The MAK-Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Azinphos-methyl

MAK Value Documentation, supplement – Translation of the German version from 2019

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

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

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

Keywords

azinphos-methyl, neurotoxicity, acetylcholinesterase inhibitor, maximum workplace concentration, MAK value, developmental toxicity, skin absorption, skin sensitization

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated azinphos-methyl [86-50-0] considering all toxicological endpoints. Available publications and unpublished study reports are described in detail. In human studies, the critical effect of acetylcholinesterase (AChE) inhibition was not observed up to 0.25 or 0.29 mg/kg body weight and day in men after oral application up to 28 days. The NOAELs (no observed adverse effect levels) from studies with rodents or dogs are in the same range. Based on these valid data, the MAK value is increased to 1 mg/m^3 for the inhalable fraction. Since a systemic effect is critical, Peak Limitation Category II is retained. As AChE inhibition is almost irreversible with a half-life of 30 hours and a steady-state is reached after 2 weeks, the excursion factor of 8 is confirmed. There is no adequate margin between the NOAELs for developmental and perinatal toxicity in rats and mice and the MAK value. Therefore, azinphos-methyl is assigned to Pregnancy Risk Group B. Starting from the lowest calculated air concentration of 1.18 mg/m^3 as the NAEC for perinatal toxicity and taking into account the absence of teratogenic effects in all studies, damage to the embryo or foetus is unlikely at concentrations of 0.1 mg/m^3 and below. Exposure to this or lower concentrations would be the prerequisite for an assignment to Pregnancy Risk Group C. Azinphos-methyl is neither genotoxic nor carcinogenic. Skin contact is expected to contribute significantly to systemic toxicity and azinphos-methyl remains designated with “H”. Azinphos-methyl has been shown to be skin sensitizing in the guinea pig. The substance is therefore labelled with “Sh”. Biomonitoring is recommended in addition to personal protective measures. A reduction of the erythrocytic AChE activity to 70% of the reference value (biological tolerance value for AChE inhibitors) must not be exceeded.

Citation Note:
Hartwig A, MAK Commission. Azinphos-methyl. MAK Value Documentation, supplement – Translation of the German version from 2019. MAK Collect Occup Health Saf. 2020 Oct;5(3):Doc052.
DOI: 10.34865/mb8650e5_3ad

Manuscript completed:
21 Mar 2018

Publication date:
09 Oct 2020

MAK value (2018)	**1 mg/m³ I (inhalable fraction)**
Peak limitation (2002)	**Category II, excursion factor 8**
Absorption through the skin (1969)	**H**
Sensitization (2018)	**Sh**
Carcinogenicity	**–**
Prenatal toxicity (2018)	**Pregnancy Risk Group B[a)]**
Germ cell mutagenicity	**–**
BAT value (1985)	**reduction of the erythrocyte acetylcholinesterase activity to 70% of the reference value**
CAS number	86-50-0

[a)] for information on the prerequisites for Pregnancy Risk Group C at 0.1 mg/m³, see Section 6

Documentation was published in 1977 (Henschler 1977, available in German only) followed by a supplement on peak limitation in 2002 (Greim 2002, available in German only).

In 2016, the Commission began using a revised approach for assessing substances with a MAK value based on systemic effects and derived from inhalation studies in animals or studies with volunteers at rest; this new approach takes into account that the respiratory volume at the workplace is higher than under experimental conditions. This supplement evaluates whether the MAK value and the pregnancy risk group for azinphos-methyl need to be re-assessed as a result of the higher respiratory volume at the workplace. Numerous studies of other end points have become available since the publication of the 1977 documentation (Henschler 1977); a brief discussion of these can be found in the evaluation by the WHO (2009).

1 Toxic Effects and Mode of Action

Azinphos-methyl induces symptoms of toxicity in animals and humans; as in the case of other organophosphate insecticide compounds, these are caused by the inhibition of acetylcholinesterase activity.

In a valid study in volunteers, neither single oral exposures up to the highest dose tested of 1 mg/kg body weight in men or 0.75 mg/kg body weight in women, nor the exposure of men to oral doses of 0.25 mg/kg body weight and day for 28 days led to signs of cholinergic effects or relevant changes in plasma ChE (cholinesterase) or erythrocyte AChE (acetylcholinesterase) activities. In animal studies with repeated oral exposure of rodents and dogs, the extrapolated no-effect concentrations were of the same magnitude as those for humans; it can therefore be assumed that there are no species-specific differences.

A large number of genotoxicity studies were carried out with azinphos-methyl that did not find evidence of a genotoxic potential. Azinphos-methyl has been evaluated as not carcinogenic on the basis of the carcinogenicity data available from studies in mice and rats.

In developmental toxicity studies of azinphos-methyl in rats, rabbits and mice, no teratogenic effects were observed up to the high doses tested of 5, 6 or 5 mg/kg body weight and day, respectively. Two generation studies in rats found reduced perinatal viability of the pups at doses of 1.48 and 1.54 mg/kg body weight and day, respectively. In a one-generation study, inhibition of the AChE activity in the brain was determined on postnatal day 5 in the pups

at the dose level of 4.87 mg/kg body weight and day. Concurrent maternal toxicity was found in the form of AChE inhibition in the erythrocytes and the brain.

Azinphos-methyl has sensitizing effects on the skin of guinea pigs.

2 Mechanism of Action

Azinphos-methyl is an organophosphate; its underlying mechanism involves the inhibition of AChE. In the organism, it is activated by microsomal liver enzymes which replace double bonded sulfur by oxygen, creating a highly potent inhibitor ("gutoxon"). In cases of acute poisoning, therapeutically administered oximes (for example pralidoxime, obidoxime) have proven to be effective reactivators of inhibited AChE (Henschler 1977).

The first clinically manifest symptoms caused by the inhibition of AChE occur only after the AChE activity has decreased to a level markedly below 50%. Critical levels of toxicity are observed after a decrease in the AChE activity to 20% of the individual norm value. The inhibition of structure-bound AChE at the cholinergic synapses is the only factor responsible for the symptoms of toxicity induced by AChE inhibitors. The acute inhibition of AChE leads to the accumulation of the transmitter in the tissue ("endogenous acetylcholine poisoning") and this in turn to a state of continuous activation. AChE is present not only at the cholinergic synapses in the central nervous system or the motor end plates, but also in erythrocytes. The inhibition of AChE activity in the erythrocytes is a surrogate for the inhibition of the peripheral AChE activity and correlates in a dose-dependent manner with inhibition at the cholinergic synapses. Cholinesterases (ChE; pseudocholinesterases), in contrast, form a group of isoenzymes which are less specific and are ubiquitous in the organism. Their inhibition does not correlate in a dose-dependent manner with that at the cholinergic synapses (Lewalter 1995).

The inhibition of the erythrocyte AChE to 70% or less of its activity prior to exposure is regarded as adverse (WHO 1986). For the reduction of AChE activity in the brain, inhibition to levels of 80% and below is considered to be adverse, as the inhibition of AChE activity in the brain must be regarded more critically from a toxicological standpoint, so that only a lower level of inhibition can be tolerated (Hartwig and MAK Commission 2017).

Studies with chickens given azinphos-methyl either as a single dose of 330 mg/kg body weight or with the diet at dose levels of up to 225 mg/kg body weight and day for 30 days did not find evidence of delayed neurotoxicity (WHO 2009).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Azinphos-methyl is rapidly absorbed by the intestinal tract, the lungs, the external mucous membranes and the skin (Henschler 1977). Oral absorption in rats is estimated to be 90% to 100%, and dermal absorption 19% (Cal EPA 2004).

In a study of dermal absorption, ^{14}C-azinphos-methyl in isopropyl alcohol was applied to the forearms of groups of 6 volunteers at concentrations of 2.6 or 9.2 µg/cm^2 or in an aqueous suspension of Guthion 25 WP (wettable powder formulation containing 25% azinphos-methyl) at a concentration of 4.7 µg/cm^2. The application site was covered for 8 hours by an aluminium dome with air holes. Blood samples were collected for 5 days, the urine and faeces for 13 days. Recovery was 102% to 105% in all groups. Dermal absorption was determined as the sum of the radioactivity in the urine, faeces and tape stripping and was 21.5% for the suspension and 27.8% for 2.6 µg azinphos-methyl/cm^2 in isopropyl alcohol, which facilitated absorption. However, azinphos-methyl is not commonly used at the workplace in a formulation with isopropyl alcohol (Cal EPA 2004). In the worst case, application by spraying

leads to exposure of the entire body for 8 hours. On the basis of the above data with the suspension and assuming a body surface of 18 000 cm^2, it is calculated that 18 mg would be absorbed.

Dermal absorption in rats, rabbits, monkeys and humans correlated highly with the excretion of the metabolite dimethyl thiophosphate (DMTP) in the urine. Dermal absorption was calculated to be 50% to 60% in rats on the basis of the DMTP levels, whereas 100% absorption was determined in both rats and rabbits on the basis of radioactively-labelled azinphos-methyl. Dermal absorption in monkeys was 30% to 40%. Dermal exposure of users who sprayed azinphos-methyl outdoors was determined to be 1.1 to 18.4 mg, depending on the length of application (1–9 hours) and the amount sprayed. A lower level of exposure, 25% of this amount, was determined only for the hands (Franklin et al. 1986). With dermal absorption of 21.5%, about 4 mg of the 18.4 mg of azinphos-methyl is absorbed (Cal EPA 2004).

Dermal exposure levels of up to 69.7 mg/person were determined for the application of azinphos-methyl by spraying (Henschler 1977). With dermal absorption of 21.5%, about 15 mg of the 69.7 mg of azinphos-methyl is absorbed.

After a single oral dose was given to rats (no other details), 99% of the dose was excreted within 48 hours, 54% to 66% of this amount with the urine and 33% to 45% with the faeces (no other details; WHO 2009).

A half-life of 30 hours was determined in volunteers after intravenous injection of 4 µg of radioactively-labelled azinphos-methyl. The total urine was collected for 5 days, wet ashed and C^{14}-labelled CO_2 was determined in a scintillation counter after being trapped in an ethanolamine solution (Feldmann and Maibach 1974; Henschler 1977). In addition to a lack of documentation of the labelling site, this study does not provide the level of radioactivity per amount of substance. The actual substance load applied during the experiments thus remains unclear.

Another study in workers proposed that azinphos-methyl metabolism occurs more rapidly. In this study, the exposure of workers to azinphos-methyl over a 5-day work week was determined on the basis of the presence of the metabolite DMTP in the postshift urine. The results consistently showed marked declines from one day to another, which is not consistent with a half-life of 30 hours (Kraus et al. 1977). However, the authors themselves point out that these were only semi-quantitative determinations, namely for the metabolites DMTP and dimethyl phosphate (DMP; results not reported); for these metabolites recovery was only 25% to 64%.

There are no data available for the half-life of oxidized azinphos-methyl, the critical metabolite.

It has been estimated that after repeated exposure, the steady state is reached after 2 weeks in humans (Cal EPA 2001). Excretion in rats seems to follow a two-compartment model. The elimination half-life of the alpha phase lasted about 10 hours, the beta phase 10 days. The slow elimination phase is attributed to the binding of azinphos-methyl and its metabolites to haemoglobin (Cal EPA 2004).

The erythrocyte AChE and plasma ChE activities, metabolites in the urine and dermal exposure were investigated in 20 workers of a peach plantation who carried out harvesting and tree pruning activities for 21 days during a 44-day period that began 30 days after the trees were treated with azinphos-methyl (2.72 kg/ha). The test readings were carried out on days 1, 2, 3 and 44. Erythrocyte AChE decreased by 7% within the first 3 days and by 19% within 44 days, while the plasma ChE activity was reduced by 9% and 12%, respectively. The average dermal exposure was 346 µg/person during logging activities (n = 6), 10 690 µg/person during thinning activities (n = 4) and 13 600 µg/person during harvesting (n = 10). The average concentrations of alkyl phosphate metabolites in the urine were 1.2, 3.8 and 5 to 7 µmol/day, respectively (Cal EPA 2001).

3.2 Metabolism

Azinphos-methyl was metabolized in a qualitatively similar manner in the mammals investigated (mice, rats, cows) and in humans (Henschler 1977).

The proposed metabolic pathway in rats is depicted in Figure 1. Azinphos-methyl is rapidly metabolized by CYP450 and glutathione transferase in the liver and other tissues, which results in the formation of the azinphos-methyl oxygen analogue gutoxon, mercaptomethylbenzazimide, glutathionyl methylbenzazimide and desmethyl isoazinphos-methyl. Further hydrolysis, methylation and oxidation of mercaptomethylbenzazimide forms benzazimide, methylthiomethylbenzazimide and its corresponding oxidized metabolites. Hydrolysis of glutathionyl methylbenzazimide may result in the formation of cysteinylmethylbenzazimide which is oxidized to its sulfoxide and sulfone (no other details; WHO 2009). DMTP and DMP are also formed. A copy of the original study was not available. Therefore, it cannot be established with certainty whether and which metabolites were isolated from the urine and whether quantification was performed.

