean irritation scores calculated from those obtained after 24, 48 and 72 hours were below the critical score (no other details) laid down by the European Union for irritating effects according to Directive 93/21, diazinon was not considered to be irritating to the eyes (Ciba-Geigy 1994).

A volume of 0.1 ml diazinon (purity: 87.7%) was instilled into the eyes of 3 male and 6 female New Zealand White rabbits. The eyes of 3 animals were rinsed with deionized water 30 seconds later. Readings were carried out 1, 24, 48 and 72 hours after instillation. The cornea was not affected. Slight transient conjunctival irritation was found, and the average irritation scores after 1 hour were 9 for the unrinsed and 5.3 for the rinsed eyes (evaluation procedure and the maximum value not specified). The test substance was regarded as slightly irritating to the eyes (US EPA 1999).

5.4 Allergenic effects

A maximization test according to Magnusson and Kligman using Hartley guinea pigs was already described in the supplement from 1995 (documentation "Diazinon" 1998). It was regarded as not evaluable as the purity of the substance was not specified. Induction was carried out via intradermal injection of a 5% aqueous preparation of technical-grade diazinon and epicutaneous treatment with a 25% preparation. After epicutaneous challenge treatment with 0.5% and 0.05% diazinon preparations, all 10 and 3 of 10 animals had produced reactions after 24 and 48 hours (no further details; Matsushita et al. 1985).

In a maximization test with 10 female and 10 male Dunkin-Hartley guinea pigs, technical-grade diazinon (purity 96.2%) was used. Intradermal induction was carried out with 10% diazinon in water and the topical induction treatment with 0.6 ml undiluted diazinon after preceding treatment with 10% sodium dodecyl sulfate. Af-

ter challenge treatment with 30 µl diazinon, the following reactions occurred: 4 of 9 female animals were found to have slight confluent erythema (grade 1) after 24 hours. After 48 hours, slight erythema was found in 2 of 9 animals, confluent erythema (grade 1) in 4 of 9 animals and moderate, confluent erythema (grade 2) in 1 of 9 animals. Slight erythema occurred in 1 of 9 male guinea pigs after 24 hours and confluent erythema (grade 1) in 2 of 9 animals. Three of 9 animals were found to have slight erythema after 48 hours (APVMA 2011). The study did not state whether a control group was included, and whether any reactions occurred in the animals of the control group. Therefore, this study with its borderline positive results cannot be evaluated conclusively.

In a modified Buehler test, 10 male guinea pigs underwent occlusive induction treatment with 0.5 ml of a 10% preparation of diazinon (purity 87.7%) in ethanol on days 1, 3 and 6. A 10% solution was used, as the undiluted substance was lethal for one animal in preliminary tests. After two weeks, challenge treatment was carried out with the same concentration. No erythema or oedema were found (US EPA 1999).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

Results from fertility studies and studies with findings in reproductive organs or sperms after administration of diazinon are shown in Table 3.

In a fertility study with male rats, no details of further ingredients of the commercial insecticide were given (documentation "Diazinon" 1998; Abd El-Aziz et al. 1994). It is therefore not possible to decide whether the effects observed are attributable to diazinon.

In male Wistar rats, 9 weeks of treatment with 3 mg diazinon/kg body weight and day by gavage led to effects on the sperms and to histological changes in the ductus epididymis (Okamura et al. 2009). As only one dose was used, a NOAEL cannot be given.

In a 2-generation study with Sprague Dawley rats, effects on the F1 generation (decreased food consumption, reduced body weight gains) and survival of the F1 offspring were observed at doses of 7.5 mg/kg body weight and day and above. The NOAEL was 0.75 mg/kg body weight and day (Syngenta 1989).

In a 4-generation study with Sprague Dawley rats, only one dose was used (Green 1970), and the documentation was inadequate. For this reason, the study is not included in this evaluation.

In male CD-1 (ICR) mice, gavage doses of diazinon of 4.1 mg/kg body weight and day and above for 4 weeks resulted in effects on the sperms, histological changes in the seminiferous tubules and reduced concentrations of gonadotropic hormones. Mating with untreated females led to a reduction in the mean foetal body weight per litter at this dose and above (ElMazoudy and Attia 2012). A NOAEL of 2.0 mg/kg body weight and day can be derived for effects on the male reproductive organs and sperms.

Table 3 Fertility studies, studies with findings in reproductive organs and generation studies after administration of diazinon

Species, strain, number per group	Exposure	Findings	References
rat, Albino, 5–8 ♂	**65 days (complete spermatogenesis cycle)**, gavage, 0, 1.5, 3 mg/kg body weight and day, commercial insecticide (oily solution) with 50% diazinon, recovery period: 21 days	≥ **1.5 mg/kg body weight**: absolute weights of testes, seminal vesicles and prostate gland ↓ (also 21 days after termination of exposure), sperm concentration ↓ (1.5 mg/kg body weight: 32%, 3 mg/kg body weight: 50%), sperm vitality ↓, proportion of motile sperms ↓ (82%, 93%), sperm abnormalities ↑ (200%, 450%) (also hardly changed 21 days after end of exposure), testosterone in the plasma ↓ (65%, 50% of the initial value) (practically unchanged during recovery period), mating with untreated ♀: pregnancy rate ↓: mating at end of exposure: pregnancy: controls: 6/8; 1.5 mg/kg body weight: 2/8; 3 mg/kg body weight: 1/8; mating 60 days after end of exposure: 1.5 mg/kg body weight: 3/8, 3 mg/kg body weight: 1/8; no details of further ingredients	Abd El-Aziz et al. 1994
rat, Wistar, 10 ♂	**9 weeks**, gavage, 0, 3 mg/kg body weight and day, in corn oil, 6 days/week, purity: 99.4%	**3 mg/kg body weight**: sperm motility ↓, percentage of broken sperms ↓, ductus epididymis: cytoplasmic vacuolation and nuclear shrinkage in the epithelial cells ↑, AChE activity in erythrocytes, plasma and testes ↓; adenine nucleotide content of sperms unchanged, no histological abnormalities in the testes	Okamura et al. 2009

Table 3 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, SD, 30 ♀	**2-generation study**, 10 weeks prior to mating then until and including the F2 generation, 0, 10, 100, 500 mg/kg feed (about 0; 0.75, 7.5, 37.5 mg/kg body weight and day assuming a body weight of 300 g and the consumption of 25 g feed/day), purity: 94.9%	**0.75 mg/kg body weight**: **NOAEL** for parenteral toxicity and effects on the offspring; ≥ **7.5 mg/kg body weight**: F1: body weight gains ↓, ♂: food consumption ↓; offspring of F1: survival ↓; **37.5 mg/kg body weight**: F0 and F1: gestation time ↑; litter size ↓, body weight gains ↓, survival of the offspring ↓; F0: dams: mortality and clinical symptoms in a few animals (no other details) F1: number of pregnancies and live births ↓; no effects found after gross pathological or microscopic examination of organs (no other details), mating behaviour, sex ratio	Syngenta 1989
rat, SD, 25 ♀	**4-generation study** , 60 days prior to mating, 0, 1 mg/kg feed (about 0.075 mg/kg body weight and day assuming a body weight of 300 g and the consumption of 25 g feed/day), purity: not specified	**0.075 mg/kg body weight**: offspring of the 3rd and 4th generations: body size ↓, delayed development; no other details; inadvertent administration down to the 4th generation	Green 1970

Table 3 (continued)

Species, strain, number per group	Exposure	Findings	References
mouse, CD-1 (ICR), 25 ♂	**4 weeks**, by gavage, 0, 2, 4.1, 8.2 mg/kg body weight and day, in distilled water, 7 days/week, purity: 98%	**2 mg/kg body weight**: NOAEL for effects on ♂ reproductive organs and sperms; ≥ **4.1 mg/kg body weight**: body weights ↓, absolute and relative weights of epididymis and prostate gland ↓, absolute weights of testes and seminal vesicles ↓, number of the spermatozoa in epididymis and of spermatids in testes ↓, sperm motility ↓, morphological sperm changes ↑, histological changes in seminiferous tubules (loss, disorder, detachment of germ cells, cytoplasmic vacuolation of germ cells, broken sperm cells), concentration of LH (4.1 mg/kg body weight: 45%; 8.2 mg/kg body weight: 49%) and FSH (56%; 65%) ↓, cholinergic symptoms (lethargy, muscular tremor, irregular movements, abdominal trembling), AChE activity in the plasma ↓ (63% of the control value; 74%); after mating with untreated ♀: mean foetal body weight/litter ↓; **8.2 mg/kg body weight**: after mating with untreated females: number of live foetuses/litter ↓, number of early and late resorptions and postimplantation losses ↑	ElMazoudy and Attia 2012

AChE: acetyl cholinesterase; FSH: follicle-stimulating hormone; LH: luteinizing hormone; SD: Sprague Dawley

Conclusions

In a 2-generation feeding study with Sprague Dawley rats given concentrations corresponding to doses of about 0, 0.75, 7.5 or 37.5 mg/kg body weight and day, effects on the F1 generation were observed at 7.5 mg/kg body weight and day and above (reduced food consumption and body weight gains) and on the survival of the offspring. The NOAEL was 0.75 mg/kg body weight and day (Syngenta 1989).

For effects on the male reproductive organs and the sperms of mice, an oral NOAEL of 2 mg/kg body weight and day can be derived. At doses of 4.1 mg/kg body weight and above, effects on different developmental stages of the sperms, histological changes in the seminiferous tubules and reduced concentrations of gonadotropic hormones were found (ElMazoudy and Attia 2012).

5.5.2 Developmental toxicity

Prenatal administration

An overview of the studies of the toxic effects on prenatal development can be found in Table 4.

Rats

In CRN rats, effects on the dams (reduced body weight gains) and foetuses (reduced esterase activity in the brain) were observed at the low dose of 40 mg/kg body weight and day and above (ACGIH 2003). A NOAEL for maternal and developmental toxicity cannot, therefore, be derived.

In a developmental toxicity study with Sprague Dawley rats, maternal toxicity was found at doses of 3.8 mg/kg body weight and day and above (reduced food intake and body weight gains, cholinergic symptoms, reduced AChE activity in the brain). At this dose, the number of visceral anomalies in the foetuses was increased. The authors discussed whether the possible inhibition of cholinesterase in the foetal brain, even if it occurs only periodically, could be detrimental to the coordination of foetal development. The NOAEL for maternal and developmental toxicity was 1.9 mg/kg body weight and day (ElMazoudy et al. 2011). The markedly lower NOAEL could be the result of a different manufacturing process and other by-products and degradation products of the diazinon made in Egypt compared with the diazinon made in the USA (Syngenta 1985). Even at doses of as little as 3.8 mg/kg body weight and day, the body weights of the dams were reduced by 10%, which is normally the value used as a criterion for establishing the highest dose. In view of the pronounced maternal toxicity, the developmental toxicity is considered to be secondary. With regard to AChE determination, there are no data

Table 4 Studies of the toxic effects on prenatal development after the administration of diazinon

Species, strain, number per group	Exposure	Findings	Remarks	References
rat, CFN, no other details	**GD 4–19**, 0, 40, 50, 60, 75 mg/kg body weight and day, gavage, in arachis oil, purity: 92.9%, examination: GD 20	**no NOAEL** for maternal and developmental toxicity; ≥ **40 mg/kg body weight**: dams: body weight gains ↓; foetuses: esterases in the brain ↓; **75 mg/kg body weight**: dams: death after 4–5 doses; no effects on litter size, foetal body weights, foetal brain weights, number of resorptions and corpora lutea		ACGIH 2003
rat, SD, 25 ♀/group	**GD 6–15**, 0, 1.9, 3.8, 7.6 mg/kg body weight and day, gavage, in saline solution, purity: 98%, produced in Egypt, examination: GD 20	**1.9 mg/kg body weight**: **NOAEL** for maternal toxicity and developmental toxicity; ≥ **3.8 mg/kg body weight**: dams: cholinergic symptoms (diarrhoea, tremor, weakness, salivation, decreased activity), body weight gains ↓, food consumption ↓, absolute and relative brain weights ↓, brain AChE ↓ (to 77% of the control value, at 7.6 mg/kg body weight: to 72%), foetuses: number of visceral anomalies ↑ (4/34 litters: 17.4%, 4/311 foetuses: 3.6%; 1 heart oedema, 2 visceral enlargements, 1 pulmonary oedema; controls: 0/24 litters, 0/331 foetuses); **7.6 mg/kg body weight**: dams: absolute and relative weights of liver and pregnant uterus ↓, foetuses: postimplantation losses ↑ (14.2 ± 1.98%, controls: 4.6 ± 0.214%), number of live foetuses/litter ↓ (13.71 ± 1.47; controls: 15.36 ± 1.08); early resorptions (10.3 ± 1.03; controls: 3.4 ± 0.68), late resorptions (3.9 ± 0.113; controls: 1.2 ± 0.016); body weights ↓ (♂: 8%, ♀: 7%), crown-rump length ↓ (♂: 18%, ♀: 19%), number of external and skeletal malformations and visceral abnormalities ↑ (external: 20/22 litters: 91%, 22/273 foetuses: 24.2%, 7 microsomias, 6 visceral hernias, 1 ophthalmological anomalies, 3 cleft lips, 2 limb anomalies, 2 tail defects, 1 dermal oedema; skeletal: 19/22 litters: 86.4%, 22/273 foetuses: 19.5%, 7 short or absent ribs, 4 reduced skull bones, 8 limb deformities, 3 reductions of vertebrae; visceral: 12/22 litters: 54.5%, 14/273 foetuses: 20.3%, 3 heart oedema, 4 distended ureter, 4 visceral enlargements, 3 pulmonary oedema); mortality, sex ratio not changed, foetal brain AChE unchanged	method of preparation, by- and degradation products not known, 3.86 mg/kg body weight and above: developmental toxicity as a result of pronounced maternal toxicity (body weight of the dams already reduced by 10% at 3.8 mg/kg body weight; a 10% reduction in body weights is normally taken as the criterion for establishing the highest dose), no details of number of investigated dams and foetuses or time of AChE determination	ElMazoudy et al. 2011

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	Remarks	References
rat, SD, 27 ♀	**GD 6–15**, 0, 10, 20 100 mg/kg body weight and day, gavage, in 0.2% carboxymethylcellulose and 0.5% Tween 80, purity: 97.4%, examination: GD 20	**20 mg/kg body weight**: **NOAEL** for maternal and developmental toxicity; **100 mg/kg body weight**: dams: food consumption and body weight gains ↓, number of resorptions and live foetuses ↓, pre- and postimplantations slightly ↑; foetuses: 9/181 14th ribs, 1 fibre-like tail, 1 hernia of umbilical cord, 1 sublingual foreign soft tissue	according to authors malformations secondary to maternal toxicity, as morphologically not associated	Syngenta 1985
rat, SD, 28–30 ♀	**GD 6–15**, 0, 15, 50, 100 mg/kg body weight and day, gavage, in carboxymethylcellulose, purity: 95%, examination: GD 20	**50 mg/kg body weight**: **NOAEL** for maternal toxicity; **100 mg/kg body weight**: **NOAEL** for developmental toxicity; **100 mg/kg body weight**: dams: food consumption and body weight gains ↓; no effects on: number of implantations, resorptions, live and dead foetuses, no teratogenic effects	no investigation of corpora lutea	Syngenta 1974

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	Remarks	References
rat, Crl:CD (SD) IGS BR, 27 ♀	**GD 6 to PND 21**, OECD Test Guideline 426, 0, 0.3, 30, 300 mg/kg feed (gestation: 0, 0.26, 2.36, 24.2 mg/kg body weight and day; GD 6–PND 21: 0, 0.039, 4.1, 39 mg/kg body weight and day), purity: 92.9%, examination: PND 0–60	**0.26 mg/kg body weight**: **NOAEL** for maternal toxicity and systemic toxicity of the offspring; **2.36 mg/kg body weight**: **NOAEL** for developmental neurotoxicity ≥ **2.36 mg/kg body weight**: dams: PND 21: erythrocyte AChE ↓ (to 12.7% and less) and brain AChE ↓ (to 75.2% and less); offspring: erythrocyte AChE ↓ (to 58.7% and less); **24.2 mg/kg body weight**: offspring body weights and body weight gains on PND 4 onwards ↓, ♂: delayed preputial separation (1.9 days later than controls), ♀: delayed vaginal opening (1.3 days later than controls), ♂: motor activity on PND 17 ↑, ♂: learning and memory in the Biel water maze test on PND 24 and 60 ↓; brain AChE ↓ (to 71.4% and less, PND 21); no effects on duration of gestation, number of live births, gestation index, offspring survival, FOB, acoustic startle response, brain weights, brain morphology and neuropathology	criticism by US EPA 2005: high deviation of motor activity and therefore difficult to interpret	Neal et al. 2004; US EPA 2005

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	Remarks	References
rat, SD, 10 ♀	**GD 6 to PND 21**, range-finding study, 0, 0.1, 0.5, 50, 300 mg/kg feed (0, 0.0125, 0.063, 6.56, 38.06 mg/kg body weight and day), purity: 94.2%, examination: FOB dams: GD 7, 14, 20, PND 1, 4, 7, 14, 20; FOB offspring: PND 4, 7, 11, 14, 17, 20	≥ **0.063 mg/kg body weight**: dams: erythrocyte AChE ↓ (to 61% and less); ≥ **6.56 mg/kg body weight**: dams: brain AChE ↓ (to 57% and less) **38.06 mg/kg body weight**: dams: during lactation body weight gains ↓, tremor in 2 animals, no pupil reflex in 2 animals; offspring: erythrocyte AChE (to 56% and less) and brain AChE ↓ (to 63% and less); no effects on survival, body weights and body weight gains during gestation, food consumption	range-finding study for the above study	US EPA 2005
rat, Wistar, 1–2 ♀	**various days during gestation**, total dose: 12.5–27.5 mg/kg body weight, gavage, vehicle: corn oil, no other details		cannot be evaluated due to small number of animals and inadequate documentation of findings	Dobbins 1967

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	Remarks	References
rat, Wistar, 4 ♂ and 4 ♀ (histochemical examinations), at least 12 ♂ and 12 ♀ (behavioural tests)	**GD 15–19**, 0, 1 mg/kg body weight and day, subcutaneous, in DMSO, purity: 99%; examination: PND 60	**1 mg/kg body weight**: ♀: passive avoidance behaviour disturbed; motor activity unchanged, number of NADPH-d$^+$/ nNOS-IR neurons in the subnuclei of the basolateral complex of the amygdala unchanged	only one dose, not possible to give a NOAEL	Vatanparast et al. 2013
rat, Sherman, 4–6 ♀	**GD 11**, 0, 100, 150, 200 mg/kg body weight and day, intraperitoneal, in arachis oil, purity: not specified, examination: GD 20	**100 mg/kg body weight**: foetuses: 6/50 distended renal pelvis; **150 mg/kg body weight**: dams: body weight gains ↓, foetuses: weight at birth ↓; **200 mg/kg body weight**: dams: 2/5 died, foetuses: 2 skeletal malformations and one hydrocephalus out of 6 foetuses	intraperitoneal administration, direct effects on embryo can therefore not be excluded	Kimbrough and Gaines 1968

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	Remarks	References
rabbit, New Zealand White, 8–9 ♀, controls: 21 ♀	**GD 5–15**, 0, 7, 30 mg/kg body weight and day, gavage, in gelatin capsules, purity: technical grade, examination: GD 28	**7 mg/kg body weight**: **NOAEL** for maternal toxicity; **30 mg/kg body weight**: **NOAEL** for developmental toxicity; **30 mg/kg body weight**: dams: 6/8 died, severe cholinergic symptoms; no effects on: average number of foetuses, foetal mortality, foetal body weights, no embryotoxic or teratogenic effects		Robens 1969
rabbit, New Zealand White, 18–22 ♀	**GD 6–18**, 0, 7, 25, 100 mg/kg body weight and day, gavage, in carboxymethylcellulose, purity: 89.2%; examination: GD 30	**25 mg/kg body weight**: **NOAEL** for maternal toxicity; **100 mg/kg body weight**: **NOAEL** for developmental toxicity; **100 mg/kg body weight**: dams: 9/22 died, tremor, convulsions, hypoactivity, body weight gains ↓, intestinal haemorrhages and erosions; no external, visceral or skeletal malformations, no effects on: number of implantations, resorptions, live or dead foetuses, body weights		Ciba-Geigy 1994; US EPA 1999

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	Remarks	References
mouse, F2 hybrids, 19–21 ♀, control group: 22 ♀	**entire pregnancy**, 0, 0.18, 9.0 mg/kg body weight and day, in peanut butter, with the diet, purity: technical grade, natural birth, examination: PND101, PND400 and PND800	**no NOAEL** for maternal and developmental toxicity; ≥ **0.18 mg/kg body weight**: offspring: ♀: serum: IgG1 ↓ (on PND 101, not PND 400 and 800); **9 mg/kg body weight**: offspring: 12/150 died as a result of respiratory infections; body weights and size gains after 7, 14 and 28 days ↓; no teratogenic effects		Barnett et al. 1980; Spyker and Avery 1977
hamster, Syrian golden hamster, 5 or 8 ♀, controls: 10 ♀	**GD 6–8, GD 7 or 8**, 0, 0.125, 0.25 mg/kg body weight and day, gavage, in corn oil, purity: technical grade, examination: GD 14 or 15	dams: no mortality, no effects on: average number of foetuses, foetal mortality, foetal body weights, no embryotoxic or teratogenic effects	it is not possible to give a NOAEL for developmental toxicity as not dosed during entire organogenesis	Robens 1969

Table 4 (continued)

Species, strain, number per group	Exposure	Findings	Remarks	References
dog, beagle, 8–10 ♀, controls: 6 ♀	**entire pregnancy**, 0, 1, 2, 5 mg/kg body weight and day, presumably gavage, purity: not specified; examination: partly delivery by caesarean section and partly natural birth, no other details	≥ **1 mg/kg body weight**: dams: extreme nervousness; **5 mg/kg body weight**: offspring: 3/34 with abnormalities in 3 different litters: 1 protruding fontanelle and absence of teeth, 1 oedematous swelling of the duodenal wall and 1 formation of fibrous strands in the abdominal cavity; percentage of stillbirths slightly ↑, but not dose-dependent (0, 1, 2, 5 mg/kg body weight: 10.5, 15.8, 9.1, 14.7%): authors explained this by substance-related nervousness at birth	no details of purity of test substance and maternal toxicity; not clear, whether substance was administered with the diet or by gavage, inadequate documentation	Earl et al. 1973
miniature swine, Hormel-Hanford, 7 ♀, controls: 27 ♀	**entire pregnancy**, 0, 5, 10 mg/kg body weight and day, presumably gavage, purity: not specified; examination: no details	**5 mg/kg body weight**: offspring: 2 cranial and extremity malformations in different litters: 1 dome-shaped skull, 1 brachygnathia with fusion of the temporal condyles and absence of teeth in the lower jaw, 1 additional metacarpal bones (which is not found spontaneously); **10 mg/kg body weight**: dams: 5/7 died, offspring: no malformations; control animals: no malformations	no details of purity of the test substance and maternal toxicity; unclear, whether substance was administered with the diet or by gavage, inadequate documentation	Earl et al. 1973

AChE: acetyl cholinesterase; DMSO: dimethyl sulfoxide; FOB: functional observational battery; NADPH-d$^+$: NADPH-diaphorase; nNOS-IR: neuronal NO-synthase immunoreactive; PND: postnatal day; SD: Sprague Dawley

available for the number of examined dams and foetuses and for the time point of the examination.

In pregnant Sprague Dawley rats, gavage administration of 100 mg/kg body weight produced the following effects: maternal toxicity (reduced food intake and body weight gains), a reduced number of resorptions and live foetuses, an increased incidence of 14th ribs in the foetuses (9 of 181), and a total of 3 malformations. The authors regarded the malformations as secondary to the maternal toxicity, as there was no morphological association between them. The NOAEL for maternal and developmental toxicity was 20 mg/kg body weight (Syngenta 1985).

In another study of the toxic effects on prenatal development with Sprague Dawley rats, reduced food consumption and body weight gains were observed in the dams at 100 mg/kg body weight. No effects on the foetuses were observed. The NOAEL for maternal toxicity was therefore 50 mg/kg body weight, and the NOAEL for developmental toxicity was 100 mg/kg body weight (Syngenta 1974).

A 2-generation feeding study in Sprague Dawley rats revealed reduced body weights and numbers of live foetuses at 7.5 mg/kg and day and above (no other details; compare Section 5.5.1; Syngenta 1989). The NOAEL for foetotoxicity was 3.75 mg/kg body weight and day, at which dose maternal toxicity occurred.