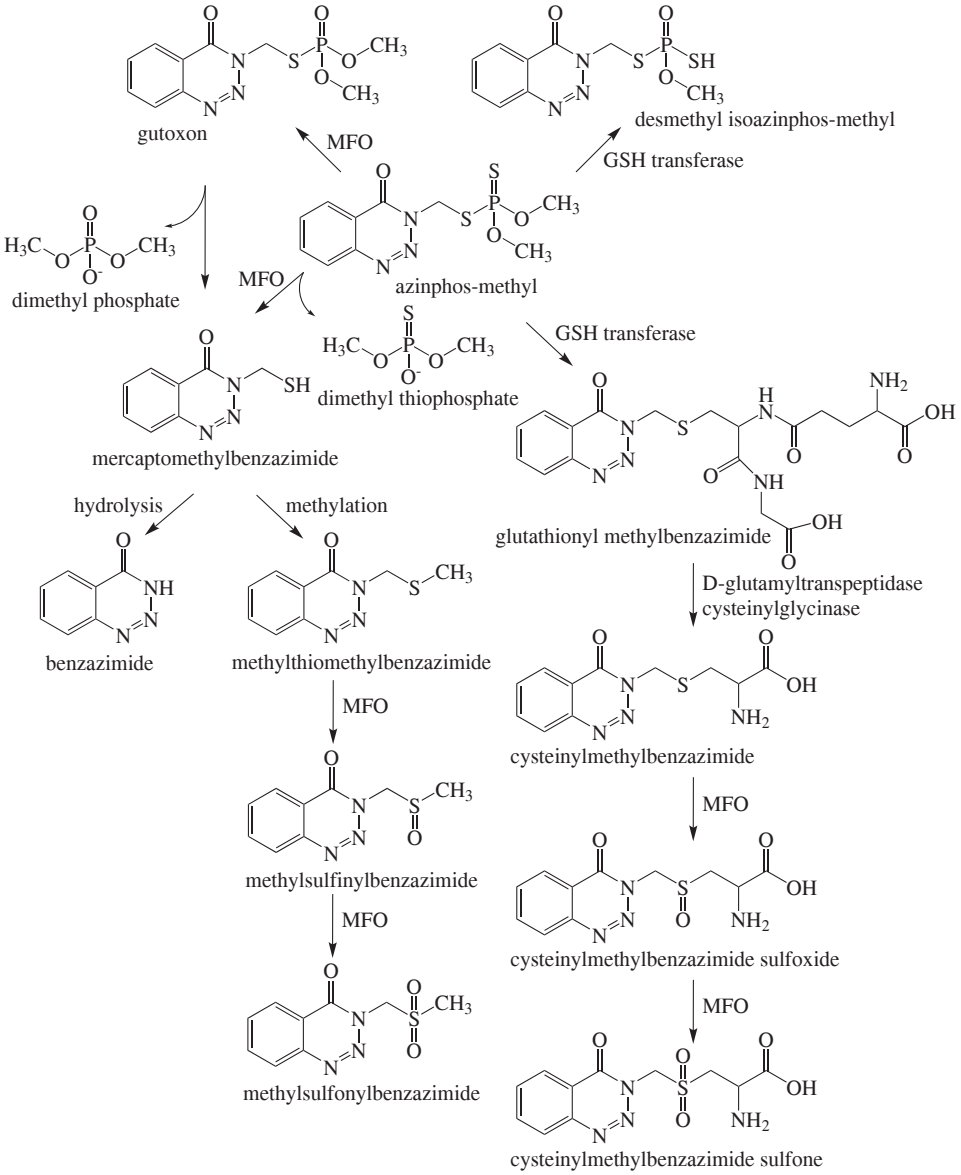

Fig. 1 Postulated metabolism of azinphos-methyl in rats (according to WHO 2009), GSH: glutathione, MFO: mixed function oxidases

4 Effects in Humans

There are no studies of reproductive toxicity, genotoxicity and carcinogenicity.

Single exposures

In a randomized double-blind study carried out according to GLP (Good Laboratory Practice) with increasing single doses of azinphos-methyl (purity 89.2%), a NOAEL for the inhibition of cholinesterase of 1 mg/kg body weight was derived for men and of 0.75 mg/kg body weight for women. The substance was given in capsule form in doses of 0 (placebo), 0.25, 0.5, 0.75 or 1 mg/kg body weight to 40 men in total (32.7 ± 9.3 years, 75.52 ± 8.36 kg body weight) and 0 (placebo) or 0.75 mg/kg body weight to 10 women in total (31.0 ± 4.6 years, 63.83 ± 6.71 kg body weight). The healthy volunteers chosen for the study were examined for 3 weeks before the testing period began. Azinphos-methyl was tolerated well at all doses and no clinically relevant decreases in plasma ChE or erythrocyte AChE activities were observed. The levels were determined after 1, 2, 4, 8, 12, 24, 48 and 72 hours and 7 and 14 days. The AChE activity was reduced by up to 27% (in this case at 0.5 mg/kg body weight) in a few volunteers at certain time points of the 350 total time points determined. A dose-response relationship or time dependency was not established for the reduced activity. Inhibition was on average below 10%. In the placebo groups the maximum inhibition was about 17%, the mean inhibition was lower than 4%. This reflects the inaccuracy of the determinations and the individual fluctuations in AChE activity. Fluctuations in AChE activity were observed also when the initial values for each volunteer were determined over several days. The NOAEL of this study is therefore equivalent to the highest dose tested of 1 mg/kg body weight for men and 0.75 mg/kg body weight for women (Bayer Corporation 1999 a).

Repeated exposure

Inhalation

There are no valid studies available that revealed effects in humans (such as cholinesterase inhibition) following exposure to a calculated concentration in air without contact with the skin (Henschler 1977). Workers of a formulating plant were exposed to dusts both by inhalation and skin contact. The authors reported that the production plant operated under the most basic of conditions. No safety or protective measures for the workers were in place. Azinphos-methyl concentrations of 0.5 to 1.0 mg/m^3 air were determined in the breathing zone and 2.3 mg/cm^2 on the barrel lids. The substance leaked from the containers when it was extracted under pressure from the ethyl compound. Therefore, the inhibition of cholinesterase activity in the blood of the workers was caused mainly by intensive contact with the skin (Henschler 1977; Simpson 1965). As a result of the impurities and the absence of protective measures against contact with the skin, it is not possible to correlate the inhibition of the cholinesterase activity in the blood of the workers with the concentration in air.

Oral administration

Volunteers were given oral azinphos-methyl doses of up to 16 mg daily for 30 days. The plasma ChE and erythrocyte AChE activities were determined before and during treatment. Enzyme activity was not significantly inhibited by exposure to azinphos-methyl (Henschler 1977).

In a study available only in the form of an abstract, groups of 5 volunteers were given capsules containing azinphos-methyl doses of 18 or 20 mg/day (equivalent to 0.26 and 0.29 mg/kg body weight and day, respectively, at 70 kg body weight) daily for 30 days. The plasma ChE or erythrocyte AChE activities were determined twice a week during the study period; no changes were found. No clinical findings of toxicity and no effects on haematological parameters or the prothrombin time were observed. Urinalysis did not reveal any effects induced by the treatment. Therefore, the NOAEL of this study was the highest dose tested of 20 mg/day (about 0.29 mg/kg body weight and day) (Rider et al. 1971).

In a study in healthy volunteers carried out according to GLP, 8 men (29.3 ± 9.6 years, 69.3 ± 5.62 kg body weight) were given gelatine capsules containing azinphos-methyl (purity 89.2%) at a dose level of 0.25 mg/kg body weight and day daily for 28 days. The dose was selected on the basis of the NOAEL of 1 mg/kg body weight and day derived from 13-week neurotoxicity studies with oral administration in rats (Miles Inc 1995; Sheets et al. 1997; see Section 5.2.2). Another 4 healthy volunteers (35.3 ± 9.5 years, 77.7 ± 12.51 kg body weight) were given a placebo. The volunteers remained in the hospital over the entire treatment period and received standard nutrition. The plasma ChE and erythrocyte AChE activity levels had been determined for each volunteer before the beginning of the exposure period (days 1, 2, 4, 6, 8, 10, 12 and 14) in order to calculate the individual fluctuations. During the 28 days of treatment with azinphos-methyl, no treatment-related clinical findings were detected during the physical examination or via vital signs, electrocardiogram, haematology, clinico-biochemical parameters or urinalysis. No changes to plasma ChE and erythrocyte AChE activities were caused by the treatment. The time points of the determinations were directly preceding and 4 hours after administration of the doses on days 1, 2, 3, 4, 5, 7, 10, 14, 17, 21, 24 and 28. The NOAEL of this study was the tested dose of 0.25 mg/kg body weight and day (Bayer Corporation 1999 b).

Summary Oral doses of 0.29 mg/kg body weight and day given to 5 male volunteers for 30 days did not lead to cholinergic signs or the inhibition of plasma ChE or erythrocyte AChE activities (Rider et al. 1971; available only as an abstract). Also in a study carried out according to GLP guidelines, oral doses of 0.25 mg/kg body weight and day given to 8 male volunteers for 28 days did not lead to cholinergic signs or changes in the plasma ChE or erythrocyte AChE activities (Bayer Corporation 1999 b).

Local effects on skin and mucous membranes

A single case was reported of a worker who was diagnosed with generalized dermatosis that was probably caused by contact with azinphos-methyl (WHO 2009).

Allergenic effects

In a patch test, a vintner who had experienced work-related, recurring eczema on the backs of her hands, forearms and face for 1.5 years reacted to 6 of 14 pesticides used in her own vineyards, but not to any of 13 pesticides that were not used. Other findings were papulovesicular reactions to fentichlor and dichlorophen contained in a skin protection ointment and to ethylenediaminetetraacetic acid (EDTA) and papular reactions to tetrachlorophenol and pentachlorophenol. After 24, 48 and 72 hours, the patient exhibited a papular response to 1% azinphos-methyl in petrolatum (Pevny 1980).

Patch tests were carried out in 347 patients from a total collective of 652 patients (of these 274 patients with contact eczema primarily on the hands and 378 patients with non-allergic skin changes) using 1% azinphos-methyl in petrolatum; none of the tests yielded positive reactions. Of the persons tested, 98 currently worked or had worked previously in the agricultural sector. More information was not provided about earlier exposure in the persons tested (Lisi et al. 1987).

There are no reports that azinphos-methyl causes sensitization of the airways.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

Exposure of male rats to azinphos-methyl aerosols by inhalation for 1 or 4 hours yielded LC_{50} values of 385 and 152 mg/m^3, respectively (Henschler 1977).

5.1.2 Oral administration

The oral LD_{50} in mice was 20 mg/kg body weight (Henschler 1977).

LD_{50} values ranging from 4.4 to 26 mg/kg body weight were reported for rats. Like with other organophosphate insecticides, the signs of acute toxicity were diarrhoea, salivation, lacrimation and vomiting (muscarinic effects), muscular tremors and paralysis (nicotinic effects), and restlessness, ataxia and convulsions (central nervous effects). These clinical signs appeared within 5 to 20 minutes after the substance was administered (WHO 2009).

In a study to investigate acute neurotoxicity, groups of 18 male and 18 female F344 rats were given gavage doses of azinphos-methyl (purity 92.8%) of 0, 2, 6 or 12 mg/kg body weight (male animals) and 0, 1, 3 or 6 mg/kg body weight (female animals). In 6 males and 6 females the cholinesterase activity was determined and the remainder were used for neurological tests. Treatment-related findings in the males were urine stains and red nasal stains (at 2 mg/kg body weight and above), muscle fasciculation, oral stains and lower body temperature (at 6 mg/kg body weight and above), tremors and uncoordinated gait (at 12 mg/kg body weight). Findings in the females were oral and urine staining at 6 mg/kg body weight and lower body temperatures. The body weights and brain weights remained unaffected at the end of the study and no effects were observed in the gross-pathological and histopathological examinations. The neurological tests yielded significant findings in both males and females at doses of 6 mg/kg body weight and above (see Table 1); however, there were only 3 remaining females in the high dose group at the time of evaluation, which negatively affected the statistical analysis and the significance of the findings. The plasma ChE and erythrocyte and brain AChE activities are shown in Table 2. In comparison with the control values, these were statistically significantly reduced in the males at doses of 2 mg/kg body weight and above and in the females at doses of 3 mg/kg body weight and above (WHO 2009).