In a study of developmental neurotoxicity with Sprague Dawley rats carried out according to OECD Test Guideline 426, effects on the dams (decreased AChE activity in the brain and erythrocytes) and the offspring (decreased AChE activity in the erythrocytes) were found at doses of 2.36 mg/kg body weight and day and above. At the high dose of 24.2 mg/kg body weight and day the following effects were observed in the offspring: reduced body weights and delayed body weight gains, delayed sexual maturity in male and female animals, impaired motor activity, learning and memory. The NOAEL for developmental neurotoxicity was 2.36 mg/kg body weight and day and for maternal toxicity 0.26 mg/kg body weight and day (US EPA 2005). The study was also published in the form of a summary (Neal et al. 2004). It was preceded by a dose-finding study, in which the erythrocyte AChE activity of the dams was reduced at 6.56 mg/kg body weight and day and above (US EPA 2005).

Because of the small number of animals and inadequate documentation of the findings, a study in Wistar rats (Dobbins 1967) cannot be evaluated. As only one dose was used in a study with Wistar rats (Vatanparast et al. 2013), a NOAEL cannot be derived. Because of the intraperitoneal administration, the study with Sherman rats (Kimbrough and Gaines 1968) is not included in this evaluation, as a direct effect on the embryos or foetuses cannot be excluded.

Rabbits

From days 5 to 15 of gestation, New Zealand White rabbits were given gavage doses of diazinon of 0, 7 or 30 mg/kg body weight and day. At the highest dose of 30 mg/kg body weight, 6 of 8 dams died. Up to this dose, no effects on the foetuses occurred. The NOAEL for maternal toxicity was 7 mg/kg body weight, and the NOAEL for developmental toxicity was 30 mg/kg body weight (Robens 1969).

In another study with the same strain of rabbits, the animals were given gavage doses of diazinon of 0, 7, 25 or 100 mg/kg body weight and day from days 6 to 18 of gestation. In the dams, the high dose of 100 mg/kg body weight and day led to cholinergic symptoms and deaths (9 of 22 animals). Up to this dose, no external, visceral or skeletal malformations or foetotoxic effects were observed. The NOAEL for maternal toxicity was 25 mg/kg body weight and the NOAEL for developmental toxicity 100 mg/kg body weight (Ciba-Geigy 1994; US EPA 1999).

Mice

From a study with hybrid mice with a limited study scope (Barnett et al. 1980; Spyker and Avery 1977), a NOAEL for maternal toxicity and developmental toxicity cannot be derived.

Other species

In a study with Syrian golden hamsters, the animals were treated on days 6 and 7 or on days 7 and 8 of gestation (Robens 1969). As the animals were not treated during the entire phase of organogenesis, the study is not included in this evaluation.

A study in dogs and miniature swine (Earl et al. 1973) is not included in this evaluation, as there are no data available for the purity of the test substance, maternal toxicity or body weights, and the documentation is inadequate.

Postnatal administration

The results of studies of the toxic effects of diazinon on postnatal development are shown in Table 5.

In several publications, a research group reported the effects of diazinon on brain development in newborn Sprague Dawley rats that had received subcutaneous treatment with diazinon from postnatal days 1 to 4. Up to the high dose of 2 mg/kg body weight and day, AChE was not inhibited below 70% of the initial values in any of the investigated brain regions (Slotkin et al. 2006 a, b, 2008 b). Inhibition to below 70% of the initial values is necessary for symptoms of cholinergic hyperstimulation to occur (Slotkin et al. 2006 b). At 0.5 mg/kg body weight and day and above, disturbed neuritic outgrowth (Slotkin et al. 2006 b), neuron loss and reactive gliosis (Slotkin et al. 2008 b), effects on the serotonin system (Slotkin et al. 2006 a, 2008 a), and upregulation of the adenylyl cyclase activity in the liver (Adigun et al. 2010) were observed. At doses of 1 mg/kg body weight and day and above, the lowest tested dose in this study, there were changes in neurotrophic factors in the brain (Slotkin et al. 2007). The authors assumed that during development diazinon acts on neurotransmitter systems that are not related to its action as a cholinesterase inhibitor (Slotkin et al. 2008 a). In view of the impairment of the serotonin system and the important role played by this system in the regulation of emotions, the same research group investigated the effects on behaviour of neonatal exposure to diazinon. Male and female Sprague Dawley rats that had been

Table 5 Studies of the toxic effects on postnatal development after administration of diazinon

Species, strain, number per group	Exposure	Findings	References
rat, SD, 10 ♂ and 10 ♀, on PND 0 all pups were randomized and redistributed to the dams in order to obtain a litter size of 9–10	PND 1–4, 0, 0.5, 1, 2 mg/kg body weight and day, vehicle: DMSO, subcutaneous, examination: PND 5	**0.5 and 1 mg/kg body weight**: 5HT1A and 5HT2 receptor expression ↑ in the forebrain or brainstem, 5HT transporters ↓ in the brainstem, 5HT transporters ↑ in the forebrain, non-monotonous dose dependency; ≥ **1 mg/kg body weight**: ♀: AChE activity ↓ (> 90%, to about 80% in 2 mg/kg body weight) in the forebrain and brainstem; **2 mg/kg body weight**: ♂: AChE activity ↓ (up to about 90%) in the forebrain and brainstem	Slotkin et al. 2006 a
rat, SD, 10 ♂ and 10 ♀, on PND 0 all pups were randomized and redistributed to the dams in order to obtain a litter size of 9–10	PND 1–4, 0, 0.5, 1, 2 mg/kg body weight and day, vehicle: DMSO, subcutaneous, examination: PND 5	≥ **0.5 mg/kg body weight**: AChE activity ↓ (> 80%) in the forebrain and brainstem, membrane protein: total protein ratio ↓ in the forebrain and brainstem (measure for neuritic outgrowth); hemicholinium-3 binding to presynaptic choline transporters (index for cholinergic neuronal activity) not affected, no downregulation of the m2-muscarinic AChE receptors (which happens with chronic cholinergic hyperstimulation)	Slotkin et al. 2006 b
rat, SD, 10 ♂ and 10 ♀, on PND 0 all pups were randomized and redistributed to the dams in order to obtain a litter size of 9–10	PND 1–4, 0, 1, 2 mg/kg body weight and day, vehicle: DMSO, subcutaneous, examination: PND 5	**1 mg/kg body weight**: fgfr1 expression ↓ in the forebrain, fgf22 expression ↓ in the brainstem, fgf14 expression ↓ in the forebrain; ≥ **1 mg/kg body weight**: fgf20 expression ↓ in the forebrain, fgf2 expression ↓ in the brainstem, fgfr4 expression ↑ in the brainstem	Slotkin et al. 2007

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, SD, 6 ♂ and 6 ♀, on PND 0 all pups were randomized and redistributed to the dams in order to obtain a litter size of 5 ♂ and 5 ♀	PND 1–4, 0, 0.5, 2 mg/kg body weight and day, vehicle: DMSO, subcutaneous, examination: PND 30, 60, 100	**0.5 mg/kg body weight**: ♂: 5HT-1 A receptors ↓ in cerebral regions and the brainstem (PND 30–100), ♀: 5HT transporters ↑ in cerebral regions and the brainstem (PND 30–100); no significant effect on 5-HT2 receptors, no significant regional selectivity for either effect	Slotkin et al. 2008 a
rat, SD, 6♂ and 6 ♀, on PND 0 all pups were randomized and redistributed to the dams in order to obtain a litter size of 5 ♂ and 5 ♀	PND 1–4, 0, 0.5, 2 mg/kg body weight and day, vehicle: DMSO, subcutaneous, examination: PND 30, 60, 100	≥ **0.5 mg/kg body weight**: neuron loss and reactive gliosis (PND 30–100), ♂: AChE activity in the cerebrocortical region ↓ (> 80%) and hippocampus ↓ (> 80%), ♂: AChE activity ↑ in the striatum (PND 30, but up to PND 100 normal or subnormal); **2 mg/kg body weight**: DNA content in the striatum ↓; no significant effects on AChE markers in the midbrain	Slotkin et al. 2008 b
rat, SD, 10 ♂ and 10 ♀, on PND 0 all pups were randomized and redistributed to the dams in order to obtain a litter size of about 10	PND 1–4, 0, 0.5, 2 mg/kg body weight and day, vehicle: DMSO, subcutaneous, examination: PND 30, 60, 100	≥ **0.5 mg/kg body weight**: liver: upregulation of AC activity and of the responses to stimulants of β-adrenergic receptors, glucagon receptors or G-proteins (PND 100); **2 mg/kg body weight**: liver: time-dependent upregulation of AC activity and of responses to β-adrenergic or glucagon receptor stimulation or G-proteins (PND 60) up to PND 100; heart: only transient effects on AC function	Adigun et al. 2010

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, SD, 6 ♂ and 6 ♀, on PND 0 pups were randomized and redistributed to the dams in order to obtain a litter size of 6 ♂ and 6 ♀	PND 1–4, 0, 0.5, 2 mg/kg body weight and day, vehicle: DMSO, subcutaneous, examination: PND 52	**0.5 mg/kg body weight**: chocolate milk anhedonia test: ♂: ↓ preference for chocolate milk over water (measure for increase in anhedonia, typical components in animal model for depression); ≥ **0.5 mg/kg body weight**: Novelty-Suppressed-Feeding test: ♂: ↓ latency period until eating; **2 mg/kg body weight**: elevated plus maze test: ♂: ↓ exploration in arms not laterally limited (measure for increased fear)	Roegge et al. 2008
rat, SD, 6 ♂ and 6 ♀, on PND 0 all pups were randomized and redistributed to the dams in order to obtain a litter size of 6 ♂ and 6 ♀	PND1–4, 0, 0.5, 2 mg/kg body weight and day, vehicle: DMSO, subcutaneous, examinations: T maze test (PNW 4–5), test for locomotor activity (PNW 6), Prepulse Inhibition test (PNW 11–12), Radial Arm maze test (PNW 19–28)	**0.5 mg/kg body weight**: Radial Arm maze test: ♂: spatial learning impaired, sensitivity ↑ to memory-impairing effects of scopolamine (muscarinic AChE antagonist), non-monotonous dose dependency; ≥ **0.5 mg/kg body weight**: T maze test: decreased response latency in early trials; ♂: prepulse inhibition test: startle reaction ↓ compared with that in controls, sex difference in controls eliminated by diazinon; no changes in locomotor activity	Timofeeva et al. 2008

Table 5 (continued)

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 4 ♂ and 4 ♀ (histochemical examinations), at least 12 ♂ and 12 ♀ (behaviour tests)	PND 1–4, 0, 1 mg/kg body weight and day, vehicle: DMSO, subcutaneous, purity: 99%, examination: PND 60–63	**1 mg/kg body weight**: passive avoidance behaviour disturbed, NADPH-d$^+$/nNOS-IR neuron in the subnuclei of the basolateral complex of the amygdala ↓; motor activity unchanged	Vatanparast et al. 2013
mouse, C3H/HeN, 8–10 ♂ and 8–10 ♀	PND 8–11, 0, 0.5, 1 mg/kg body weight and day, vehicle: 0.01% DMSO, subcutaneous, purity: 99%, examination: PND 46–50, 81–85	≥ **0.5 mg/kg body weight**: ability to distinguish between new and familiar objects ↓ (PND 49 and 84), NGF mRNA ↓ (PND 50 and 85), NR1 mRNA, NR2B mRNA, CaMKIV mRNA,CREB-1 mRNA ↓ (only PND 50, not PND 85)	Win-Shwe et al. 2012

AC: adenylyl cyclase; AChE: acetyl cholinesterase; CaMKIV: calcium/calmodulin-dependent protein kinase type IV; ChE: cholinesterase; CREB-1: cAMP response element binding protein; DMSO: dimethylsulfoxide; fgf: fibroblast growth factor, fgfr: fibroblast growth factor receptor; 5-HT: 5-hydroxytryptamine; NADPH-diaphorase; NGF: nerve growth factor; NR1, NR2B: subunits of the NMDA receptor (N-methyl-D-aspartate); nNOS-IR: neuronal NO-synthase immunoreactive; SD: Sprague Dawley; PND: postnatal day; PNW: postnatal week

subjected to subcutaneous treatment with diazinon in doses of 0, 0.5 or 2 mg/kg body weight and day from postnatal days 1 to 4 were found in various tests on postnatal day 52 to have changed emotional reactivity at both dose levels (Roegge et al. 2008). In a study with the same dosing scheme, the dose of 0.5 mg/kg body weight also resulted in neurocognitive deficits in adult rats (Timofeeva et al. 2008). At the only subcutaneous dose tested of 1 mg/kg body weight and day in Wistar rats, avoidance behaviour was disturbed after the same period of exposure (Vatanparast et al. 2013). After subcutaneous injection of 0.5 mg/kg body weight and day and above, a reduced ability to distinguish between new and familiar objects was found in C3H/HeN mice between postnatal days 8 and 11 (Win-Shwe et al. 2012).

In vitro

In a chicken embryo assay, 200 µg diazinon produced malformations of the limbs on day 3 of development. The authors suggested that these malformations could be related to the reduction of pyrimidine nucleotides in the embryo (Filkins 1997). (Filkins 1997).

Conclusions

Visceral anomalies occurred in rats at doses of 3.8 mg/kg body weight and day and above, which were interpreted as secondary effects caused by pronounced maternal toxicity. A NOAEL of 1.9 mg/kg body weight and day for developmental toxicity and maternal toxicity was obtained. The discrepancy between the low NOAEL and LOAEL for developmental and maternal toxicity of 1.9 mg/kg and 3.8 mg/kg body weight and day, respectively, in the study of ElMazoudy et al. (2011) and the NOAEL for developmental toxicity of 20 or 100 mg/kg body weight and day in the studies of Syngenta (1974, 1985) of the toxic effects on prenatal development in rats could be the result of a different manufacturing process for diazinon including other by-products and degradation products.

From the developmental neurotoxicity study with Sprague Dawley rats (US EPA 2005) a NOAEL for developmental neurotoxicity of 2.36 mg/kg body weight and day was obtained. As initial effects on the offspring, delayed body weight gains, delayed sexual maturity, impairment of motor activity as well as in learning and memory were found at the high dose of 24.2 mg/kg body weight and day. The NOAEL for maternal toxicity was 0.26 mg/kg body weight and day. In the dams and also in the offspring, the inhibition of AChE activity in plasma, erythrocytes and the brain began at 2.36 mg/kg body weight and day. Only at the high dose of 24.2 mg/kg body weight and day were the effects on AChE activity in the offspring as pronounced as those in the dams at 2.36 mg/kg body weight and day and above. Effects on motor activity and learning were first observed at 24.2 mg/kg body weight and day and above.

In developmental toxicity studies with rabbits, gavage administration of diazinon caused maternal toxicity (cholinergic symptoms, mortality) at doses of 30 mg/kg body weight and day and above (Ciba-Geigy 1994; Robens 1969; US EPA 1999). Up to the high dose of 100 mg/kg body weight and day, no effects on the foetuses

were found (Ciba-Geigy 1994; US EPA 1999). The NOAEL for maternal toxicity was 25 mg/kg body weight and day and the NOAEL for developmental toxicity was 100 mg/kg body weight and day in rabbits.

As regards the toxic effects on postnatal development, a dose without effects cannot be derived. The lowest effective dose level, administered subcutaneously from postnatal days 1 to 4, which produced effects on brain development in rats (Slotkin et al. 2006 a, b, 2007, 2008 a, b) and changes in behavioural tests in both adult rats (Roegge et al. 2008; Timofeeva et al. 2008) and adult mice (Win-Shwe et al. 2012), is 0.5 mg/kg body weight and day. The results of these studies are not relevant for assessing the effects of diazinon at the workplace because of the time at which postnatal treatment was initiated.

5.6 Genotoxicity

5.6.1 In vitro

The results of in vitro genotoxicity studies are shown in Table 6. Most of these studies were already described in the supplement from 1995 (documentation "Diazinon" 1998). More recent studies are Colović et al. 2010, Salazar-Arredondo et al. 2008, Shirasu et al. 1976, Tisch et al. 2002, 2007 and Wong et al. 1989.

A differential killing test with Bacillus subtilis yielded negative results for diazinon (Shirasu et al. 1976). In several bacterial gene mutation tests, diazinon was not found to be mutagenic in Salmonella strains TA98, TA100, TA102, TA1535, TA1536, TA1537 and TA1538, nor in Escherichia coli WP2hcr and WP2uvrA both with and without the addition of a metabolic activation system (Ciba-Geigy 1994; Moriya et al. 1983; Wong et al. 1989). In a sperm chromatin structure assay, diazinon led to reduced DNA integrity at 500 µM and above. Diazoxon, a metabolite of diazinon, had the same effect at 300 µM and above (Salazar-Arredondo et al. 2008). In two comet assays, at 0.5 mM and above, the number of DNA strand breaks was increased in the mucosal cells of the inferior and middle nasal turbinate (Tisch et al. 2002) and, at 0.05 mM and above, in mucosal epithelial cells from tonsils (Tisch et al. 2007). In both studies, the cells were taken from patients who had undergone surgery of the nose or tonsils. In the second study, patients with chronic tonsillitis were involved (Tisch et al. 2007). In the first study, it is also conceivable that the patients had chronic inflammation or allergy in the nasal region (Tisch et al. 2002). It is unclear what effects inflammation has on the results of a comet assay in isolated cells. In a UDS test, diazinon did not lead to unscheduled DNA repair synthesis up to concentrations of 120 µg/ml (Ciba-Geigy 1994). In several SCE (sister chromatid exchange) tests, diazinon caused a disturbance in cell division and delayed the cell cycle (Chen et al. 1981; Kuroda et al. 1992; Sobti et al. 1982). In human lymphoid cells (Ciba-Geigy 1994; Sobti et al. 1982), V79 cells (Chen et al. 1981; Kuroda et al. 1992) and CHO (Chinese hamster ovary) cells (Nishio and Uyeki 1981) an increase in sister chromatid exchange was found, or questionable positive findings were obtained in primary human lym-

Table 6 Genotoxicity of diazinon in vitro

End point	Test system	Concentration	Cytotoxicity	Results		Remarks	References
				–m.a.	+m.a.		
Bacteria							
rec test	Bacillus subtilis H17 Rec^+ and M45 Rec^-	no details of concentrations, in DMSO, purity: not specified	no data	–	not reported		Shirasu et al. 1976
gene mutation	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538, Escherichia coli WP2hcr	up to 5000 µg/plate or up to cytotoxic concentrations (no other details), in DMSO, purity: not specified, commercially obtainable in Japan	no data	–	–		Moriya et al. 1983
gene mutation	Salmonella typhimurium TA98, TA100, TA1535, TA1537, Escherichia coli WP2uvrA	3130–50 000 µg/ml, in DMSO, purity: 88%	no data	–	–		Ciba-Geigy 1994
gene mutation	Salmonella typhimurium TA1535, TA1536, TA1537, TA1538	50–1000 µg/plate, in DMSO, purity: analytical grade (no other details)	no data	–	–		Ciba-Geigy 1994
gene mutation, plate incorporation	Salmonella typhimurium TA98, TA102, TA1535, TA1537	20–80 µg/g, no details of volume, 6 concentrations tested, in DMSO, purity: technical grade: 90%–95%	LC_{50}: 80 µg/g	–	+TA98	average number of spontaneous revertants in TA98 +m.A.: 30; number of revertants/µg +m.A.: 0.39 ± 0.07	Wong et al. 1989

Table 6 (continued)

End point	Test system	Concentration	Cytotoxicity	Results		Remarks	References
				−m.a.	+m.a.		
Mammalian cells							
DNA integrity, sperm chromatin structure assay	sperm samples from 3 healthy volunteers	50–750 µM diazinon or diazoxon, in DMSO, 1 hour, purity: chemical grade (no other details)	% living cells: ≥ 86.9% (diazinon), ≥ 81.1% (diazoxon)	+ at 300 µM (diazoxon) and above + at 500 µM (diazinon) and above	not reported		Salazar-Arredondo et al. 2008
DNA strand breaks, comet test	mucosal cells of the inferior and middle nasal turbinate of 21 patients (16 ♂, 5 ♀) who had undergone nasal surgery (functional endoscopic sinus operation, turbinoplastic)	0.5–1.0 mM, in DMSO, 1 hour, purity: 99.5%	controls: inferior and middle nasal turbinate: 5% and 8%; 1 hour diazinon: 17% and 28%	(+) at 0.5 mM and above	not reported	possible chronic inflammation or allergy in the nasal region	Tisch et al. 2002
DNA strand breaks, comet test	mucosal epithelial cells from tonsils of 85 patients (64 ♂, 21 ♀), who had undergone surgery for chronic tonsillitis	0.05–1 mM, in DMSO, 1 hour, purity: 95.4%	cell vitality > 75%	(+) at 0.05 mM and above	not reported	patients with chronic inflammation	Tisch et al. 2007
UDS	primary rat hepatocytes	1.1–120 µg/ml, in DMSO, purity: 88%	no data	–	not reported		Ciba-Geigy 1994

Table 6 (continued)

End point	Test system	Concentration	Cytotoxicity	Results		Remarks	References
				–m.a.	+m.a.		
SCE	human lymphoid cells LAZ-007	–S9: 0.02–20 µg/ml, +S9: 20 µg/ml, in ethanol, purity: chemical grade (no other details)	–S9: cytotoxicity at 0.02 µg/ml: 20%, at 20 µg/ml: 60%, mitotic index ↓, metaphase 2 dose-dependent increase, metaphase 3 dose-dependent decrease; +S9: at 20 µg/ml: 60%	–	(+)	study inadequately conducted	Sobti et al. 1982
SCE	human lymphoid cells CCL-156	12.5–200 µg/ml, in DMSO, purity: 87.5%	no data	–	–		Ciba-Geigy 1994
SCE	primary human lymphocytes	0.0668–2000 µg/ml, in DMSO, purity: 88%	no data	(+)	(+)	results –S9 not reproducible, +S9 SCE slightly ↑ (not double the control incidence)	Ciba-Geigy 1994
SCE	V79 cells	0.05–0.4 µg/ml, in methanol, purity: 99%	0.09 µg/ml: mitotic index 50%, 0.38 µg/ml: secondary mitotic index 50%	–	not reported		Kuroda et al. 1992

Table 6 (continued)

End point	Test system	Concentration	Cytotoxicity	Results		Remarks	References
				−m.a.	+m.a.		
SCE	V79 cells	10–80 µg/ml, in DMSO, purity: 99.2%	at 80 µg/ml: complete inhibition of mitosis	–	not reported		Chen et al. 1981
SCE	CHO cells	0.03–1.0 mM (9.1–304 µg/ml), in DMSO, purity: 89%	no data	–	not reported	solubility exceeded at 304 µg/ml, diazoxon (metabolite of diazinon) at 1 mM SCE ↑	Nishio and Uyeki 1981
MN	fibroblasts and lymphocytes of a healthy non-smoker	2×10^{-5}–0.2 mM, in ethanol, purity: 97.3%	at about 0.2 mM	+ at 2×10^{-5} mM and above	not reported		Colović et al. 2010
CA	CHL cells	100 µg/ml, in ethanol or DMSO, purity: not specified	−S9: at 100 µg/ml	–	(+)	study inadequately conducted, only one concentration investigated, effect not reproducible	Matsuoka et al. 1979
CA	primary human lymphocytes	5–30 µg/ml, in DMSO, purity: technical product (no other details)	mitotic index 5.4 at 5 µg/ml, 0.9 at 30 µg/ml	(+)	not reported	study inadequately conducted and inadequate documentation (2.1% DMSO positive in the test)	Lopez et al. 1986

Table 6 (continued)

End point	Test system	Concentration	Cytotoxicity	Results		Remarks	References
				–m.a.	+m.a.		
CA	primary human lymphocytes	30 µg/ml, in DMSO, purity: not specified	no data	(+)	not reported	unusual exposure schedule: for 1 hour every 6 hours, study inadequately conducted, only one concentration investigated, effect not reproducible	Lopez and Carrascal 1987
gene mutation $TK^{+/-}$	L5178Y mouse lymphoma cells	6.25–100 µg/ml, in DMSO, purity: not specified, sample from NTP	> 200 µg/ml in the 1st test > 100 µg/ml in the 2nd test	(+)	not reported	cytotoxic effects, mutation incidence and positive control not reproducible	McGregor et al. 1988
gene mutation $TK^{+/-}$	L5178Y mouse lymphoma cells	–S9 : 12–120 µg/ml, +S9: 6–60 µg/ml, solvent: not specified, purity: 97.2%	no data	–	–		Ciba-Geigy 1994

CA: chromosomal aberration test; CHO: Chinese hamster ovary; DMSO: dimethyl sulfoxide; MN: micronucleus test; NTP: National Toxicology Program; SCE: sister chromatid exchange test; $TK^{+/-}$: thymidine kinase mutation test; UDS: autoradiographic test for unscheduled DNA synthesis

phocytes (Ciba-Geigy 1994) and human lymphoid cells (Sobti et al. 1982). These results are not included in this evaluation because of the inadequate study methods (Sobti et al. 1982) or because the results were not reproducible (Ciba-Geigy 1994). Diazoxon, a metabolite of diazinon, produced an increase in SCE at 1 mM without the addition of a metabolic activation system (Nishio and Uyeki 1981). Already in the supplement from 1995 (documentation "Diazinon" 1998) the three chromosomal aberration tests were not considered valid because of the inadequate study methods or because the results were not reproducible (Lopez et al. 1986; Lopez and Carrascal 1997; Matsuoka et al. 1979). In a micronucleus test with human fibroblasts and lymphocytes, diazinon produced an increase in the number of micronuclei at 2×10^{-5} mM and above. Cytotoxicity was observed only at 0.2 mM and above (Colović et al. 2010). The $TK^{+/-}$ test with diazinon in L5178Y mouse lymphoma cells yielded negative results both with and without the addition of a metabolic activation system (Ciba-Geigy 1994). Another $TK^{+/-}$ test cannot be evaluated as a result of cytotoxicity, the mutation incidence was not reproducible and no positive control was used (McGregor et al. 1988).