Tab. 1 Findings (same day) yielded by neurological tests after single oral doses of azinphos-methyl in rats (WHO 2009)

Parameters	Dose (mg/kg body weight)					
	2	6	12	1	3	6
	males			females		
number of animals tested	12	12	12	12	12	3
autonomic effects						
lacrimation	0	3	1	0	0	1*
salivation	0	4	4	0	0	1*
neuromuscular effects						
incoordination (home cage)	0	1	3	0	0	1*
incoordination (open field)	0	6*	7*	0	0	1*
repetitive chewing (home cage)	0	3	7*	0	0	1*
repetitive chewing (open field)	0	8*	10*	0	0	1*
posture	0	3*	6*	0	1	1*
minimal movement	1	3	6	0	0	1
central nervous effects						
muscle fasciculation (home cage)	0	8*	12*	0	0	1*
muscle fasciculation (open field)	0	12*	12*	0	0	1*
tremor (home cage)	0	3	9*	0	0	1*
tremor (open field)	0	6*	9*	0	0	1*

*$p \leq 0.05$;
N.B.: only 3 remaining females in the high dose group at the time of evaluation

Tab. 2 Cholinesterase activity after single oral doses of azinphos-methyl in rats (WHO 2009)

Dose (mg/kg body weight)	Cholinesterase activity (% inhibition relative to the control values)		
	plasma	erythrocytes	brain
males			
2	32*	33*	15
6	57*	67*	74*
12 (4 animals died on the day of treatment)	50*	63*	88*
females			
1	11	17	5
3	36*	65*	51*
6 (all animals died on the day of treatment)	–	–	–

*$p \leq 0.05$

5.1.3 Dermal application

There are no data available.

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Only one study is available that investigated the toxic effects induced by repeated exposure by inhalation; this was briefly described in the 1977 documentation (Henschler 1977) and is discussed in more detail below.

Male and female SPF Wistar rats were exposed to azinphos-methyl aerosol at concentrations of 0, 0.195, 1.24 and 4.72 mg/m^3 for 6 hours a day, on 5 days a week, for 3 months. Azinphos-methyl was dissolved in a 1:1 mixture of ethanol and polyethylene glycol 400 and the solution was nebulized; 97% of the drops had a diameter of 1 ± 0.5 µm. The animals underwent whole-body exposure in dynamic inhalation chambers. No changes in appearance and behaviour were observed up to the high concentration. A significant reduction in body weight was found only in the males exposed to 4.72 mg/m^3. Exposure did not have an effect on the absolute and relative organ weights (thyroid gland, thymus, heart, lungs, liver, spleen, kidneys, adrenal glands, gonads). No organ damage was determined in the histological examination. The haematological examination and urinalysis did not yield substance-related findings. Also the serum enzyme activities (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase) and the urea, creatinine and bilirubin values remained unchanged. At the highest exposure concentration of 4.72 mg/m^3, relevant inhibition of the plasma ChE and erythrocyte AChE activities in the males and females was observed as of exposure week 4 (see Table 3). The AChE activity in the brain of rats remained unchanged up to the highest concentration tested (Kimmerle 1976). The NOAEC (no observed adverse effect concentration) of this study was 1.24 mg/m^3. However, the study does not meet today's criteria as regards the scope of the examination. As the inhalation study was carried out with whole-body exposure, additional oral and dermal exposure is to be assumed. For this reason, a MAK value cannot be derived from this study.

Tab. 3 Cholinesterase activity after exposure of rats to azinphos-methyl by inhalation for 3 months (Kimmerle 1976)

Week of examination	Azinphos-methyl concentration (mg/m³)							
	0		0.195		1.24		4.72	
	cholinesterase activity (µ equivalent of acetylcholine)							
	plasma	erythrocytes	plasma	erythrocytes	plasma	erythrocytes	plasma	erythrocytes
					males			
initial value	2.36	3.80	2.26	3.81	2.30	3.32	2.39	3.52
2	2.20	3.77	2.27	3.98	2.39	3.51	1.93[a)]	2.83
4	3.26	3.64	3.12	3.90	3.09	3.36	2.96	1.90[a)]
6	2.32	3.73	2.38	3.74	2.38	3.14	2.26	2.39[a)]
8	2.37	3.73	2.31	3.66	2.49	3.26	1.92[a)]	2.54[a)]
10	2.34	4.08	2.32	3.91	2.28	3.49	1.96[a)]	2.89[a)]
12	2.46	4.04	2.41	3.99	2.44	3.36	2.07	2.27[a)]
					females			
initial value	3.30	3.50	3.32	3.40	3.28	3.27	3.35	3.34
2	3.32	3.44	3.35	3.65	3.31	3.55	3.09[a)]	2.83
4	4.24	3.06	4.20	3.21	4.14	3.06	3.08[a)]	1.90[a)]
6	3.96	3.93	3.96	3.79	3.86	3.83	3.30	2.39[a)]
8	3.60	3.63	3.65	3.85	3.58	3.62	3.93[a)]	2.54[a)]
10	4.00	3.88	4.04	4.05	3.98	4.02	3.58	2.89[a)]
12	4.05	3.55	4.02	3.56	4.00	3.97	3.45	2.27[a)]

[a)] greater than 20% inhibition

5.2.2 Oral administration

The WHO (2009) included an in-depth discussion of the studies with oral administration that are relevant to the evaluation. No new studies have since been published. An overview of the studies is shown in Table 4.

Tab. 4 Studies of repeated toxicity after oral administration of azinphos-methyl

Species, strain, number per group	Exposure	Findings	References
rat, Wistar, 5 ♂, 5 ♀	**28 days**, 0, 5, 20, 50 mg/kg in the diet, ♂: 0, 0.35, 1.3, 3.37 mg/kg body weight and day, ♀: 0, 0.46, 1.54, 3.96 mg/kg body weight and day, purity 93.3%, dose-finding study	**1.3/1.54 mg/kg body weight**: **NOAEL** ♂, ♀; **1.54 mg/kg body weight and above**: ♀: erythrocyte AChE activity ↓ (17%–22%, not adverse[a)]), **3.96 mg/kg body weight**: ♀: brain AChE activity ↓ (> 30%), plasma ChE activity ↓ (> 30%)	Bayer AG 1983

Tab. 4 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, Fischer, 18 ♂, 18 ♀ of these 12 ♂, 12 ♀ for behavioural tests, of these 6 ♂, 6 ♀ for neuropathological examination, 6 ♂, 6 ♀ for the determination of ChE activity	**13 weeks**, ♂: 0, 15, 45, 120 mg/kg in the diet (0, 0.91, 2.81, 7.87 mg/kg body weight and day), ♀: 0, 15, 45, 90 mg/kg in the diet (0, 1.05, 3.23, 6.99 mg/kg body weight and day), purity 92.2%, neurotoxicity study	**0.91/1.05 mg/kg body weight (LOAEL) and above**: ♂/♀: erythrocyte AChE ↓ (> 30%); **3.23 mg/kg body weight and above**: ♂/♀: brain AChE and plasma ChE activity ↓ (> 30%), ♀: perianal stains, red-coloured lacrimation, increased reactivity, uncoordinated gait, tremor; **7.87/6.99 mg/kg body weight**: ♂/♀: body weights ↓ (9% to 10%), feed consumption ↓, ♂: perianal stains, red-coloured lacrimation, increased reactivity, uncoordinated gait, tremor	Miles Inc 1995; Sheets et al. 1997
rat, CD, 10 ♂, 10 ♀	**13 weeks**, 0, 0.215, 0.86, 3.44 mg/kg body weight and day, gavage, converted to pure azinphos-methyl: 0, 0.2, 0.8 and 3.2 mg/kg body weight and day, respectively, purity 93%	**0.2 mg/kg body weight**: **NOAEL**; **0.8 mg/kg body weight and above**: ♂/♀: salivation, ♀: erythrocyte AChE activity ↓ (29%); **3.2 mg/kg body weight**: ♂/♀: brain AChE and plasma ChE activity ↓ (> 30%)	MCW Ltd 1987
rat, Wistar, 60 ♂, 60 ♀, of these 10 ♂, 10 ♀ for interim necropsy	**2 years**, 0, 5, 15, 45 mg/kg in the diet, ♂: 0, 0.25, 0.75, 2.33 mg/kg body weight and day, ♀: 0, 0.31, 0.96, 3.11 mg/kg body weight and day, purity 87.2%	**0.75/0.96 mg/kg body weight and day**: **NOAEL**; **2.33/3.11 mg/kg body weight**: ♂/♀: plasma ChE activity ↓ (> 30%), erythrocyte and brain AChE activity ↓ (> 30%), ♂: body weight gains ↓ (7%)	Bayer AG 1987 c
mouse, CD1, 50 ♂, 50 ♀	**2 years**, 0, 5, 20, 40 mg/kg in the diet, ♂: 0, 0.79, 3.49, 11.33 mg/kg body weight and day, ♀: 0, 0.98, 4.12, 14.30 mg/kg body weight and day, high dose of 80 mg/kg reduced to 40 mg/kg after 1 week because of severe toxicity, purity 88.6%	**0.79/0.98 mg/kg body weight**: **NOAEL**; **3.49/4.12 mg/kg body weight and above**: ♂/♀: erythrocyte AChE activity ↓ (> 30%), ♂: plasma ChE activity ↓ (> 30%); **11.33/14.30 mg/kg body weight and above**: ♂/♀: brain AChE activity ↓ (> 30%), ♀: plasma ChE activity ↓ (> 30%)	Mobay Chemical Corp 1985
dog, beagle, 4 ♂, 4 ♀, according to OECD Test Guideline 452	**52 weeks**, 0, 5, 25, 125 mg/kg in the diet, ♂: 0, 0.15, 0.69, 3.84 mg/kg body weight and day, ♀: 0, 0.16, 0.78, 4.33 mg/kg body weight and day, purity 91.9%	**0.15/0.16 mg/kg body weight**: **NOAEL**; **0.69/0.78 mg/kg body weight and above**: ♂/♀: erythrocyte AChE activity ↓ (> 30%), ♀: plasma ChE activity ↓ (> 30%); **3.84/4.333 mg/kg body weight and above**: diarrhoea, body weights ↓ (2 ♂ animals), ♂/♀: brain AChE activity ↓ (significant, but only > 20%), liver *N*-demethylase ↑; ♂: plasma ChE activity ↓ (> 30%), cytochrome P450 enzyme activity slightly ↑, albumin levels ↓	Bayer AG 1990 a

[a] The current BAT value was established as a reduction in the erythrocyte acetylcholinesterase activity to 70% of the reference value (Lewalter 1995), which means that inhibition of erythrocyte acetylcholinesterase activity is considered adverse only when it reaches a level of 30% or higher.

Rat

In a **28-day** dose-finding study that investigated the most sensitive end point, the inhibition of cholinesterase activity, groups of 5 male and 5 female Wistar rats were exposed to azinphos-methyl (purity 93.3%) with the diet. The concentrations in the feed were 0, 5, 20 or 50 mg/kg (according to the authors, equivalent to doses of 0, 0.35, 1.3 or 3.37 mg/kg body weight and day, respectively, in the males, and 0, 0.46, 1.54 or 3.96 mg/kg body weight and day, respectively, in the females). No effects on outward appearance, behaviour, mortality, body weight gains, feed consumption and gross-pathological findings were observed. The erythrocyte AChE activity was unaffected in the

males of the medium dose group and significantly reduced in the females (17% to 22%). In the females of the high dose group, a statistically significant reduction in the plasma ChE activity of 44% to 61% compared with the levels in the control animals was observed. In the males of the high dose group, inhibition was markedly lower over the entire study period; however, in contrast to the females, this effect could already be observed in the medium dose group. Also, the reduction in brain AChE activity was significant only in the females of the high dose group (see Table 5; Bayer AG 1983). As the erythrocyte AChE activity was not adversely reduced (up to 22%) in the females of the medium dose group, a NOAEL of 1.3 mg/kg body weight and day was established for the males and of 1.54 mg/kg body weight and day for the females.

Tab. 5 Cholinesterase activity in rats after oral exposure to azinphos-methyl for 28 days (Bayer AG 1983; WHO 2009)

Tissue/day of examination	Azinphos-methyl (mg/kg body weight and day)					
	0.35	1.3	3.37	0.46	1.54	3.96
	cholinesterase activity (% inhibition relative to control values)					
	males			females		
plasma						
1	0	8	10	0	0	15
4	0	21	25	0	0	44*
14	0	21	33*	0	0	53**
28	0	26*	26*	0	0	61**
erythrocytes						
1	0	0	0	0	0	0
4	0	0	0	0	+15[a)]	0
14	0	0	22**	0	17*	34**
28	0	0	14**	0	22**	35**
brain						
28	0	0	9	0	0	53**

*$p < 0.05$; **$p < 0.01$
[a)] activity was increased

In a **13-week** neurotoxicity study, groups of 18 male and 18 female Fischer rats were given azinphos-methyl (purity 92.2%) with the diet; concentrations of 0, 15, 45 or 120 mg/kg feed were given to the males (according to the authors, doses of 0, 0.91, 2.81 or 7.87 mg/kg body weight and day, respectively) and concentrations of 0, 15, 45 or 90 mg/kg feed were given to the females (according to the authors, doses of 0, 1.05, 3.23 or 6.99 mg/kg body weight and day, respectively). Twelve rats from each group were used for behavioural tests and 6 of these underwent neuropathological examination. The remaining 6 animals were tested for cholinesterase activity. Mortality was not observed. The treatment-related clinical signs or neurotoxic symptoms found in the males of the high dose group and in the females of the medium dose group and above were perianal stains, red-coloured lacrimation, increased reactivity, uncoordinated gait and tremor. The symptoms grew stronger with increasing exposure, but no evidence of cumulative toxicity was observed beyond week 4. Body weights (9% to 10%) and feed consumption were reduced in the high dose group. The erythrocyte AChE activity was inhibited by more than 30% in all treated animals and inhibition of the brain AChE and plasma ChE activities was found at the medium dose and above (see Table 6). The gross-pathological examination, brain weights, ophthalmology and histopathological examination of the neural tissue and skeletal muscle did not reveal substance-related findings. Cholinergic signs were observed only after inhibition of the AChE activity in the brain by more than 70% or in the erythrocytes by 65% to 80%. This was observed at doses of 3.2 mg/kg body weight and day and above. The NOAEL for the inhibition of AChE in the brain was 0.91 and 1.05 mg/kg body weight and day, respectively; a NOAEL could not be established for the inhibition of

AChE in the erythrocytes (Miles Inc 1995; Sheets et al. 1997). The dose chosen for the clinical trial performed by Bayer Corporation (1999 b) was based on this study (see Section 4.2.2).