5.6.2 In vivo

The results of in vivo genotoxicity tests are shown in Table 7. Most of the studies were already described in the supplement from 1995 (documentation "Diazinon" 1998) so that only those by Çakir and Sarikaya 2005 and Tsitsimpikou et al. 2012 are new.

In the SMART (somatic mutation and recombination test, wing spot test) in Drosophila melanogaster, a positive result was obtained at diazinon concentrations of 1 µl/ml and above. According to the authors, diazinon was found to be very toxic for the larvae of Drosophila melanogaster (no other details; Çakir and Sarikaya 2005). The number of evaluated wings was between 50 and 110 (at 3 µl/ml). The number of wings counted was barely sufficient; 110 wings must be counted for a doubling of the spontaneous incidence (0.5 to 0.8 spots per wing) to be identified with a probability of error of 5%. No details of the toxic effects of diazinon on the larval stages are available. As diazinon was not found to be mutagenic in a large number of mutagenicity tests in bacteria and mammalian cells, the positive result in the SMART does not seem plausible as an indication of mutagenic activity. In New Zealand White rabbits, diazinon led to an increase in oxidative DNA damage (AP positions) and increased telomerase activity in the liver and kidneys after the administration of 2.64 or 5.28 mg/kg body weight for three months and one month with an eight-month interruption (Tsitsimpikou et al. 2012). As this was a non-standardized study procedure, and a small number of animals was used, the positive result is regarded as questionable. The following studies with negative results were already described in the supplement from 1995 (documentation "Diazinon" 1998). In three in vivo SCE tests in hamsters (single doses up to 26 mg/kg body weight)

Table 7 Genotoxicity of diazinon in vivo

Test system		Dose	Results	Remarks	References
Somatic cells					
SMART, wing spot test	Drosophila melanogaster, *multiple wing hair (mwh/mwh), flare-3: (flr3/In (3LR), TM3 BdS)*	1 µl/ml (2 hours, long-term, no other details), 3 µl/ml (2.4 hours), 5 µl/ml (2 hours), in distilled water, purity: 95%	+ ≥ 1 µl/ml (with long-term treatment; + ≥ 3 µl/ml	≥ 1 µl/ml (with long-term treatment): small random spots, total number of *mwh* spots, total number of spots ↑; ≥ 3 µl/ml: small and large random spots, total number of *mwh* spots, total number of spots ↑; no "twin spots"	Çakir and Sarikaya 2005
oxidative DNA damage (AP positions)	rabbit, New Zealand White, 2 ♀	0, 2.64, 5.28 mg/kg body weight and day, treatment every 2 days, oral (no other details), 2 dose periods of 3 and 1 month, interrupted by an 8-month phase without treatment, in distilled water, purity: not specified	+ ≥ 2.64 mg/kg body weight in liver and kidneys	≥ 2.64 mg/kg body weight: telomerase activity ↑ in liver and kidneys; 5.28 mg/kg body weight: liver and kidneys: focal inflammation and fibrosis, number of animals too small, unusual dosing scheme	Tsitsimpikou et al. 2012
SCE	hamster, bone marrow cells, no other details	6.5, 13, 26 mg/kg body weight, no details of control animals, single doses, 24 hours later 10 mg colcemid/kg body weight, no other details	–		Ciba-Geigy 1994

Table 7 (continued)

Test system		Dose	Results	Remarks	References
SCE	mouse, ICI, bone marrow cells, no other details	10, 50, 100 mg/kg body weight, no details of control animals, single doses, gavage, examination after 24 hours, purity: 88%	–		Ciba-Geigy 1994
SCE	mouse, CD-1, ♀, no other details	150, 160, 175 mg/kg body weight, no details of control animals, gavage, examination after 29 hours, purity: 88%	–		Ciba-Geigy 1994
nuclear anomalies	hamster, bone marrow cells, no other details	6.5, 13, 26 mg/kg body weight, no details of control animals, two doses at an interval of 24 hours, gavage, examination 24 hours after 2nd dose, no other details	–		Ciba-Geigy 1994
MN	mouse, no other details	120 mg/kg body weight, no details of control animals, single doses, examination after 16, 24, 48 hours, 30, 60, 120 mg/kg body weight, single doses, examination after 24 hours, purity: 87.5%, according to OECD Test Guideline 474	–		Ciba-Geigy 1994

Table 7 (continued)

Test system		Dose	Results	Remarks	References
Germ cells					
SCE	mouse, spermatogonia, no other details	10.5, 21, 63 mg/kg body weight and day, 5 days, no other details	–		Ciba-Geigy 1994
SCE	mouse, spermatocytes, no other details	5 treatments over 10 days, 3 hours prior to investigation 10 mg colcemid, no other details	–		Ciba-Geigy 1994
DLT	mouse, no other details	15, 45 mg/kg body weight, single doses, gavage, purity: 95%	–		Ciba-Geigy 1994

AP positions: positions in the DNA containing neither purines nor pyrimidine; DLT: dominant lethal test; MN: micronucleus test; SCE: sister chromatid exchange test; SMART: somatic mutation and recombination test

and mice (single doses up to 175 mg/kg body weight), diazinon did not cause an increase in the incidence of sister chromatid exchange in bone marrow cells (no other details; Ciba-Geigy 1994). In hamsters given gavage doses of diazinon of up to 26 mg/kg body weight for 2 days, the treatment did not lead to an increase in nuclear anomalies in bone marrow cells (no other details; Ciba-Geigy 1994). In a micronucleus test with mice carried out according to the OECD Test Guideline, single diazinon doses of up to 120 mg/kg body weight did not produce an increase in the incidence of micronuclei (Ciba-Geigy 1994). In the spermatogonia and spermatocytes of mice, diazinon doses of up to 63 mg/kg body weight, given over 5 days, did not lead to an increase in the incidence of sister chromatid exchange (no other details; Ciba-Geigy 1994). A dominant lethal test in mice with single gavage doses of up to 45 mg/kg body weight likewise yielded negative results (no other details; Ciba-Geigy 1994).

Conclusions

Diazinon was not found to be mutagenic in bacteria and mammalian cells (Ciba-Geigy 1994; Moriya et al. 1983; Wong et al. 1989). In the supplement of 1995, the fact was emphasized that the clastogenicity of diazinon has been less well investigated in vitro than other end points of genotoxicity (documentation "Diazinon" 1998). The positive results in a micronucleus test with human fibroblasts and lymphocytes (Colović et al. 2010) provide evidence of a possible clastogenic potential of diazinon in vitro. Further valid tests which support or refute these results are not available. No in vivo confirmation has been found for the assumed clastogenicity in vitro, however. Thus, the negative result of a micronucleus test in mice (Ciba-Geigy 1994), which is not available in the original but was carried out according to OECD Test Guideline 474, supports the fact that diazinon is not clastogenic in vivo. Both diazinon and its metabolite diazoxon led in vitro to a reduction in DNA integrity in sperms (Salazar-Arredondo et al. 2008); however, neither sister chromatid exchange nor dominant lethal mutations were induced in vivo in germ cells (Ciba-Geigy 1994).

5.7 Carcinogenicity

5.7.1 Short-term studies

All short-term studies were already described in the supplement from 1995 (documentation "Diazinon" 1998). In none of the studies is the purity of the substance specified.

Diazinon caused an increase in the frequency of cell transformations in rat embryo cells infected with the Rauscher leukaemia virus (Traul et al. 1981). An increased number of cell transformations was demonstrated in C3H/10Tl/2 cells only after prolonged cultivation and the replating of confluent cells (Schechtman et al. 1987). An increased incidence of pulmonary adenomas was found only in female Strain A mice after diazinon doses of 5 mg/kg body weight (Maronpot et al.

1986). The administration of diazinon for 6 weeks did not result in an increase in GST-P-(glutathione S-transferase, placental form)-positive foci in the liver up to doses of 100 mg/kg body weight and day, nor did single intraperitoneal injections of 200 mg diethyl nitrosamine/kg body weight (Kato et al. 1995).

5.7.2 Long-term studies

The carcinogenicity studies (see Table 8) with diazinon were already described in the supplement from 1995 (documentation "Diazinon" 1998).

Table 8 Carcinogenicity studies with diazinon

Author:	NCI 1976			
Substance:	diazinon (purity: 98%)			
Species:	rat, F344, 50 ♂ and 50 ♀ per dose group, 25 ♂ and 25 ♀ per control group			
Administration route:	with the diet			
Dose:	0, 400, 800 mg/kg diet (about 0, 20, 40 mg/kg body weight and day assuming a body weight of 400 g and a food intake of 20 g)			
Duration:	103 weeks, recovery period 1–2 weeks			
Toxicity:	≥ **20 mg/kg body weight**: ♂ and ♀: tachypnoea, ♂: hyperactivity, ♀: bloating, vaginal bleeding and discharge; **40 mg/kg body weight**: ♀: hyperactivity, discoloured urine; body weights not changed, MTD not attained			
	Dose (mg/kg body weight and day)			
		0	20	40
Survivors after 78 weeks:	♂	24/25 (96%)	49/50 (98%)	49/50 (98%)
	♀	23/25 (92%)	44/50 (88%)	44/50 (88%)
	Dose (mg/kg body weight and day)			
		0	20	40
Tumours after 105 weeks: lymphomas or leukaemia	♂	5/25 (20%)	25/50 (50%)*	12/50 (24%)
Endometrium: stroma polyps	♀	2/23 (9%)	8/43 (19%)	11/49 (22%)
Historical control data of 72 control groups from 6 laboratories: lymphomas or leukaemia ♂: mean value (range): 16.9% (0–54%); endometrium: stroma polyps ♀: 14.9% (0–38%) (Tarone et al. 1981)				

* p = 0.011 (Fisher's exact test);
MTD: maximum tolerated dose

Table 8 (continued)

Author:	Ciba-Geigy 1994
Substance:	diazinon (purity: 87.7%), stabilizer: epoxidized soy oil
Species:	rat, Sprague Dawley, 30–40 ♂ and 30–40 ♀ per dose group
Administration route:	with the diet
Dose:	0, 0.1, 1.5, 125, 250 mg/kg diet (♂: 0, 0.004, 0.06, 5, 10 mg/kg body weight and day; ♀: 0, 0.005, 0.07, 6, 12 mg/kg body weight and day); vehicle control 26.5 mg epoxidized soy oil/kg diet; controls untreated animals
Duration:	98–99 weeks: 10–20 animals per sex and group; interim killing after 52 weeks: 8–10 animals per sex and group; interim killing after 52 weeks exposure and subsequent 4-week recovery period without exposure: 9–10 animals per sex in control groups and high dose group
Toxicity:	**0.06 (♂)/0.07 (♀) mg/kg body weight**: **NOAEL** for brain AChE ↓; ≥ **0.06 (♂)/0.07 (♀) mg/kg body weight**: serum ChE ↓ (♂: to 49.3% of the control value; ♀: 70.4%); ≥ **5 (♂)/6 (♀) mg/kg body weight**: erythrocyte AChE ↓ (♂: 79.3%; ♀: 74.4%), brain AChE ↓ (♂: 76%; ♀:71.4%) ↓; **10 (♂)/12 (♀) mg/kg body weight**: body weight and food intake ↑ (palatability ↑ due to soy oil); no substance-related mortality, no unusual gross pathological or microscopic findings, MTD not attained

No increased incidences of substance-related tumours

Author:	NCI 1976
Substance:	diazinon (purity: 98%)
Species:	mouse, B6C3F1, 50 ♂ and 50 ♀ per dose group, 25 ♂ and 25 ♀ per control group
Administration route:	with the diet
Dose:	0, 100, 200 mg/kg diet (about 0, 12.5, 25 mg/kg body weight and day assuming a body weight of 20 g and a food intake of 2.5 g)
Duration:	103 weeks, recovery period 2–3 weeks
Toxicity:	≥ **12.5 mg/kg body weight**: ♂ and ♀: hyperactivity; body weights not changed, MTD not attained

		Dose (mg/kg body weight and day)		
		0	12.5	25
Survivors after 78 weeks:	♂	21/25 (84%)	45/50 (90%)	49/50 (98%)
	♀	24/25 (96%)	50/50 (100%)	49/50 (98%)

		Dose (mg/kg body weight and day)		
		0	12.5	25
Tumours after 106 weeks: hepatocellular carcinomas	♂	4/21 (19%)	20/46 (43%)*	10/48 (21%)

Table 8 (continued)

Historical control data of 54 control groups from 5 laboratories: hepatocellular carcinomas: mean value (range): ♂: 32.1 (7%–58%) (Tarone et al. 1981)	
* p = 0.046 (Fisher's exact test); MTD: maximum tolerated dose	
Author:	Ciba-Geigy 1994
Substance:	diazinon (purity: 87.7%)
Species:	mouse, CD-1, 60 ♂ and 60♀ per dose group
Administration route:	with the diet
Dose:	0, 4, 20, 100 mg/kg diet (about 0, 0.5, 2.5, 12.5 mg/kg body weight and day assuming a body weight of 20 g and a food intake of 2.5 g)
Duration:	♂: 18 months, ♀: 19 months
Toxicity:	mortality ↑ (survivors in dose groups 0, 0.5, 2.5, 12.5 mg/kg body weight: ♂ after 79 weeks: 32%, 28%, 42%, 22%, ♀ after 83 weeks: 38%, 23%, 27%, 22%); no unusual findings for body weights, or in gross pathological and microscopic examinations
no increase in the incidences of substance-related tumours; according to Ciba-Geigy (1994), some data were lacking and there were contradictions in the study	

Rats

In carcinogenicity studies with rats, with dose levels below the MTD, the incidences of lymphomas or leukaemia were within the range of historical control animals (NCI 1976; Tarone et al. 1981) or the tumour incidences were not increased (Ciba-Geigy 1994).

Mice

A carcinogenicity study with mice was carried out at dose levels below the MTD (NCI 1976), and another study is not included in the evaluation because of a lack of data and contradictory results (Ciba-Geigy 1994; documentation "Diazinon" 1998).

Conclusions

No evidence of a carcinogenic potential of diazinon was obtained in carcinogenicity studies with F344 rats and B6C3F1 mice at dose levels below the MTD.

5.8 Other effects

In Jurkat cells, diazinon concentrations of 125 µM and above reduced the induction of several cytokines such as interferon γ or interleukin 4 without having any effect on cell viability. In A549 cells, diazinon concentrations of 62.5 µM and above reduced the activation of the NF-κB binding sequence (nuclear factor κ of activated B cells) without affecting cell viability (Oostingh et al. 2009).

In PC12 cells, diazinon concentrations of 30 μM induced tryptophan hydroxylase, the rate-limiting enzyme for serotonin biosynthesis, and suppressed the expression of serotonin transporter genes and serotonin receptors (Slotkin and Seidler 2008). In the same cells and at the same concentration, diazinon also had effects on the gene expression of subtypes of protein kinase C and its modulators (Slotkin and Seidler 2009 a), on glutamate receptors and on genes responsible for oxidative stress responses (Slotkin and Seidler 2009 b), on regulators of the cell cycle and apoptosis (Slotkin and Seidler 2012 a, b), and on the induction of the gene expression of the splice variant acetylcholinesterase-S (Jameson et al. 2007). In MCF7 cells, diazinon upregulated calreticulin and TGF-β3 (transforming growth factor β3) (Mankame et al. 2006 a, b) and reduced the DNA excision repair capacity (Mankame et al. 2006 a).

6 Manifesto (MAK value/classification)

The critical effect of diazinon is inhibition of the AChE activity at the cholinergic synapses.

MAK value. No data for humans suitable for the derivation of a MAK value are available. In a 3-week inhalation study there was an adverse reduction in AChE activity in the brain of female rats after nose-only exposure to 1.57 mg/m^3 and above (as aerosol). The NOAEC was 0.46 mg/m^3 (Ciba-Geigy 1994). Inhalation studies with longer-term exposure are not available.

However, there are several oral studies with long-term exposure.

As in the two long-term feeding studies in rats and dogs (Ciba-Geigy 1994; US EPA 1999), due to the doses selected, the LOAEL was higher than the NOAEL by a factor of 83 and 225, respectively, the true NAEL for both these studies could be markedly higher. For this reason, these studies are not used for the derivation of a MAK value.

In a 13-week feeding study in rats, the NOAEL for reduced AChE activity in erythrocytes and the brain of the females was 0.3 mg/kg body weight and day (LOAEL 15 mg/kg body weight and day) (Ciba-Geigy 1994). In a 106-week gavage study with monkeys, a decrease in the AChE activity in erythrocytes to between 56% and 82% of the control value was found in the males at 0.5 mg/kg body weight. The NOAEL was 0.05 mg/kg body weight (Ciba-Geigy 1994; US EPA 1999). The BAT value for the reduction in erythrocyte AChE activity has been established at 70%. It can be concluded from the study with monkeys that an inhibition of 70% is attained at one third of the LOAEL. The no adverse effect concentration (NAEC) for monkeys is therefore estimated to be 0.17 mg/kg body weight. In female beagle dogs, the NOAEL for the reduced activity of AChE in erythrocytes and the brain was found to be 0.75 mg/kg body weight and day in a 4-week feeding study (US EPA 1999). As, in female beagle dogs, the oral absorption of single doses of 4.0 mg/kg [^{14}C]diazinon was at least 85% (ATSDR 1996), the oral absorption of 85% is assumed for rats, dogs and monkeys. The following toxicokinetic data are taken into consideration for the

extrapolation of these NOAELs of 0.3 (rats), 0.75 (dogs) and 0.17 (NAEC monkeys) mg/kg body weight and day to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the corresponding species-specific correction values (1:4, 1:1.4 and 1:2), the assumed oral absorption of 85%, the body weight (70 kg) and respiratory volume (10 m^3) of the person, and the assumed 100% absorption by inhalation. The concentrations in air calculated from this are 0.6, 4.5 and 0.7 mg/m^3, respectively. A possible increase in AChE inhibition over time cannot be excluded, but does not occur consistently. In the 52-week study with dogs (Ciba-Geigy 1994; US EPA 1999) one third of the dose used in the 4-week study (US EPA 1999) is necessary to obtain the same level of inhibition of AChE in the erythrocytes. This does not apply to AChE inhibition in the brain. In the 99-week study in rats, one third of the dose used in the 13-week study produced a similar level of AChE inhibition in the erythrocytes and more pronounced inhibition in the brain of male rats, but not in female rats (Ciba-Geigy 1994). Studies with three species are available, so that a particular sensitivity in humans is not to be expected. On the basis of the lowest calculated concentration in air from the 13-week feeding study with rats (0.6 mg/m^3) and taking into account the possible enhancement of the effects over time, the MAK value of 0.1 mg/m^3 I has been confirmed. The MAK value is furthermore also supported by the 3-week inhalation study in rats in which a NOAEC of 0.46 mg/m^3 was obtained.

Peak limitation. The critical effect for the derivation of the MAK value is systemic toxicity, for which reason the classification of diazinon in Peak Limitation Category II remains valid. The elimination half-time of the dialkyl phosphate metabolites of diazinon in the urine of humans after single oral diazinon doses of 11 µg/kg body weight (36 nmol/kg body weight) is about 2 hours (Garfitt et al. 2002), so that the corresponding excursion factor of 2 has been retained in accordance with the procedures used by the Commission (see documentation "Peak limitation" 2011).

Prenatal toxicity. In the evaluation of the embryotoxicity of diazinon, both prenatal toxicity and postnatal neurotoxicity resulting from the inhibition of the cholinesterase activity must be taken into account.

Prenatal toxicity: In humans, an inverse correlation between the diazinon concentration in the umbilical plasma and both the birth weight and body size was found in newborn babies (Whyatt et al. 2005). In a study of the toxic effects on prenatal development in rats, visceral anomalies occurred at 3.8 mg/kg body weight and day and above as the only effect (ElMazoudy et al. 2011), which were interpreted by the Commission as secondary to the pronounced maternal toxicity. The NOAEL for developmental and maternal toxicity is 1.9 mg/kg body weight and day. The NOAEL and the LOAEL for developmental neurotoxicity in rats are 2.36 and 24.2 mg/kg body weight and day, respectively, while the NOAEL for maternal toxicity is 0.26 mg/kg body weight and day (US EPA 2005). In rabbits, the NOAEL for maternal toxicity (cholinergic symptoms, mortality) is 25 mg/kg body weight and day (Ciba-Geigy 1994; Robens 1969; US EPA 1999) and the NOAEL for developmental toxicity 100 mg/kg body weight and day, the highest dose tested (Ciba-Geigy

1994; US EPA 1999). The following toxicokinetic data are taken into consideration for the extrapolation of the NOAELs of 1.9 mg/kg body weight and day for rats and 100 mg/kg body weight and day for rabbits to a concentration in the workplace air: the species-specific correction values (1:4 and 1:2.4) for the rat and the rabbit, the assumed oral absorption (85%), the body weight (70 kg) and respiratory volume (10 m^3) of the person and the assumed 100% absorption by inhalation. The concentrations calculated from this are 2.8 mg diazinon/m^3 and 248 mg diazinon/m^3, respectively, which are higher than the MAK value of 0.1 mg/m^3 by factors of 28 and 2480, respectively.

Postnatal AchE inhibition: In the developmental neurotoxicity study, only at the high dose of 24.2 mg/kg body weight and day were the effects on AChE activity in the offspring as pronounced as those in the dams at 2.36 mg/kg body weight and day and above (US EPA 2005). The offspring are therefore less sensitive to AChE inhibition than the dams. A study in which the AChE-inhibiting effects of 5 organophosphates in the dams and the offspring were compared in 2-generation studies likewise revealed that AChE inhibition in the newborn pups (< 10%) was significantly lower than in the dams (about 50%). The AChE inhibition in the offspring increases with the increasing dietary intake of organophosphates during the lactation phase, and reaches about 30% at the end of lactation (Astroff et al. 1998).

As the margin between the calculated concentrations without effects and the MAK value is sufficiently large, developmental neurotoxicity is induced at higher doses than the effects on prenatal development, and the newborn offspring are less sensitive to the inhibition of acetylcholinesterase than the dams after prenatal administration, the classification of diazinon in Pregnancy Risk Group C has been retained.

Carcinogenicity. Diazinon is not mutagenic in bacteria, and confirmation of the suspected in vitro clastogenicity has not been found in vivo. Carcinogenicity studies in F344 rats and B6C3F1 mice at doses below the MTD (up to 40 mg/kg body weight and day in rats; up to 12.5 mg/kg body weight and day in mice) (NCI 1976), did not produce any evidence of a carcinogenic potential of diazinon. Diazinon is therefore not classified in one of the categories for carcinogenic substances.

Germ cell mutagenicity. Diazinon is not mutagenic in bacteria. Confirmation of the suspected in vitro clastogenicity has not been found in vivo. Diazinon and its metabolite diazoxon reduced the DNA integrity in sperms in vitro; however, neither SCE nor dominant lethal mutations are induced in germ cells in vivo. On the basis of the available data, diazinon is considered to be not genotoxic. Therefore, germ cell mutagenicity is not suspected, and the substance is not classified in one of the categories for germ cell mutagens.