Tab. 6 Cholinesterase activity in rats after oral exposure to azinphos-methyl for 13 weeks (Miles Inc 1995; Sheets et al. 1997; WHO 2009)

Tissue/week of examination	Azinphos-methyl (mg/kg body weight and day)					
	0.91	2.81	7.87	1.05	3.23	6.99
	cholinesterase activity (% inhibition relative to control values)					
	males			females		
plasma						
4	7	42*	75*	14*	59*	83*
13	15*	44*	69*	13	60*	81*
erythrocytes						
4	37*	88*	98*	41*	88*	91*
13	37*	84*	95*	38*	78*	95*
brain						
13	8*	46*	82*	16*	72*	85*

*$p < 0.05$

Groups of 10 male and 10 female CD rats were given gavage doses of azinphos-methyl (purity 93%) of 0, 0.215, 0.86 or 3.44 mg/kg body weight and day for **13 weeks**. Mortality did not occur. Salivation was observed in the males treated with the medium dose and above beginning in week 3; up to 80% of the animals were affected after 8 weeks. Salivation was observed only sporadically in the females; however, this effect was observed in 2 animals of the medium dose group in week 7 and in 40% of the animals of the high dose group in week 9. There were no substance-related findings in the ophthalmoscopic examination, or for body weight gains, and feed and water consumption. The plasma butyryl ChE and plasma AChE activities and the erythrocyte and brain AChE activities were significantly inhibited at all doses. The inhibition of the erythrocyte AChE activity was critical at 29% in the females of the medium dose group; this inhibition was markedly higher in the high dose group (see Table 7) as was the inhibition of the brain AChE activity. Substance-related effects on the organ weights were not observed, and the histopathological examination did not yield any findings. This study established a NOAEL of 0.2 mg/kg body weight and day (MCW Ltd 1987). The erythrocyte AChE activity was inhibited by 29% in the females of the 0.86 mg/kg group. The level of inhibition that is considered critical is 30% (see Section 2); the actual LOAEL (lowest observed adverse effect level) is therefore probably only a little over this dose.

Tab. 7 Cholinesterase activity in rats after oral exposure to azinphos-methyl for 13 weeks (MCW Ltd 1987; WHO 2009)

Tissue	Azinphos-methyl (mg/kg body weight and day)					
	0.215	0.86	3.44	0.215	0.86	3.44
	acetyl cholinesterase activity (% inhibition relative to control values)					
	males			females		
plasma	18**	14*	42***	+3[a)]	7	49***
erythrocytes	8*	17***	77***	9***	29***	78***
brain	3	9*	67***	4	3	64***

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$
[a)] activity was increased

In a long-term study in Wistar rats, groups of 60 males and 60 females were given azinphos-methyl (purity 87.2%) with the diet for 2 years at concentrations of 0, 5, 15 or 45 mg/kg feed (according to the authors, equivalent to doses of 0, 0.25, 0.75 or 2.33 mg/kg body weight and day, respectively, in the males, and doses of 0, 0.31, 0.96 or 3.11 mg/kg body weight and day, respectively, in the females). At interim necropsy after 1 year, 10 animals per sex and dose group were examined. No clinical signs were observed and the survival of the animals remained unchanged by the treatment. The body weight gains of the males were slightly (7%) reduced in the high dose group in comparison with the values for the controls. No substance-induced findings were determined by haematology or urinalysis. The (A)ChE activities in the erythrocytes, plasma and brain were markedly reduced in the high dose group in both sexes in comparison with the levels in the controls (see Table 8). No substance-induced effects were found in the gross-pathological examination, organ weight analysis and histopathological examination. The NOAEL was 15 mg/kg feed (0.75 and 0.96 mg/kg body weight and day, respectively, in the males and females) on the basis of the inhibition of the AChE activity in the brains of both sexes and the reduced body weight gains in the males of the high dose group (Bayer AG 1987 c).

Tab. 8 Cholinesterase activity in rats after oral exposure to azinphos-methyl for 2 years (Bayer AG 1987 c; WHO 2009)

Tissue/time of examination	Azinphos-methyl (mg/kg body weight and day)					
	0.25	0.75	2.33	0.31	0.96	3.11
	cholinesterase activity (% of control values)					
	males			females		
plasma						
1 month	93	95	62**	88	88	44**
3 months	91	102	60**	88	65**	35**
6 months	88	95	57**	92	71*	34**
1 year	84	87	54**	90	65**	33**
1.5 years	87	90	55**	100	74*	46**
2 years	113	88	51**	102	81	38**
erythrocytes						
1 month	97	84**	76**	105	87	74*
3 months	101	88**	77**	110**	88**	72**
6 months	97	90	80**	109*	86**	77**
1 year	102	82*	73**	101	81**	69**
1.5 years	96	83**	73**	94	80**	73**
2 years	88**	78**	63**	98	84**	71**
brain						
1 year	130**	137**	109	112	90	50**
2 years	117	112	68**	102	79**	45**

*$p < 0.05$; **$p < 0.01$

Mouse

In a long-term study, groups of 50 male and 50 female CD1 mice were given azinphos-methyl (purity 88.6%) with the diet for 2 years at concentrations of 0, 5, 20 or 40/80 mg/kg feed (according to the authors, equivalent to doses of about 0, 0.79, 3.49 or 11.33 mg/kg body weight and day, respectively, in the males and 0, 0.98, 4.12 or 14.30 mg/kg body weight and day, respectively, in the females). The high dose was initially 80 mg/kg, but was reduced to 40 mg/kg feed after 1 week because of severe toxicity. As of this point, no further clinical signs were seen in the animals of

this dose group. Feed consumption and body weight gain were not adversely affected up to the dose of 40 mg/kg. No unusual findings were determined in the haematological examination. The (A)ChE activities in the plasma, erythrocytes and brain remained unchanged in the low dose group in comparison with the levels in the controls. A dose-dependent reduction in the erythrocyte AChE activity in both sexes and in the plasma ChE activity in the males was observed at doses of 20 mg/kg and above. Adverse effects on the AChE activity in the brain were detected in both sexes and on the ChE activity in the plasma of the females at the high dose (see Table 9).

The histopathological examinations did not reveal any substance-related findings. The NOAEL of this study was 5 mg/kg feed (0.79 and 0.98 mg/kg body weight and day, respectively, in the males and females) and was established on the basis of the reduction in AChE activity found at the higher doses (Mobay Chemical Corp 1985).

Tab. 9 Cholinesterase activity in mice after oral exposure to azinphos-methyl for 104 weeks (Mobay Chemical Corp 1985; WHO 2009)

Tissue/time of examination	Azinphos-methyl (mg/kg body weight and day)							
	0	0.79	3.49	11.33	0	0.98	4.12	14.30
	cholinesterase activity[a] (% of control values)							
	males				females			
plasma (µmol/ml per min)								
6 months	3.11	3.33 (107)	2.57 (83)	1.62 (52)	5.76	5.45 (95)	3.08 (53)	1.48 (26)
1 year	3.88	4.81 (124)	2.63 (68)	1.32 (34)	6.51	5.44 (84)	3.27 (50)	1.50 (23)
2 years	4.33	3.95 (91)	2.97 (69)	1.89 (44)	4.98	4.93 (99)	3.86 (89)	1.65 (38)
erythrocytes (µmol/ml per min)								
6 months	1.33	1.11 (84)	0.88 (66)	0.67 (50)	1.16	1.03 (89)	0.67 (58)	0.63 (54)
1 year	1.04	0.99 (95)	0.45 (43)	0.20 (19)	0.87	0.81 (78)	0.39 (45)	0.20 (19)
2 years	0.95	0.80 (84)	0.54 (56)	0.35 (37)	0.79	0.62 (78)	0.40 (51)	0.32 (41)
brain (µmol/g per min)								
2 years	14.7	12.9 (88)	12.3 (84)	5.4 (37)	14.4	13.6 (94)	10.6 (74)	4.7 (33)

[a] no statistical evaluation provided

Dog

In a 52-week study carried out according to OECD Test Guideline 452, groups of 4 male and 4 female beagle dogs were given azinphos-methyl (purity 91.9%) in the diet at concentrations of 0, 5, 25 or 125 mg/kg (according to the authors, equivalent to doses of 0, 0.15, 0.69 or 3.84 mg/kg body weight and day, respectively, in the males and 0, 0.16, 0.78 or 4.33 mg/kg body weight and day, respectively, in the females). In the high dose group, there were more cases of diarrhoea and body weight losses were determined in 2 males; the feed consumption of the animals was not affected adversely. The haematological examination and urinalysis did not yield substance-related findings. The plasma ChE and erythrocyte AChE activities were reduced in the medium dose group and above and the AChE activity in the brain was reduced in the high dose group (see Table 10). In the high dose group, the liver *N*-demethylase activity in both sexes was slightly increased in comparison with that in the controls, and the cytochrome P450 enzyme activity was slightly increased and the albumin levels reduced in the males. Organ weight analyses and the gross pathological and histopathological examinations did not yield substance-related

findings. The NOAEL of this study was 0.15 or 0.16 mg/kg body weight and day and was established on the basis of erythrocyte AChE inhibition (Bayer AG 1990 a).

Tab. 10 Cholinesterase activity in dogs after oral exposure to azinphos-methyl for 52 weeks (Bayer AG 1990 a; WHO 2009)

Tissue/time of examination	Azinphos-methyl (mg/kg body weight and day)					
	0.15	0.69	3.84	0.16	0.78	4.33
	cholinesterase activity (% inhibition relative to control values)					
	males			females		
plasma						
week 1	11	12	37	+18[a)]	14	52*
week 13	13	15	53**	+2[a)]	17	58**
week 26	14	12	58**	+10[a)]	33	57**
week 52	11	12	53*	12	30	53**
erythrocytes						
week 1	+9[a)]	22	66**	11	2	86**
week 13	8	40**	87**	16	43**	92**
week 26	8	32	88**	21	38**	91**
week 52	+5[a)]	27	86**	15	35*	86**
brain						
week 52	1	10	27**	1	1	20*

*p < 0.05; **p < 0.01
[a)] activity was increased

Chicken

In hens given azinphos-methyl with the diet for 30 days, the NOAEL was 18.7 mg/kg body weight and day; blood cholinesterase (no other details) was reduced at 37.5 and 75 mg/kg body weight and day, respectively (to 15% and 27%) (WHO 2009).

Summary: The most sensitive toxic effect in rodents and dogs, also after repeated oral exposure, was the inhibition of (A)ChE activity. Hens were less sensitive. Reduced body weights and neurotoxic clinical signs were observed at higher doses. These were muscarinic effects such as diarrhoea and salivation that correlate with an inhibition of brain AChE activity by more than 80% (WHO 2009).

In summary, the NOAELs for critical levels of AChE inhibition established by long-term studies were in a range from about 0.75 to 0.86 mg/kg body weight and day in rats and about 0.79 to 0.98 mg/kg body weight and day in mice. The NOAEL was 0.16 mg/kg body weight and day in a 1-year study with dogs and was derived on the basis of AChE inhibition in the erythrocytes (WHO 2009).

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

There are no data available.

5.4 Allergenic effects

In a maximization test, 20 male Dunkin Hartley guinea pigs were given intradermal injections of a 1% azinphos-methyl formulation (vehicle: 2% Cremophor EL in physiological saline) for induction. This was followed after a week by open treatment with 10% sodium lauryl sulfate in petrolatum and 1 day later by occlusive treatment with a 12.5% azinphos-methyl formulation (vehicle: see above) for 48 hours. Reactions were observed in 19 of the 20 animals 24 hours after the challenge treatment with the 12.5% formulation. Severe erythema or oedema was found in 9 animals, and in the remaining 10 animals moderate or slight erythema were observed. The reactions were still visible after 48 hours. At this time point, scabbing was observed in 11 animals. Only 1 of 20 animals reacted to the vehicle. In the control group, which was pre-treated with the vehicle and complete Freund's adjuvant, a weak reaction to the test formulation and to the vehicle was observed in 5 of 10 animals in each case. Data for the purity of the test substance was not included in the report; it was probably a technical product with a purity of 92.4% to 92.8% (APVMA 2006).

In a Buehler test, a similar 12.5% formulation of technical azinphos-methyl (purity 92.4% to 92.8%) was used for 3 occlusive induction treatments in 12 male Dunkin Hartley guinea pigs. A 6% formulation was chosen for the challenge treatment and a 0.6% formulation for the second challenge treatment, both in the same vehicle. Slight erythema (grade 1 on a scale with a maximum of 3) and moderate erythema (grade 2) were observed 24 hours after the first challenge treatment in 3 of 12 animals in each case; only 2 weak reactions (grade 1) were produced in the 12 animals of the control group. In the animals with an initial grade 2 reaction, slight erythema (grade 1) remained after 72 hours. The second challenge treatment produced a weak reaction (grade 1) in 1 animal of the pre-treated group and in 2 of the 12 animals of the control group at the 48-hour reading. The reaction in 2 other animals of the 12 control group animals was not included in the evaluation, as the entire area of shaved skin was reddened (APVMA 2006).

In a Buehler test, 3 occlusive applications of a 25% formulation of a batch of technical azinphos-methyl (purity 88.8%) in ethanol/water (1:1) produced low-grade reactions (slight or barely discernible erythema, grade 1) in 6 of 15 male Dunkin Hartley guinea pigs and a moderate reaction in 1 animal (moderate erythema, grade 2) 24 hours after the challenge treatment with the same formulation. The reactions persisted in 6 of the 7 animals up to the second day (5 × grade 1, 1 × grade 2). Weak reactions (grade 1) were detectable also in 2 of 5 controls but only after 24 hours (APVMA 2006).

In another Buehler test, a ready-to-use product containing 19.3% azinphos-methyl (purity not specified) was used in a 50% dilution in physiological saline solution for induction treatment and in a 25% and 12% dilution for the challenge treatment. A second challenge was carried out with the product in a 6% and 1% dilution and readings were taken after 30, 54 and 78 hours. The 25% formulation led to slight and discrete to moderate and confluent erythema in a total of 9 of 20 female Dunkin Hartley guinea pigs; these effects persisted in 8 animals after 78 hours. A positive reaction to the 12% formulation was observed in 5 animals (after 78 hours) and the second challenge treatment with the 6% and 1% test formulations led in both cases to a reaction in 2 of the 20 animals only after 30 hours. No reactions to any of the formulations were observed at any of the readings in the 10 animals of the control group (APVMA 2006).

Another ready-to-use product containing 35% azinphos-methyl yielded a positive result in a Buehler test with 15 male Dunkin Hartley guinea pigs after 3 occlusive applications of a 5% formulation in ethanol/water (1:1). At the readings taken 24 and 48 hours after the challenge treatment using the same formulation, slight (10 animals) or moderate erythema (1 animal) was found in 11 of the 15 animals. No reactions were produced in the 5 control animals (Author not named 1996).

A similar product (azinphos-methyl content: 36%–38%) was tested in a Buehler test with 10 female and 10 male Dunkin Hartley guinea pigs, apparently in undiluted form. Very slight erythema was observed in 2 females and 2 males and slight erythema (grade 1) in 7 animals (3 females, 4 males) 24 hours after the challenge treatment. After 48 hours, the erythema persisted in the 7 animals, while very slight erythema was detected in 3 males and 1 female. No reactions were produced in the 10 control animals at any of the readings (APVMA 2006).

In a modified Buehler test, 500 mg of solid, light brown azinphos-methyl (purity not specified) that was moistened with the vehicle (no other details) was applied for induction treatment, which was carried out on alternating days. As a result of its lethality, the amount of substance applied was halved for the last 4 of the 10 induction treatments and the challenge treatment, which was carried out 2 weeks after the last induction treatment. A reaction was not induced in any of the 6 surviving animals 24 and 48 hours after the challenge treatment (APVMA 2006).

A negative result was reported in a Buehler test carried out in 15 Dunkin Hartley guinea pigs with a ready-to-use product containing 13% azinphos-methyl. Slight erythema (grade 1) was observed in 1 of the pre-treated animals after 48 hours, but not after 24 hours. No information was provided whether the test was carried out with the undiluted product (APVMA 2006).

Summary: The predominantly positive results demonstrate that technical-grade azinphos-methyl has a slight to moderate contact sensitizing potential. However, whether more highly purified grades also have contact sensitizing effects could not be assessed on the basis of the study data.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a 2-generation study (2 litters per generation), groups of 12 male and 24 female Wistar rats were fed diets containing azinphos-methyl (purity 87.2%) at concentrations of 0, 5, 15 or 45 mg/kg feed (according to the authors, doses of 0, 0.33–0.42, 1.02–1.22 and 3.46–7.37 mg/kg body weight and day, respectively, in the males and 0, 0.48–0.67, 1.48–2.02 and 4.84–10.27 mg/kg body weight and day, respectively, in the females). At the high dose, there was a decrease in the fertility of the F0 and F1b dams and the total number of offspring. In the high dose group, the survival index, both up to postnatal day 5 and up to postnatal day 21, was significantly reduced in the F1a, F1b and F2a litters; this effect was equivocal in the medium dose group (see Table 11). Also in the high dose group, there was increased mortality of the dams in the F0 generation and reduced mean survival of the pups in the F1a, F1b and F2a litters on postnatal day 5. As a consequence, only 5 females were available for mating in the F1b generation. In all generations, there was no evidence of treatment-induced macrostructural malformations. Food intake remained unaffected. The clinical effects were reduced body weights in the high dose group and cholinergic signs. The NOAEL for fertility was 15 mg/kg feed (doses of 1.02–1.22 mg/kg body weight and day in the males and 1.48–2.02 mg/kg body weight and day in the females, respectively) (Bayer AG 1987 a).

Tab. 11 Parameters for the offspring of rats of a 2-generation study given azinphos-methyl with the diet (Bayer AG 1987 a)

Parameters	Azinphos-methyl (mg/kg diet)[a]			
	0	5	15	45
F1a				
living/dead offspring at birth (number)	252/1	247/0	204/8	197/9
male/female offspring (%)	52/48	53/47	49/51	52/48
litter size day 0/day 5 (number)	11.5/11.1	11.2/10.5	10.1/8.7*	10.1/3.9**
survival index (%)	96.8	93.9	86.6**	38.7**
offspring on day 5[b]/week 4 (number)	175/169	167/156	139/134	62/17
lactation index (%)	96.6	93.4	96.4	27.4**
body weight on day 0/week 3 (g)	5.8/36.7	5.7/37.5	5.9/35.9	5.4/25.8**
F1b				
living/dead offspring at birth (number)	235/1	236/11	175/1	133/0
male/female offspring (%)	52/48	50/50	51/49	55/45
litter size day 0/day 5 (number)	10.6/10.5	9.8/9.5	9.7/9.7	8.9/2.8**
survival index (%)	98.3	97.3	98.9	31.6**
offspring on day 5[b]/week 4 (number)	165/161	164/162	128/117	39/18
lactation index (%)	97.6	98.8	91.4*	46.2**
body weight on day 0/week 3 (g)	5.7/39.9	5.8/39.2	5.9/37.8	5.2**/27.2**
F2a				
living/dead offspring at birth (number)	295/3	270/0	230/0	43/0
male/female offspring (%)	55/45	55/45	54/46	51/49
litter size day 0/day 5 (number)	11.7/11.5	11.2/10.8	11.0/10.7	8.6*/7.0*
survival index (%)	98.1	95.9	97.8	81.4**
offspring on day 5[b]/week 4 (number)	176/173	185/174	152/134	29/21
lactation index (%)	98.3	94.1	88.7*	72.4**
body weight on day 0/week 3 (g)	5.7/37.3	5.7/35.6	5.7/36.0	5.4/22.4**
F2b				
living/dead offspring at birth (number)	223/1	244/2	214/3	25/0
male/female offspring (%)	51/49	55/45	49/51	56/44
litter size day 0/day 5 (number)	10.6/10.1	11.0/10.0	9.6/8.5	6.2/6.2
survival index (%)	95.5	90.1*	88.6*	100
offspring on day 5[b]/week 4 (number)	143/133	165/138	137/123	22/20
lactation index (%)	93.0	83.6*	89.8	90.9
body weight on day 0/week 3 (g)	5.8/40.2	5.9/39.6	5.6/37.8	5.8/27.0**

[a] 5, 15 or 45 mg/kg feed is equivalent to doses of 0.33–0.42, 1.02–1.22 and 3.46–7.37 mg/kg body weight and day, respectively, in males and of 0.48–0.67, 1.48–2.02 and 4.84–10.27 mg/kg body weight and day, respectively, in females

[b] after reduction

*$p < 0.05$; **$p < 0.01$

Another 1-generation study was carried out to confirm the slight effects on the viability of the pups fed 15 mg/kg with the diet. If possible, it was to be determined whether the effects were attributable to the treatment of the male or female animals and whether they were associated with cholinesterase inhibition. Groups of 18 male and

46 female Wistar rats were given azinphos-methyl (purity: 92%) in the diet at concentrations of 0, 5, 15 or 45 mg/kg (equivalent to doses of 0, 0.43, 1.30 and 3.73 mg/kg body weight and day, respectively, in the males and of 0, 0.55, 1.54 and 4.87 mg/kg body weight and day, respectively, in the females). The treated animals were mated and the pups were reared until postnatal day 28. In addition, treated males were mated with untreated females. In the high dose group, 2 females had to be taken out of the study as a consequence of severe toxicity (cholinergic effects, poor general condition, bloody noses, inertia and a stumbling gait). No clinical signs were detected in the males at the same dose. There were no treatment-related effects on fertility parameters (mating index, fertility index, gestation index and length of gestation). A slight decrease in the survival index up to postnatal day 5 was observed in the pups of the medium dose group that had two treated parents; this was not accompanied by reduced body weights in the offspring. In the high dose group, a significant decrease in the survival index of the offspring was determined on postnatal day 5; this was accompanied by reduced body weights. These effects were not observed after treatment of only the male animals. Examination of the cholinesterase activity of the parents revealed the inhibition of plasma ChE and erythrocyte AChE activity in all treated animals and the inhibition of brain AChE activity in the males of the high dose group and in the females of the medium dose group and above (see Table 12) (Bayer AG 1990 b).

Tab. 12 Cholinesterase activity in the F0 and F1 offspring of rats given azinphos-methyl with the diet (Bayer AG 1990 b)

Azinphos-methyl (mg/kg diet)[a)]	Cholinesterase activity (% inhibition relative to control values)						
	male F0 animals		female F0 animals			F1 pups	
	end of mating	end of treatment	postcoital day 11	postnatal day 5	postnatal day 28	postnatal day 5	postnatal day 28
plasma							
5	2.3	n. d.	n. d.	26*	5	n. d.	n. d.
15	14***	25*	18	46***	39**	n. d.	n. d.
45	43***	62***	60***	66***	63***	n. d.	n. d.
erythrocytes							
5	19**	n. d.	n. d.	25**	47***	n. d.	n. d.
15	69***	46***	52***	75***	84***	n. d.	n. d.
45	94***	71***	81***	91***	89***	n. d.	n. d.
brain							
5	1	10*	n. d.	n. d.	12	n. d.	n. d.
15	n. d.	4	21**	38**	48***	1	14
45	19**	55***	69***	66***	68***	17*	46***

[a)] 5, 15 or 45 mg/kg feed is equivalent to doses of 0.43, 1.30 and 3.73 mg/kg body weight and day, respectively, in males and of 0.55, 1.54 and 4.87 mg/kg body weight, respectively, in females,
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; n. d.: not determined

There were no treatment-related clinical signs observed in the pups at birth or during the 4-week rearing period. Histopathological findings were not observed. In the high dose group, the brain AChE activity in the offspring was reduced by 17% and 46% in comparison with the control levels on postnatal days 5 and 28, respectively (Bayer AG 1990 b). In this study, the NOAEL for the parental toxicity of azinphos-methyl was 5 mg/kg in the diet, which is equivalent to a dose of 0.55 mg/kg body weight and day in the females. At the next-higher dose, erythrocyte AChE activity was inhibited by more than 46% (at the end of pre-treatment) and up to 91.1% (postnatal day 5). The NOAEL for perinatal toxicity was 0.55 mg/kg body weight and day also for the offspring and the NOAEL for decreased AChE activity in the brain was 1.54 mg/kg body weight and day.

Summary: Both the multi-generation studies in rats and other studies with repeated oral administration revealed cholinergic toxicity in the high dose groups and reduced body weights and the inhibition of AChE activities. The NOAEL for parental toxicity was 0.55 mg/kg body weight and day. The NOAEL for perinatal toxicity was 0.55 mg/kg body weight and day and the NOAEL for reduced brain AChE activity in the offspring was 1.54 mg/kg body weight and day.