Absorption through the skin. In vivo experiments with test persons and animal studies have provided clear evidence of the dermal absorption of diazinon. On the basis of an experimentally determined flux with volunteers (1.13 µg/cm^2 and hour; Garfitt et al. 2002), the absorption of 2.3 mg diazinon is to be expected after the exposure of both hands and forearms (area 2000 cm^2) to an ethanolic solution of diazinon (concentration 250 g/l) for one hour. On the other hand, after inhalation

exposure for 8 hours at the level of the MAK value, the absorption of 1 mg is to be expected. Even if the conditions selected in the above study (ethanolic solution of the active ingredient, occlusive application) may have favoured absorption of the active ingredient through the skin, dermal absorption must nevertheless be assumed to make a toxicologically relevant contribution to systemic exposure. Diazinon therefore remains designated with an "H" (for substances which can be absorbed through the skin).

Sensitization. No clinical findings of sensitization or positive patch test results are available for diazinon. Although some of the results from experimental studies indicate a contact sensitization potential, there is no conclusive picture. As, in addition, the test substance used is not sufficiently characterized and details of possible impurities are not given in any of these studies, the contact sensitization potential of diazinon must be called in question. There are no data available for sensitization of the airways. Diazinon is therefore not designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

7 References

Abd El-Aziz MI, Sahlab AM, Abd El-Khalik M (1994) Influence of diazinon and deltamethrine on reproductive organs and fertility of male rats. Dtsch Tieraerztl Wochenschr 101: 230–232

Abu-Qare AW, Abou-Donia MB (2001) Inhibition and recovery of maternal and fetal cholinesterase enzyme activity following a single cutaneous dose of methyl parathion and diazinon, alone and in combination, in pregnant rats. J Appl Toxicol 21: 307–316

ACGIH (American Conference of Governmental Industrial Hygienists) (2003) Diazinon. in: Documentation of TLVs and BEIs, ACGIH, Cincinnati, OH, USA

Adigun AA, Wrench N, Seidler FJ, Slotkin TA (2010) Neonatal organophosphorus pesticide exposure alters the developmental trajectory of cell-signaling cascades controlling metabolism: differential effects of diazinon and parathion. Environ Health Perspect 118: 210–215

Alluwaimi AM, Hussein Y (2007) Diazinon immunotoxicity in mice: modulation of cytokines level and their gene expression. Toxicology 236: 123–131

Anthony J, Banister E, Oloffs PC (1986) Effect of sublethal levels of diazinon: histopathology of liver. Bull Environ Contam Toxicol 37: 501–507

APVMA (Australian Pesticides and Veterinary Medicines Authority) (2011) Chemical Review Program. Consolidated Human Health Risk Assessment for Diazinon. Office of Chemical Safety, Department of Health and Ageing, Canberra, Australia

Astroff AB, Freshwater KJ, Eigenberg DA (1998) Comparative organophosphate-induced effects observed in adult and neonatal Sprague-Dawley rats during the conduct of multigeneration toxicity studies. Reprod Toxicol 12: 619–645

ATSDR (Agency for Toxic Substances and Disease Registry) (1996) Toxicological profile for diazinon. US Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA, http://www.atsdr.cdc.gov/toxprofiles/tp.asp?id=22&tid=3

Barnett JB, Spyker-Cranmer JM, Avery DL, Hoberman AM (1980) Immunocompetence over the lifespan of mice exposed in utero to carbofuran or diazinon: I. Changes in serum immunoglobulin concentrations. J Environ Pathol Toxicol 4: 53–63

de Blaquière GE, Waters L, Blain PG, Williams FM (2000) Electrophysiological and biochemical effects of single and multiple doses of the organophosphate diazinon in the mouse. Toxicol Appl Pharmacol 166: 81–91

Bruce RB, Howard JW, Elsea JR (1955) Toxicity of 0,0-diethyl-0-(2-iso- propyl-6-methyl-4-pyrimidyl) phosphorotioate (Diazinon). J Agr Food Chem 3: 1017–1021

Çakir S, Sarikaya R (2005) Genotoxicity testing of some organophosphate insecticides in the Drosophila wing spot test. Food Chem Toxicol 43: 443–450

Chen HC, Hsueh Jl, Sirianni SR, Huang CC (1981) Induction of sister-chromatid exchanges and cell cycle delay in cultured mammalian cells treated with eight organophosphorous pesticides. Mutat Res 88: 307–316

Ciba-Geigy (1994) Toxicological evaluation of Diazinon G 24480. Ciba-Geigy Limited, 19.08.1994, Basel, Schweiz, unpublished report

Colović M, Krstić D, Petrović S, Leskovac A, Joksić G, Savić J, Franko M, Trebse P, Vasić V (2010) Toxic effects of diazinon and its photodegradation products. Toxicol Lett 193: 9–18

Davies DB, Holub BJ (1980 a) Comparative subacute toxicity of dietary diazinon in the male and female rat. Toxicol Appl Pharmacol 54: 359–367

Davies DB, Holub BJ (1980 b) Toxicological evaluation of dietary diazinon in the rat. Arch Environ Contam Toxicol 9: 637–650

DFG (Deutsche Forschungsgemeinschaft) (2010) List of MAK and BAT Values 2010: Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area. Report 46, Wiley-VCH, Weinheim

Dobbins PK (1967) Organic phosphate insecticides as teratogens in the rat. J Fla Med Assoc 54: 452–456

Duysen EG, Cashman JR, Schopfer LM, Nachon F, Masson P, Lockridge O (2012) Differential sensitivity of plasma carboxylesterase-null mice to parathion, chlorpyrifos and chlorpyrifos oxon, but not to diazinon, dichlorvos, diisopropylfluorophosphate, cresyl saligenin phosphate, cyclosarin thiocholine, tabun thiocholine, and carbofuran. Chem Biol Interact 195: 189–198

Earl FL, Melveger BE, Reinwall JE, Bierbower GW, Curtis JM (1971) Diazinon toxicity–comparative studies in dogs and miniature swine. Toxicol Appl Pharmacol 18: 285–295

Earl FL, Miller E, van Loon EJ (1973) Reproductive, teratogenic, and neonatal effects of some pesticides and related compounds in beagle dogs and miniature swine. in: Deichmann WB (Ed.): Pesticide and the environment: A continuing controversy, Stratton, New York, USA, 253–266

Edson EF, Noakes DN (1960) The comparative toxicity of six organophosphorus insecticides in the rat. Toxicol Appl Pharmacol 2: 523–539

Ellison CA, Tian Y, Knaak JB, Kostyniak PJ, Olson JR (2012) Human hepatic cytochrome P450-specific metabolism of the organophosphorus pesticides methyl parathion and diazinon. Drug Metab Dispos 40: 1–5

Elmazoudy RH, Attia AA, Abdelgawad HS (2011) Evaluation of developmental toxicity induced by anticholinesterase insecticide, diazinon in female rats. Birth Defects Res B Dev Reprod Toxicol 92: 534–542

ElMazoudy RH, Attia AA (2012) Endocrine-disrupting and cytotoxic potential of anticholinesterase insecticide, diazinon in reproductive toxicity of male mice. J Hazard Mater 209–210: 111–120

EU (Europäische Union) (2000) Statutory instruments. European communities (classification, packaging, labelling and notification of dangerous substances) regulations, 2000

Filkins K (1997) Pesticides. in: Frazier LM, Hage ML (Eds) Reproductive hazards of the workplace, John Wiley and Sons Ltd, New York, USA; 192–256

García-Repetto R, Martinez D, Repetto M (1996) A biodisposition study of diazinon in the Wistar rat. Toxic Subst Mech 15: 415–423

Garfitt SJ, Jones K, Mason HJ, Cocker J (2002) Exposure to the organophosphate diazinon: data from a human volunteer study with oral and dermal doses. Toxicol Lett 134: 105–113

Green VA (1970) Effects of pesticides on rat and chick embryo. in: Hemphill DD (Ed.) Trace substances in environmental health – III, University of Missouri, Columbia, Missouri, USA, 183–209

Greim H, Lehnert G (Eds) (1995) Acetylcholinesterase inhibitors. Biological exposure values for occupational toxicants and carcinogens, Vol 2, VCH, Weinheim, 15–24 https://doi.org/10.1002/3527600418.bb0astrinhe0002

Handy RD, Abd-El Samei HA, Bayomy MF, Mahran AM, Abdeen AM, El-Elaimy EA (2002) Chronic diazinon exposure: pathologies of spleen, thymus, blood cells, and lymph nodes are modulated by dietary protein or lipid in the mouse. Toxicology 172: 13–34

IFA (Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung) (Institute for Occupational Safety and Health of the German Social Accidence Insurance) (2013) Diazinon, GESTIS databank, www.dguv.de/ifa/de/gestis/stoffdb/index.jsp

Jameson RR, Seidler FJ, Slotkin TA (2007) Nonenzymatic functions of acetylcholinesterase splice variants in the developmental neurotoxicity of organophosphates: chlorpyrifos, chlorpyrifos oxon, and diazinon. Environ Health Perspect 115: 65–70

Kalender S, Ogutcu A, Uzunhisarcikli M, Açikgoz F, Durak D, Ulusoy Y, Kalender Y (2005) Diazinon-induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. Toxicology 211: 197–206

Kalender Y, Uzunhisarcikli M, Ogutcu A, Acikgoz F, Kalender S (2006) Effects of diazinon on pseudocholinesterase activity and haematological indices in rats: The protective role of Vitamin E. Environ Toxicol Pharmacol 22: 46–51

Kappers WA, Edwards RJ, Murray S, Boobis AR (2001) Diazinon is activated by CYP2C19 in human liver. Toxicol Appl Pharmacol 177: 68–76

Kato T, Ogiso T, Kato K, Sano M, Hasegawa R, Shirai T, Ito N (1995) Lack of promoting activity of four pesticides on induction of preneoplastic liver cell foci in rats. Teratog Carcinog Mutagen 15: 251–257

Kimbrough RD, Gaines TB (1968) Effect of organic phosphorus compounds and alkylating agents on the rat fetus. Arch Environ Health 16: 805–808

Király J, Szentesi I, Ruziska M, Czeizel A (1979) Chromosome studies in workers producing organophosphate insecticides. Arch Environ Contam Toxicol 8 : 309–319

Kuroda K, Yamaguchi Y, Endo G (1992) Mitotic toxicity, sister chromatid exchange, and rec assay of pesticides. Arch Environ Contam Toxicol 23: 13–18

Lee CH, Kamijima M, Kim H, Shibata E, Ueyama J, Suzuki T, Takagi K, Saito I, Gotoh M, Hibi H, Naito H, Nakajima T (2007) 8-Hydroxydeoxyguanosine levels in human leukocyte and urine according to exposure to organophosphorus pesticides and paraoxonase 1 genotype. Int Arch Occup Environ Health 80: 217–227

Lisi P, Caraffini S, Assalve D (1987) Irritation and sensitization potential of pesticides. Contact Dermatitis 17: 212–218

Lopez DE, Carrascal E (1987) Sensitivity of human lymphocyte chromosome to diazinon at different times during cell cycle. Bull Environ Contam Toxicol 38: 125–130

Lopez D, Aleixandre C, Merchan M, Carrascal E (1986) In vitro induction of alterations in peripheral blood lymphocytes by different doses of diazinon. Bull Environ Contam Toxicol 37: 517–522

Mankame T, Hokanson R, Fudge R, Chowdhary R, Busbee D (2006 a) Altered gene expression in human cells treated with the insecticide diazinon: correlation with decreased DNA excision repair capacity. Hum Exp Toxicol 25: 57–65

Mankame T, Hokanson R, Fudge R, Chowdhary R, Busbee D (2006 b) Alteration of gene expression in human cells treated with the agricultural chemical diazinon: possible interaction in fetal development. Hum Exp Toxicol 25: 225–233

Maronpot RR, Shimkin MB, Witschi HP, Smith LH, Cline JM (1986) Strain A mouse pulmonary tumor test results for chemicals previously tested in the National Cancer Institute carcinogenicity tests. J Nat Cancer Inst 76: 1101–1112

Matsuoka A, Hayashi M, Ishidate Jr M (1979) Chromosomal aberration tests on 29 chemicals combined with S9 mix in vitro. Mutat Res 66: 277–290

Matsushita T, Aoyama K, Yoshimi K, Fujita Y, Ueda A (1985) Allergic contact dermatitis from organophosphorus insecticides. Ind Health 23: 145–153

McGregor DB, Brown A, Cattanach P, Edwards I, McBride D, Riach C, Caspary WJ (1988) Responses of the L5178Y tk+/tk– mouse lymphoma cell forward mutation assay: III, 72 coded chemicals. Environ Mol Mutagen 12: 85–154

Melis R, Montanelli P, Melis G (1959) Hazards caused by the presence of residual parathion & diazinone in olive oil destined for nutritional use: experimental research in the albino rat (Italian) Ann Sanita Pubbl 20: 5–71