5.5.2 Developmental toxicity

Rat

Two studies were carried out to investigate the developmental toxicity induced by azinphos-methyl (purity: 90.6%) in CD rats. In the first study, groups of 20 to 22 animals were given oral doses (gavage) of 0, 1.25, 2.5 or 5 mg/kg body weight and day for 10 days, beginning on gestation day 6. In the second study, treatment of the animals continued up to postnatal day 21. The first study did not reveal any anomalies or malformations in the foetuses after examination on gestation day 20. In this study, decreased body weight gains, reduced feed consumption and increased salivation and lacrimation, more frequent urination and tremor were observed in the dams only in the high dose group. In addition, several dams (no other details) exhibited signs of cholinergic effects. The NOAEL for developmental toxicity in rats was 5 mg/kg body weight and day, for maternal toxicity 2.5 mg/kg body weight and day. In contrast, the dams in the second study reacted with greater sensitivity to azinphos-methyl during the final stage of gestation and more marked signs of cholinergic effects, including mortality, were observed in the high dose group given 5 mg/kg body weight and day. This resulted in decreased foetal weights at birth and on postnatal day 4 and reduced survival of the foetuses from birth to postnatal day 4 and during the remaining lactation period (postnatal day 4 to 21); only 1 of 13 litters of this group survived the lactation period. The pups of this group exhibited signs of neuromuscular problems on postnatal day 22 (Short et al. 1980; US EPA 1978).

Groups of 33 CD rats were given gavage doses of azinphos-methyl (purity 87.7%) of 0, 0.5, 1.0 or 2 mg/kg body weight and day in an aqueous Emulphor solution from gestation days 6 to 15. Five animals of each group were examined on gestation day 16, the remaining were reared until gestation day 20. In addition to the standard parameters of a study carried out according to OECD Test Guideline 414, the plasma ChE activity and erythrocyte and brain AChE activities were determined in the parent animals on gestation days 16 and 20 and in the foetuses on gestation day 20. No substance-related changes in appearance, behaviour, feed consumption and body weight gains were observed in the parent animals. On gestation day 16, the plasma ChE activity and the erythrocyte and brain AChE activities were reduced in comparison with the levels in the control animals only in the dams of the high dose group. On gestation day 20, this reduction was statistically significant only in the brains of the dams. The foetal tissue was not examined for this effect on gestation day 16; however, no reduction in the AChE activities was determined in the brains of the foetuses on gestation day 20 (see Table 13). Azinphos-methyl did not cause embryotoxic, foetotoxic or teratogenic effects up to 2 mg/kg body weight and day. The NOAEL for developmental toxicity was therefore 2 mg/kg body weight and day, the NOAEL for maternal toxicity was 1 mg/kg body weight and day (Astroff and Young 1998; Bayer AG 1987 b).

Tab. 13 Cholinesterase activity in rats (and their foetuses) given azinphos-methyl with the diet from gestation days 6 to 15 (Astroff and Young 1998; Bayer AG 1987 a, b)

Tissue	Cholinesterase activity (% of control)		
	azinphos-methyl in mg/kg body weight and day		
	0.5	1.0	2.0
gestation day 16			
plasma	90	95	63
erythrocytes	90	90	21*
brain	107	101	61*
gestation day 20			
plasma	102	97	92
erythrocytes	103	106	77
brain	102	92	72*
foetal brain	91	100	96

*$p \leq 0.05$

In a study carried out according to OECD Test Guideline 414, groups of 22 Sprague Dawley rats were given azinphos-methyl doses of 0, 0.4, 1.2 or 3.6 mg/kg body weight and day (purity 92.7%) by gavage from gestation days 6 to 15. Necropsy of the animals was performed on gestation day 20. No mortality occurred. Feed consumption, body weights and body weight gains were not affected by treatment. The gravid uterus weights, number and sex of the living foetuses and the number of resorptions remained unchanged in comparison with the values for the controls. In the high dose group, increased incidences of delayed ossification of the occipital bone, pubic bone or hyoid bone were observed. In addition, an increase in the incidence (7/149 foetuses compared with 0/147 foetuses in the control group, 4.7%; $p < 0.01$; 7/21 litters compared with 0/21 litters in the control group, 4.9%, $p < 0.01$) of supernumerary ribs (14th ribs) was found in the high dose group, which was outside the historical control range of the test laboratory (0–3.1% of the foetuses). The NOAEL for developmental toxicity was 1.2 mg/kg body weight and day; maternal toxicity was not observed up to the highest dose tested of 3.6 mg/kg body weight and day (MCW Ltd 1988 a). The primary effect induced by azinphos-methyl, AChE inhibition, was not examined. It is possible that the effects observed in the foetuses may have been secondary effects arising from AChE inhibition in the dams. The findings of delayed ossification of the skull and the additional 14th rib in the foetuses cannot be used in the evaluation because AChE inhibition was not examined and no other signs of maternal toxicity were found. The study is not included in the evaluation of developmental toxicity.

Rabbit

In a prenatal developmental toxicity study in Himalayan rabbits, groups of 11 or 12 animals were given azinphos-methyl (purity 92.4%) doses of 0, 0.3, 1.0 or 3,0 mg/kg body weight and day by gavage from gestation days 6 to 18. Delivery by caesarean section was performed on gestation day 29. Neither maternal nor foetotoxic or embryotoxic effects were observed (Bayer AG 1975). AChE activities were not determined.

In another prenatal developmental toxicity study in American Dutch rabbits, groups of 20 animals were given azinphos-methyl doses (purity 87.7%) of 0, 1, 2.5 or 6 mg/kg body weight and day by gavage from gestation days 6 to 18. The plasma ChE and erythrocyte AChE activities were examined on gestation days 19 and 28, the AChE activity in the brain only on gestation day 28. Ataxia was observed in 4 animals of the high dose group, which was accompanied by tremor in 2 of the animals. On gestation day 19, the plasma ChE and erythrocyte AChE activities were reduced at the medium dose and above in comparison with the levels in the control animals. This finding was reversible on day 28; however, the brain AChE activity was reduced in the high dose group. There were no signs of foetotoxic and embryotoxic effects or teratogenicity. According to the authors, the NOEL (no observed effect

level) for maternal toxicity was 1 mg/kg body weight and day on the basis of the induction of the above-mentioned effects at the medium and high doses. The NOAEL for developmental toxicity in rabbits was 6 mg/kg body weight and day (Bayer AG 1988).

A third study of developmental toxicity was carried out in New Zealand White rabbits. Groups of 15 to 18 animals were given azinphos-methyl (purity 92.7%) doses of 0, 1.5, 4.75 or 15 mg/kg body weight and day by gavage from gestation days 7 to 19. The erythrocyte AChE and plasma ChE activities were determined before mating and after 11 days of treatment. In the groups given doses of 0, 1.5, 4.75 or 15 mg/kg body weight and day, 1, 2, 1 and 3 animals, respectively, died: in the high dose group because of incorrect administration in the lungs and in the other animals as a result of an infection (pneumonia or gastroenteritis). There were no treatment-related clinical signs or changes in body weight gains. After 11 days of treatment (gestation day 17), there was a significant 27% decrease in erythrocyte AChE activity in the high dose group. At this time point, the plasma ChE activity was significantly reduced in all treated animals without clear dose dependency (22%, 29% and 26% at 1.5, 4.75 and 15 mg/kg body weight and day, respectively). No treatment-related findings were observed for the corrected uterine weights, the number of living foetuses, the number of resorptions, the placental weights and the crown-rump length. In the high dose group, there was a significant increase in the number of foetuses with a body weight of less than 30 g. In addition, the incidence of reduced ossification of tubular bone epiphyses was increased at 4.75 mg/kg body weight and day and above. Expressed on a foetal basis, effects were observed in 36 of 85 (42.4%) and 54 of 122 (44.3%) foetuses at 4.75 and 15 mg/kg body weight and day, respectively, compared with in 21/117 (17.9%) foetuses in the control group (historical controls: 10.7%–35.9%). Expressed on a litter basis, effects were found in 8 of 12 (40.1%) and 11 of 14 (41.4%) litters at 4.75 and 15 mg/kg body weight and day, respectively, compared with in 10 of 14 (18.7%) litters in the control group (historical controls: 10.3%–31.6%). The NOAEL for developmental toxicity was therefore 1.5 mg/kg body weight and day; concurrent maternal toxicity was observed (MCW Ltd 1988 b). It is difficult to evaluate the toxic effects on development (delays in ossification and the increased number of foetuses with a body weight below 30 g) in view of the infection and the possible secondary influence of AChE inhibition in the dams. The incorrect administration in the high dose group is to be regarded as a methodological shortcoming. For this reason, the study is not included in the evaluation of developmental toxicity.

Mouse

Groups of 22 to 23 CD-1 mice were given oral azinphos-methyl (purity 90.6%) doses of 0, 1.25, 2.5 or 5.0 mg/kg body weight and day for 10 days beginning on gestation day 6. No substance-related increase in variations or malformations was determined after examination on gestation day 18. Signs of toxicity such as increased salivation and lacrimation, more frequent urination and tremor were observed in the dams of the high dose group. The NOAEL for developmental toxicity in mice was thus 5 mg/kg body weight and day and for maternal toxicity 2.5 mg/kg body weight and day (Short et al. 1980; US EPA 1978).

Generation studies

The two generation studies in rats (see Section 5.5.1; Bayer AG 1987 a, 1990 b) observed initial toxic effects on the offspring (decreased survival index on postnatal day 5) and the one-generation study found effects on brain AChE activity in the pups on postnatal day 5 at 1.48 and 1.54 mg/kg body weight and day, respectively. The one-generation study reported concurrent maternal toxicity in the form of inhibition of the ChE activity in the plasma to 46% of the activity levels determined in the controls, and of the AChE activity in the erythrocytes to 75% and in the brain to 38% of the control levels. The NOAEL for perinatal toxicity were 0.48 and 0.55 mg/kg body weight and day, respectively, and the NOAEL for the effects on brain AChE activity in pups was 1.54 mg/kg body weight and day.

Summary The NOAELs for developmental toxicity were 2 mg/kg body weight and day for rats, 6 mg/kg body weight and day for rabbits and 5 mg/kg body weight and day for mice; these were the highest doses tested. The AChE activity in the brain of the dams was not found to be inhibited up to 1 mg/kg body weight and day in rats and up to 2.5 mg/kg body weight and day in rabbits. Corresponding studies of the AChE activity in mice are not available. In a developmental toxicity study in rats with exposure to azinphos-methyl at a dose of 2 mg/kg body

weight and day, the brain AChE activity in the foetuses was not inhibited on gestation day 20. However, the level of inhibition was statistically significant in the dams (Astroff and Young 1998; Bayer AG 1987 b). The NOAELs for effects on brain AChE activity in the foetuses of rats were 2 mg/kg body weight and day in the developmental toxicity study (gestation day 20) and 1.54 mg/kg body weight and day in the one-generation study (postnatal day 5). Reduced viability of the pups was observed on postnatal day 5 in the two generation studies in rats at 1.48 and 1.54 mg/kg body weight and day and above, respectively. NOAELs of 0.48 and 0.55 mg/kg body weight and day, respectively, were thus established for perinatal toxicity in pups up to postnatal day 5.

5.6 Genotoxicity

5.6.1 In vitro

Azinphos-methyl was examined in numerous in vitro test systems (see Table 14).

Tab. 14 Genotoxicity tests with azinphos-methyl in vitro

End point	Test system	Concentration [µg/plate][a)]	Effective concentration[a)]	Cytotoxicity[a)]	Results		References
					– m. a.	+ m. a.	
gene mutation	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	0–160	–	no data	–	–	WHO 2009
	Salmonella typhimurium TA98, TA100, TA1535, TA1537	4–2500	–	no data	–	–	WHO 2009
	Salmonella typhimurium TA98, TA100, TA1535, TA1537	75–9600	–	no data	–	–	WHO 2009
	Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538	33–4000	–	no data	–	–	WHO 2009
	Saccharomyces cerevisiae S138 and S211α	33.3–10 000 µg/ml	–	no data	–	–	WHO 2009
	Saccharomyces cerevisiae D3	no data, highest concentration with maximum cytotoxicity of 50%	no data	no data	+	+	Waters et al. 1982
	Escherichia coli W3110, Escherichia coli p3478	625–10 000	–	no data	–	–	WHO 2009
sister chromatid exchange	V79 cells	5–25 µg/ml	–	no data	–	–	WHO 2009
	V79 cells	2.5–20 µg/ml	–	no data	–	–	WHO 2009

Tab. 14 *(continued)*

End point	Test system	Concentration [µg/plate][a)]	Effective concentration[a)]	Cytotoxicity[a)]	Results		References
					– m. a.	+ m. a.	
DNA repair synthesis	rat hepatocytes	0.25–50.3 µg/ml	–	no data	–	not tested	WHO 2009
gene mutation, $TK^{+/-}$ test	mouse lymphoma cells	tested up to cytotoxicity	no data	no data	–	+[b)]	Waters et al. 1982
chromosomal aberrations	CHO cells (K1)	60–120 µg/ml	60 µg/ml	at 60 µg/ml and above mitotic rate < 50% of the controls, at 80 µg/ml and above arrest in the S phase	+ with cytotoxicity	not tested	Alam et al. 1974
	human lymphocytes	1–100 µg/ml (–m. a.) 5–500 µg/ml (+m. a.)	500 µg/ml (+m. a.)	500 µg/ml (+m. a.)	–	+ with cytotoxicity	Bayer AG 1986

[a)] unless otherwise specified, concentrations in [µg/plate],
[b)] no data whether a distinction was made between large and small colonies
m. a.: metabolic activation

A study used a total of 14 test systems to investigate azinphos-methyl and other pesticides. Azinphos-methyl yielded negative results in 12 tests for mutation or DNA damage. The results of a test for mitotic recombination in Saccharomyces cerevisiae D3 were positive. Five concentrations (no other details) were tested both with and without the addition of a metabolic activation system. For cytotoxic substances, the highest concentration was chosen that produced 50% toxicity. Positive results were obtained in the $TK^{+/-}$ test in mouse lymphoma cells only with the addition of metabolic activation. The results were considered positive if there was a two-fold, concentration-related increase in the mutation frequency. The tests were performed up to cytotoxic concentrations (Waters et al. 1982). It is unclear whether a distinction was made between large and small colonies.