Messarah M, Amamra W, Boumendjel A, Barkat L, Bouasla I, Abdennour C, Boulakoud MS, Feki AE (2013) Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythrocytes. Toxicol Ind Health 29: 77–88

Mink PJ, Adami HO, Trichopoulos D, Britton NL, Mandel JS (2008) Pesticides and prostate cancer: a review of epidemiologic studies with specific agricultural exposure information. Eur J Cancer Prev 17: 97–110

Moriya M, Ohta T, Watanabe K, Miyazawa T, Kato K, Shirasu Y (1983) Further mutagenicity studies on pesticides in bacterial reversion assay systems. Mutat Res 116: 185–216

NCI (National Cancer Institute) (1976) Bioassay of diazinon for possible carcinogenicity. DHEW Publication No. (NIH) 79-1392, NCI, National Institutes of Health, Bethesda, Maryland

Neal BH, Mandella R, Gordon EB (2004) Diazinon: A developmental neurotoxicity study in rats (abstract). Neurotoxicology 25: 721–722

Nishio A, Uyeki EM (1981) Induction of sister chromatid exchanges in chinese hamster ovary cells by organophosphate insecticides and their oxygen analogs. J Toxicol Environ Health 8: 939–946

Okamura A, Kamijima M, Ohtani K, Yamanoshita O, Nakamura D, Ito Y, Miyata M, Ueyama J, Suzuki T, Imai R, Takagi K, Nakajima T (2009) Broken sperm, cytoplasmic droplets and reduced sperm motility are principal markers of decreased sperm quality due to organophosphorus pesticides in rats. J Occup Health 51: 478–487

Oostingh GJ, Wichmann G, Schmittner M, Lehmann I, Duschl A (2009) The cytotoxic effects of the organophosphates chlorpyrifos and diazinon differ from their immunomodulating effects. J Immunotoxicol 6: 136–145

Pahwa P, Karunanayake CP, Dosman JA, Spinelli JJ, McLaughlin JR; Cross-Canada Group (2011) Soft-tissue sarcoma and pesticides exposure in men: results of a Canadian case-control study. J Occup Environ Med 53: 1279–1286

Pastor S, Gutiérrez S, Creus A, Cebulska-Wasilewska A, Marcos R (2001) Micronuclei in peripheral blood lymphocytes and buccal epithelial cells of Polish farmers exposed to pesticides. Mutat Res 495: 147–156

Poet TS, Wu H, Kousba AA, Timchalk C (2003) In vitro rat hepatic and intestinal metabolism of the organophosphate pesticides chlorpyrifos and diazinon. Toxicol Sci 72: 193–200

Poet TS, Kousba AA, Dennison SL, Timchalk C (2004) Physiologically based pharmacokinetic/pharmacodynamic model for the organophosphorus pesticide diazinon. Neurotoxicology 25: 1013–1030

Richter ED, Kowalski M, Leventhal A, Grauer F, Marzouk J, Brenner S, Shkolnik I, Lerman S, Zahavi H, Bashari A, Peretz A, Kaplanski H, Gruener N, Ishai PB (1992) Illness and excretion of organophosphate metabolites four months after household pest extermination. Arch Environ Health 47: 135–138

Robens JF (1969) Teratologic studies of carbaryl, diazinon, norea, disulfiram, and thiram in small laboratory animals. Toxicol Appl Pharmacol 15: 152–163

Roegge CS, Timofeeva OA, Seidler FJ, Slotkin TA, Levin ED (2008) Developmental diazinon neurotoxicity in rats: later effects on emotional response. Brain Res Bull 75: 166–172

Salazar-Arredondo E, de Jesús Solís-Heredia M, Rojas-García E, Hernández-Ochoa I, Quintanilla-Vega B (2008) Sperm chromatin alteration and DNA damage by methyl-parathion, chlorpyrifos and diazinon and their oxon metabolites in human spermatozoa. Reprod Toxicol 25: 455–460

Schechtman LM, Kiss E, McCarvill J, Nis R, Kouri RE, Lubet RA (1987) A method for the amplification of chemically induced transformation in C3H10T1/2 clone 8 cells: Its use as a potential screening assay. J Nat Cancer Inst 79: 487–498

Shirasu Y, Moriya M, Kato K, Furuhashi A, Kada T (1976) Mutagenicity screening of pesticides in the microbial system. Mutat Res 40: 19–430

Slotkin TA, Seidler FJ (2008) Developmental neurotoxicants target neurodifferentiation into the serotonin phenotype: Chlorpyrifos, diazinon, dieldrin and divalent nickel. Toxicol Appl Pharmacol 233: 211–219

Slotkin TA, Seidler FJ (2009 a) Protein kinase C is a target for diverse developmental neurotoxicants: transcriptional responses to chlorpyrifos, diazinon, dieldrin and divalent nickel in PC12 cells. Brain Res 1263: 23–32

Slotkin TA, Seidler FJ (2009 b) Oxidative and excitatory mechanisms of developmental neurotoxicity: transcriptional profiles for chlorpyrifos, diazinon, dieldrin, and divalent nickel in PC12 cells. Environ Health Perspect 117: 587–596

Slotkin TA, Seidler FJ (2012 a) Does mechanism matter? Unrelated neurotoxicants converge on cell cycle and apoptosis during neurodifferentiation. Neurotoxicol Teratol 34: 395–402

Slotkin TA, Seidler FJ (2012 b) Developmental neurotoxicity of organophosphates targets cell cycle and apoptosis, revealed by transcriptional profiles in vivo and in vitro. Neurotoxicol Teratol 34: 232–241

Slotkin TA, Tate CA, Ryde IT, Levin ED, Seidler FJ (2006 a) Organophosphate insecticides target the serotonergic system in developing rat brain regions: disparate effects of diazinon and parathion at doses spanning the threshold for cholinesterase inhibition. Environ Health Perspect 114: 1542–1546

Slotkin TA, Levin ED, Seidler FJ (2006 b) Comparative developmental neurotoxicity of organophosphate insecticides: effects on brain development are separable from systemic toxicity. Environ Health Perspect 114: 746–751

Slotkin TA, Seidler FJ, Fumagalli F (2007) Exposure to organophosphates reduces the expression of neurotrophic factors in neonatal rat brain regions: similarities and differences in the effects of chlorpyrifos and diazinon on the fibroblast growth factor superfamily. Environ Health Perspect 115: 909–916

Slotkin TA, Ryde IT, Levin ED, Seidler FJ (2008 a) Developmental neurotoxicity of low dose diazinon exposure of neonatal rats: effects on serotonin systems in adolescence and adulthood. Brain Res Bull 75: 640–647

Slotkin TA, Bodwell BE, Levin ED, Seidler FJ (2008 b) Neonatal exposure to low doses of diazinon: long-term effects on neural cell development and acetylcholine systems. Environ Health Perspect 116: 340–348

Sobti RC, Krishan A, Pfaffenberger CD (1982) Cytokinetic and cytogenetic effects of some agricultural chemicals on human lymphoid cells in vitro: organophosphates. Mutat Res 102: 89–102

Spyker JM, Avery DL (1977) Neurobehavioral effects of prenatal exposure to the organophosphate Diazinon in mice. J Toxicol Environ Health 3: 989–1002

Swan SH (2006) Semen quality in fertile US men in relation to geographical area and pesticide exposure. Int J Androl 29: 62–68

Syngenta (1974) Reproduction study – G 24480 (Diazinon Techn.), rat segment II (test for teratogenic or embryotoxic effects). Ciba-Geigy Corporation, No. PH 2.632, Ciba-Geigy Corporation, Basel, Schweiz, unpublished report

Syngenta (1985) A teratology study of Diazinon technical in Charles River rats. The Safety Evalutation Facility, Ciba-Geigy Corporation, Toxicology/Pathology Report No. 52-83, Ciba-Geigy Corporation, Greensboro, NC, USA, unpublished

Syngenta (1989) A two generation reproductive study in albino rats, EPA guidelines No. 83-4. Research Department, Ciba-Geigy Corporation, Laboratory study number: 852218, Ciba-Geigy Corporation, Greensboro, NC, USA, unpublished report

Tarone RE, Chu KC, Ward JM (1981) Variability in the rates of some common naturally occurring tumors in Fischer 344 rats and (C57BL/6 N x C3H/HeN)F1 (B6C3F1) mice. J Natl Cancer Inst 66: 1175–1181

Timofeeva OA, Roegge CS, Seidler FJ, Slotkin TA, Levin ED (2008) Persistent cognitive alterations in rats after early postnatal exposure to low doses of the organophosphate pesticide, diazinon. Neurotoxicol Teratol 30: 38–45

Tisch M, Schmezer P, Faulde M, Groh A, Maier H (2002) Genotoxicity studies on permethrin, DEET and diazinon in primary human nasal mucosal cells. Eur Arch Otorhinolaryngol 259: 150–153

Tisch M, Faulde M, Maier H (2007) Genotoxic effects of insecticides in current use on mucosal epithelial cells from human tonsil tissue (German). HNO 55: E15–22

Traul KA, Takayama K, Kachevsky V, Hink RJ, Wolff JS (1981) A rapid in vitro assay for carcinogenicity of chemical substances in mammalian cells utilizing an attachment-independence endpoint. J Appl Toxicol 1: 190–195

Tsitsimpikou C, Tzatzarakis M, Fragkiadaki P, Kovatsi L, Stivaktakis P, Kalogeraki A, Kouretas D, Tsatsakis AM (2012) Histopathological lesions, oxidative stress and genotoxic effects in liver and kidneys following long term exposure of rabbits to diazinon and propoxur. Toxicology 307: 109–114

US EPA (United States Environmental Protection Agency) (1999) Memorandum. Subject: Diazinon: Toxicology Chapter for the RED as revised 3/30/00 in response to the Novartis Crop Protection, Inc. responses submitted February 9, 2000 to the RED. US EPA, Washington, DC, USA

US EPA (2005) Memorandum. Subject: 057801: Diazinon, Review of the developmental neurotoxicity study. PC Code No.: 057801, DP Barcode No.: D299890, July 19, 2005, US EPA, Washington, DC, USA

US EPA (2006) Reregistration eligibility decision for diazinon. US EPA, Washington, DC, USA, http://www.epa.gov/oppsrrd1/REDs/diazinon_red.pdf

Vatanparast J, Naseh M, Baniasadi M, Haghdoost-Yazdi H (2013) Developmental exposure to chlorpyrifos and diazinon differentially affect passive avoidance performance and nitric oxide synthase-containing neurons in the basolateral complex of the amygdala. Brain Res 1494: 17–27

Wester RC, Sedik L, Melendres J, Logan F, Maibach HI, Russell I (1993) Percutaneous absorption of diazinon in humans. Food Chem Toxicol 31: 569–572

WHO (World Health Organization) (1986) Organophosphorus insectisides: a general introduction. IPCS – Environmental health criteria 63, WHO, Geneva, Switzerland

WHO (1993) Diazinon. Pesticide residues in food. 1993 evaluations. Part II toxicology, WHO, Geneva, Switzerland, http://www.inchem.org/documents/jmpr/jmpmono/v93pr04.htm

Whyatt RM, Rauh V, Barr DB, Camann DE, Andrews HF, Garfinkel R, Hoepner LA, Diaz D, Dietrich J, Reyes A, Tang D, Kinney PL, Perera FP (2004) Prenatal insecticide exposures and birth weight and length among an urban minority cohort. Environ Health Perspect 112: 1125–1132

Whyatt RM, Camann D, Perera FP, Rauh VA, Tang D, Kinney PL, Garfinkel R, Andrews H, Hoepner L, Barr DB (2005) Biomarkers in assessing residential insecticide exposures during pregnancy and effects on fetal growth. Toxicol Appl Pharmacol 206: 246–254

Williams MW, Fuyat HN, Fitzhugh OG (1959) The subacute toxicity of four organic phosphates to dogs. Toxicol Appl Pharmacol 1: 1–7

Win-Shwe TT, Nakajima D, Ahmed S, Fujimaki H (2012) Impairment of novel object recognition in adulthood after neonatal exposure to diazinon. Arch Toxicol 87: 753–162

Wong PK, Wai CC, Liong E (1989) Comparative study on mutagenicities of organophosphorus insecticides in Salmonella. Chemosphere 18: 2413–2422

Wu HX, Evreux-Gros C, Descotes J (1996) Diazinon toxicokinetics, tissue distribution and anticholinesterase activity in the rat. Biomed Environ Sci 9: 359–369

completed 26.02.2014