Azinphos-methyl induced chromosomal aberrations in CHO-K1 cells from Chinese hamsters at concentrations of 60 to 120 µg/ml; concentrations of 80 µg/ml and above led to an arrest in the S phase, 60 µg/ml to delayed growth (Alam et al. 1974).

In a chromosomal aberration test, human lymphocytes were exposed to concentrations of up to 100 µg/ml in the absence of S9 mix and up to 500 µg/ml in the presence of S9 mix. Clastogenicity with concurrent cytotoxicity was induced only at the high concentration of 500 µg/ml in the presence of S9 mix (Bayer AG 1986).

The small number of in vitro tests with positive results are at variance with the large number of genotoxicity studies in vivo that yielded negative results (see Table 15).

Tab. 15 Genotoxicity tests with azinphos-methyl in vivo (WHO 2009)

End point	Species	Dose	Result
micronucleus test	mouse	2 × 2.5 or 2 × 5 mg/kg body weight, oral in Cremophor/water	–
		5 mg/kg body weight, single, intraperitoneal in Cremophor/water	–
chromosomal aberration test	rat	6.28 mg/kg body weight, single, oral in methyl cellulose	–
dominant lethal test	mouse	4 mg/kg body weight, single, oral in Cremophor/water	–
		0.125 or 0.25 mg/kg body weight, single, intraperitoneal in corn oil	–

5.6.2 In vivo

Azinphos-methyl yielded negative results in two micronucleus tests in mice, one after intraperitoneal administration and the other after administration of up to 2 oral doses of 5 mg/kg body weight. A chromosomal aberration test in the bone marrow of rats given oral doses of about 6 mg/kg body weight likewise yielded negative results. In addition, two dominant lethal tests were carried out in mice, one with intraperitoneal administration, one with the administration of oral doses of up to 4 mg/kg body weight; both yielded negative results (see Table 15; WHO 2009).

Summary Azinphos-methyl was investigated for genotoxicity in a large number of in vitro and in vivo test systems and is considered not to be genotoxic (WHO 2009).

5.7 Carcinogenicity

Rat

In a carcinogenicity study carried out by the National Cancer Institute (NCI), 50 Osborne Mendel rats per sex and dose group were fed azinphos-methyl (purity 90%) with the diet for 80 weeks. The control group consisted of 10 untreated male and 10 untreated female rats. The animals were then observed for another 34 to 35 weeks. The males were given concentrations of 0, 78 or 156 mg/kg feed; according to the authors, these were equivalent to doses of about 0, 6.25 and 12.5 mg/kg body weight and day, respectively. The females were given concentrations of 0, 62.5 or 125 mg/kg feed (about 0, 3.12 and 6.25 mg/kg body weight and day, respectively). The typical symptoms of organophosphate poisoning were observed in several animals of the high dose group; these included hyperactivity, tremor and dyspnoea. The body weight gains, which were reported only in diagram form, were delayed relative to the control values in both sexes in the high dose group. The number of surviving male animals was 6/10 (60%), 35/50 (70%) and 27/50 (54%) at 0, 6.25 and 12.5 mg/kg body weight and day, respectively, and of surviving female animals 7/10 (70%), 34/50 (68%) and 25/50 (50%) at 0, 3.12 and 6.25 mg/kg body weight and day, respectively. Therefore, a sufficient number of animals survived to allow the investigation of subsequent tumour development. Increased incidences of tumours of the pancreatic islet cells and of the follicular cells of the thyroid gland were observed only in male rats (see Table 16). The increase in tumour incidences was statistically significant compared with the incidences in the laboratory controls. The tumour incidences of the concurrent control animals cannot be included in the evaluation because, at only 10 animals per sex, the number of control animals was much too small. The significant increase in the incidence of pancreatic and thyroid tumours determined by including data from laboratory control animals led the authors to report that the findings suggested, but did not prove, that azinphos-methyl induces carcinogenic effects (NCI 1978). On the basis of this study, the Commission concluded that azinphos-methyl is a substance with a suspected carcinogenic effect, which was, however, not confirmed by other studies.

Tab. 16 Study of the carcinogenicity of azinphos-methyl in rats

Author:	NCI 1978
Substance:	azinphos-methyl (purity 90%)
Species:	**rat**, Osborne Mendel, 50 ♂, 50 ♀ per dose, 10 ♂, 10 ♀ in the control group
Administration route:	diet
Dose:	males: 0, 6.25 or 12.5 mg/kg body weight and day; females: 0, 3.12 or 6.25 mg/kg body weight and day
Duration:	continuous dosing: 80 weeks, observation period: 34–35 weeks
Toxicity:	12.5/6.25 mg/kg body weight: typical symptoms of organophosphate poisoning such as hyperactivity, tremor and dyspnoea, and delayed body weight gains

Tab. 16 *(continued)*

		dose (mg/kg body weight and day) for ♂/♀		
		0	6.25/3.12	12.5/6.25
surviving animals	♂	6/10 (60%)	35/50 (70%)	27/50 (54%)
	♀	7/10 (70%)	34/50 (68%)	25/50 (50%)
tumours				
pancreas:				
islet cell adenomas[a)]	♂	0/9 (0%)	1/47 (2%)	4/45 (9%)
islet cell carcinomas[a)]	♂	0/9 (0%)	0/47 (0%)	2/45 (4%)
thyroid gland:				
cystadenomas[b)]	♂	0/9 (0%)	7/44 (16%)	10/43 (23%)
cystadenocarcinomas[b)]	♂	0/9 (0%)	1/44 (2%)	0/43 (0%)
papillary cystadenocarcinomas[b)]	♂	0/9 (0%)	0/44 (0%)	1/43 (2%)

laboratory control animals (only combined data available):
[a)] pancreas, islet cell adenomas and carcinomas: 2/92 (2%)
[b)] thyroid gland, cystadenomas, cystadenocarcinomas, papillary cystadenocarcinomas: 0/86 (0%)

In a second carcinogenicity study, groups of 60 male and 60 female Wistar rats were given azinphos-methyl (purity 87.2%) with the diet for 2 years (see also Section 5.2.2). The concentrations tested were 0, 5, 15 or 45 mg/kg feed; according to the authors, these were equivalent to doses of 0, 0.25, 0.75 and 2.33 mg/kg body weight and day, respectively, in the males and 0, 0.31, 0.96 and 3.11 mg/kg body weight and day, respectively, in the females. At interim necropsy after 1 year, 10 animals per sex and dose group were examined. No clinical signs were observed and the number of surviving animals remained unchanged by the treatment. The body weight gains of the males in the high dose group were slightly (7%) delayed in comparison with those in the controls. The gross-pathological findings, the organ weights and the histopathological examinations did not yield substance-related findings (Bayer AG 1987 c).

Mouse

In a carcinogenicity study carried out by the National Cancer Institute (NCI), groups of 50 B6C3F1 mice per sex and dose group were given azinphos-methyl (purity 90%) with the diet for 80 weeks. The control group, however, was made up of only 10 animals per sex. The animals were subsequently observed for another 12 to 13 weeks. The males were given concentrations of 0, 31.3 or 62.5 mg/kg feed, equivalent to doses of about 0, 4.7 or 9.4 mg/kg body weight and day, respectively. The females were given 0, 62.5 or 125 mg/kg feed (according to the authors, equivalent to doses of about 0, 9.4 or 18.75 mg/kg body weight and day, respectively). The typical symptoms of organophosphate poisoning were observed in several animals of the high dose group; these included hyperactivity, tremor and dyspnoea. The body weight gains, which were reported only in diagram form (numerical values not provided), were delayed relative to the control values in both sexes in the high dose group. The number of surviving animals was similar in all groups; males: 8/10 (80%), 45/50 (90%) and 42/50 (84%) at 0, 4.7 and 9.4 mg/kg body weight and day, respectively; females: 7/10 (70%), 44/50 (88%) and 42/50 (84%) at 0, 9.4 and 18.75 mg/kg body weight and day, respectively. The incidence of hepatocellular carcinomas was significantly increased in the males of the high dose group (see Table 17) compared with the incidence in the low dose group and the concurrent controls, but not when compared with the incidence in the laboratory controls. At 10 animals per sex, the number of control animals (study controls) was too small for a statistical evaluation. Therefore, additional control animals from the laboratory were included to carry out statistical comparisons (laboratory controls). These animals were the subjects of other experiments concurrently performed at the laboratory. This increased the number of male and female B6C3F1 mice in the control group to 140 and 130 animals, respectively. The animals of the laboratory control group were bred

by the same breeder, were fed the same diet and housed in the same facilities (NCI 1978; WHO 2009). A valid statistical evaluation cannot be carried out because of the insufficient number of concurrent control animals. As male B6C3F1 mice have a high spontaneous incidence of hepatocellular carcinomas, the findings are not considered to be of relevance by the Commission, or by the authors of the study and the WHO (2009).

Tab. 17 Study of the carcinogenicity of azinphos-methyl in mice

Author:		NCI 1978		
Substance:		azinphos-methyl (purity 90%)		
Species:		**mouse**, B6C3F1, 50 ♂, 50 ♀ per dose, 10 ♂, 10 ♀ in the control group		
Administration route:		diet		
Dose:		males: 0, 4.7 or 9.4 mg/kg body weight and day; females: 0, 9.4 or 18.75 mg/kg body weight and day		
Duration:		continuous dosing: 80 weeks, observation period: 12–13 weeks		
Toxicity:		9.4/18.75 mg/kg body weight: typical symptoms of organophosphate poisoning such as hyperactivity, tremor, and dyspnoea, and delayed body weight gains		
		dose (mg/kg body weight and day) for ♂/♀		
		0	4.7/9.4	9.4/18.75
surviving animals	♂	8/10 (80%)	45/50 (90%)	42/50 (84%)
	♀	7/10 (70%)	44/50 (88%)	42/50 (84%)
tumours				
liver:				
hepatocellular adenomas	♂	2/8 (25%)	8/49 (16%)	7/50 (14%)
hepatocellular carcinomas	♂	0/9 (0%)	2/49 (6%)	12/50 (24%)*

*p = 0.006 significant linear trend in the Cochran-Armitage test; laboratory controls 27/128 (21%)

In a second carcinogenicity study, groups of 50 male and 50 female CD1 mice were given azinphos-methyl (purity 88.6%) with the diet for 2 years (see also Section 5.2.2). The concentrations were 0, 5, 20 or 40/80 mg/kg feed (according to the authors, equivalent to doses of about 0, 0.79, 3.49 and 11.33 mg/kg body weight and day, respectively, in the males and 0, 0.98, 4.12 and 14.30 mg/kg body weight and day, respectively, in the females). At the beginning of the study, the high dose group was given 80 mg/kg feed, which was reduced to 40 mg/kg feed after 1 week because of severe toxicity including hyperactivity, tremor, dyspnoea and delayed body weight gains. No further signs of toxicity were observed in the animals of this concentration group with the exception of reduced feed consumption and delayed body weight gains. There was no increase in tumour incidences. The histopathological examinations did not yield substance-related findings (Mobay Chemical Corp 1985).

Summary Two carcinogenicity studies were carried out in mice (Mobay Chemical Corp 1985; NCI 1978) and two in rats (Bayer AG 1987 c; NCI 1978); only one of the carcinogenicity studies in rats (NCI 1978) found an increased incidence of tumours of the endocrine organs. As a result, azinphos-methyl is considered to be, at most, a substance with suspected carcinogenic effects. In view of the study design and the 3 carcinogenicity studies that yielded negative results, the increased tumour incidences are considered to have little relevance.

6 Manifesto (MAK value/classification)

The critical effect is acetylcholinesterase (AChE) inhibition.

MAK value. The most sensitive end point after exposure to azinphos-methyl is the inhibition of AChE activity in humans and animals.

The NOAEC of a 3-month inhalation study in rats was 1.24 mg/m³ (0.62 mg/m³ taking the increased respiratory volume into account). However, the inhalation study was carried out with whole-body exposure, so that additional oral absorption is to be assumed. The NOAEC for exposure by inhalation alone is probably higher.

No effects were observed in 4-week studies in volunteers up to the highest oral dose tested of 0.29 mg/kg body weight and day. The NOAELs from studies with long-term oral administration in rats, mice and dogs resulted in NOAEL values of the same order of magnitude (see Table 18).

Tab. 18 NOAELs in rats, mice, dogs and volunteers from studies with repeated oral exposure that are relevant to the evaluation, and toxicokinetic conversion of the NOAELs to a concentration in air

NOAEL for	NOAEL (mg/kg body weight and day)	Toxicokinetic conversion[a)] of the NOAEL to mg/m³	LOAEL (mg/kg body weight and day)	Toxicokinetic conversion[a)] of the LOAEL to mg/m³	References
rat, 2 years, diet	0.75	1.84	2.33	5.71	Bayer AG 1987 c
mouse, 2 years, diet	0.98	1.37	4.1	5.74	Mobay Chemical Corp 1985
dog, 1 year, oral	0.16	1.12	0.74	5.18	Bayer AG 1990 a
volunteers, 28 days, oral, n = 8, male, GLP study	0.25	1.75	not determined	not determined	Bayer Corporation 1999 b
volunteers, 30 days, oral, n = 5, male, available as an abstract only	0.29	2.03	not determined	not determined	Rider et al. 1971

[a)] taking into account the species-specific correction value, the confirmed oral absorption of 100% and assumed 100% absorption by inhalation, the body weight of 70 kg and the respiratory volume of the person of 10 m³ (see List of MAK and BAT Values)

As valid data are available for humans, these are used for the derivation of the MAK value. A concentration of 1.75 to 2.03 mg/m³ is calculated from the NOAEL for volunteers (0.25 to 0.29 mg/kg body weight and day) taking into account a body weight of 70 kg and a respiratory volume of 10 m³. A MAK value of 1 mg/m³ I (inhalable fraction) has been derived from this taking into consideration the preferred value approach. This MAK value is lower than the concentrations in air calculated from the NOAELs from animal studies with oral exposure.

Peak limitation. The critical effect is AChE inhibition, which is a systemic effect. The substance remains therefore classified in Peak Limitation Category II.

The deactivation of AChE by organophosphates is practically irreversible and lasts for several days. After intravenous injection of volunteers with radioactively-labelled azinphos-methyl, the study of Feldmann and Maibach (1974; see Section 3.1) determined a half-life of 30 hours arising from the almost completely unbreakable bond between the critical azinphos-methyl metabolite and the enzyme. This also explains why the steady state of azinphos-methyl is reached in humans only after 2 weeks (Cal EPA 2001). In spite of the rapid onset of the phosphorylation of the enzyme by the organophosphate, the degree of inhibition is dependent on the cumulative dose and not short-term peak exposures. This supports the excursion factor of 8.

A single oral dose of azinphos-methyl reduced the AChE activity in a small number of volunteers without a dose-response relationship or time dependency. The mean inhibition values were below 10% also after exposure to a dose of 1 mg/kg body weight and below 4% after administration of a placebo (Bayer Corporation 1999 a). This reflects the inaccuracy of the determinations and the individual fluctuations in AChE activity. Assuming not the maximum, but an average inhibition of AChE activity because of the uncertainty of the determinations, adverse AChE inhibition (> 30%) is not to be expected at an excursion factor of 8, which is equivalent to exposure to a concentration of 8 mg/m³ for 15 minutes.

Prenatal toxicity. Both prenatal toxicity and perinatal and postnatal neurotoxic effects resulting from AChE inhibition must be included in the evaluation of the prenatal toxicity induced by azinphos-methyl.

There are no studies of developmental toxicity in humans.

In a developmental toxicity study in rats, the brain AChE activity in the foetuses was not inhibited on gestation day 20. However, at 61% of the control value, inhibition in the dams was statistically significant at an azinphos-methyl dose of 2 mg/kg body weight and day (Astroff and Young 1998; Bayer AG 1987 b). This finding was confirmed also by a 1-generation study in rats (Bayer AG 1990 b). The pups reacted, therefore, with less sensitivity than the dams as regards AChE inhibition.

The NOAELs for rats, rabbits and mice from developmental toxicity and generation studies that are relevant to the evaluation are shown in Table 19.

Tab. 19 NOAELs for rats, mice and rabbits from prenatal developmental toxicity and generation studies that are relevant to the evaluation; toxicokinetic conversion of the NOAELs to a concentration in air and the ratios of the calculated values (NAEC) to the MAK value of 1 mg/m³ I

NOAEL for	NOAEL (mg/kg body weight and day)	Toxicokinetic conversion[a] to mg/m³	Ratio of NAEC to the MAK value of 1 mg/m³ I	References
developmental toxicity	**rat, gavage**			
	5 (highest dose)	8.75	9	Short et al. 1980
	rabbit, gavage			
	6 (highest dose)	17.5	18	Bayer AG 1988
	mouse, gavage			
	5 (highest dose)	5	5	Short et al. 1980
brain AChE activity	**rat**			
of foetuses on gestation day 20	2 (highest dose), **gavage**	3.5	4	Astroff and Young 1998; Bayer AG 1987 b
of pups on postnatal day 5	1.54 (medium dose), **diet**	3.77[b]	4	Bayer AG 1990 b
perinatal toxicity	**rat, diet (generation studies)**			
	0.48 (lowest dose)	1.18[b]	1	Bayer AG 1987 a
	0.55 (lowest dose)	1.35[b]	1	Bayer AG 1990 b

[a] taking into account the species-specific correction value, the confirmed oral absorption of 100% and assumed 100% absorption by inhalation, the body weight of 70 kg and the respiratory volume of the person of 10 m³ (see List of MAK and BAT Values)
[b] additional conversion of 7-day exposure of the animals to the 5-day working week of humans (7/5)

With one exception, the margins between the calculated no-effect concentrations in air for the respective end points (see Table 19) and the MAK value are not sufficiently large. For this reason, azinphos-methyl has been classified in Pregnancy Risk Group B.

Information on the prerequisites for Pregnancy Risk Group C As azinphos-methyl induces developmental toxicity, but not teratogenicity, it can be assumed on the basis of the lowest calculated no-effect concentration in air that prenatal toxicity will not be induced after exposure to a concentration of 0.1 mg/m³ (see Table 19).

Carcinogenicity. Azinphos-methyl is not genotoxic. There are 2 carcinogenicity studies available in mice (Mobay Chemical Corp 1985; NCI 1978) and 1 in rats (Bayer AG 1987 c), none of which revealed a substance-related increase in tumour incidences. A second carcinogenicity study in rats (NCI 1978) demonstrated an increased incidence of tumours in endocrine organs. However, this is not regarded as evidence of carcinogenic effects, but that the substance has, at best, carcinogenic potential. In view of the study design and the 3 carcinogenicity studies that yielded

negative results, the increased tumour incidences are considered to be of little relevance. For this reason, azinphos-methyl has not been classified in a category for carcinogens.

Germ cell mutagenicity. Azinphos-methyl was not found to be mutagenic in vitro in bacteria and in mammalian cells. The positive results obtained by in vitro studies that investigated the clastogenic effects observed with concurrent cytotoxicity have to be considered together with the negative results in studies that investigated the induction of micronuclei in mice or chromosomal aberrations in the bone marrow of rats. In addition, two dominant lethal tests in mice yielded negative results. For this reason, azinphos-methyl has not been classified in a category for germ cell mutagens.

Absorption through the skin. Absorption of 18 mg was calculated from determinations in humans after exposure of the entire body surface to radioactively labelled azinphos-methyl in a commercial formulation for 8 hours. In this case, the standard exposure conditions of 1 hour and a 2000 cm^2 area of the body are not assumed because of its application as an insecticide spray. Under actual application conditions, absorption through the skin can be estimated to be about 4 to 18 mg.

The systemically tolerable dose in humans was 0.25 mg/kg body weight, which is equivalent to an amount of 17.5 mg at a body weight of 70 kg.

On the basis of the study data, it can be assumed that the amount absorbed after application by spraying is more than 25% of the systemic NOAEL for humans after long-term exposure. The substance therefore retains the "H" designation (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Only 1 report concluded that azinphos-methyl has a sensitizing potential and 1 patch test yielded positive results. For this reason, the evaluation of the sensitizing effects on the skin is almost completely based on the findings of studies in guinea pigs, which were, however, not completely conclusive. Nonetheless, the largely positive results demonstrate that technical-grade azinphos-methyl has a slight to moderate contact sensitizing potential and the substance has been designated with "Sh" (for substances which cause sensitization of the skin). As a result of the incomplete characterization of the test substances applied, no conclusions can be drawn from the studies whether more purified grades would lead to contact sensitizing effects. There are no data relating to the induction of sensitizing effects on the airways by azinphos-methyl. For this reason, the substance has not been designated with "Sa" (for substances which cause sensitization of the airways).

References

Alam MT, Corbeil M, Chagnon A, Kasatiya SS (1974) Chromosomal anomalies induced by the organic phosphate pesticide guthion in Chinese hamster cells. Chromosoma 49: 77–86. DOI: 10.1007/bf00284989

APVMA (Australian Pesticides and Veterinary Medicines Authority) (2006) The reconsideration of approvals of the active constituent azinphos-methyl, registrations of products containing azinphos-methyl and their approved labels. Preliminary review findings. Volume 2: Technical Report – Toxicology. APVMA, Canberra. https://apvma.gov.au/sites/default/files/publication/14386-azinphos-methyl-prelim-review-tox.pdf, accessed 16 Mar 2017

Astroff AB, Young AD (1998) The relationship between maternal and fetal effects following maternal organophosphate exposure during gestation in the rat. Toxicol Ind Health 14: 869–889. DOI: 10.1177/074823379801400608

Author not named (1996) Draft assessment report on the active substance azinphos-methyl prepared by the rapporteur Member State Germany in the framework of Council Directive 91/414/EEC, September 1996, Part III. https://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/02_eu_berichte/Azinphos-DAR-Part3.pdf, accessed 16 Mar 2017, document no longer available

Bayer AG (1975) Azinphos-methyl, studies for embryotoxic and teratogenic effects on rabbits following oral administration. Bayer report no. 5455, 3 Jun 1975, Institut für Toxikologie, Bayer AG, Wuppertal, unpublished

Bayer AG (1983) Azinphos-methyl, toxicity study on rats with particular attention to cholinesterase activity (28-day feeding study as a range-finding test for a 2-year study). Bayer report no. 11813, 18 May 1983, Institut für Toxikologie, Bayer AG, Wuppertal, unpublished

Bayer AG (1986) Azinphos-methyl, cytogenetic study with human lymphocyte cultures in vitro to evaluate for harmful effect on chromosomes. Bayer report no. 15145, 20 Oct 1986, Institut für Toxikologie, Bayer AG, Wuppertal, unpublished

Bayer AG (1987 a) Azinphos-methyl, two generation study on rats. Bayer report no. R3956 / T6006415, 10 Mar 1987, Institut für Toxikologie, Bayer AG, Wuppertal, unpublished

Bayer AG (1987 b) A teratology study with azinphos-methyl in the rat. Bayer report no. MTD0043 / toxicology report no. 973, 22 Dec 1987, Institut für Toxikologie, Bayer AG, Wuppertal, unpublished

Bayer AG (1987 c) Azinphos-methyl, study of chronic toxicity and carcinogenicity to Wistar rats (administration in the feed for up to 2 years) in three sections. Bayer report no. 16290 / T2015169, 10 Dec 1987, Institut für Toxikologie, Bayer AG, Wuppertal, unpublished

Bayer AG (1988) A teratology study in the rabbit with azinphos-methyl. Bayer report no. MTD0070 / toxicology report no. 1030, 27 Jun 1988, Institut für Toxikologie, Bayer AG, Wuppertal, unpublished

Bayer AG (1990 a) 52-Week oral toxicity (feeding) study with azinphos-methyl in the dog. RCC Project 204388, Bayer Project T2027698, 31 May 1990, Bayer AG, Wuppertal, unpublished

Bayer AG (1990 b) Azinphos-methyl, investigation of inhibition of cholinesterase activity in plasma, erythrocytes and brain in a 1-generation study. Report no.: 19594, Study no.: T0027362, 8 Oct 1990, Bayer AG, Fachbereich Toxikologie, Wuppertal, unpublished

Bayer Corporation (1999 a) A randomised double blind ascending single oral dose study with azinphos-methyl to determine the no effect level on plasma and RBC cholinesterase activity. ICR Report No. 013219, Addendum 20 Jul 1999, Bayer Corporation, Agriculture Division, South Metcalf, Stilwell, KS, unpublished

Bayer Corporation (1999 b) A randomised double blind placebo controlled study with azinphosmethyl to determine the no effect level on plasma and RBC cholinesterase activity after repeated doses. ICR Report No. 013580, 15 Apr 1999, Bayer Corporation, Agriculture Division, South Metcalf, Stilwell, KS, unpublished

Cal EPA (California Environmental Protection Agency) (2001) Estimation of exposure of persons in California to pesticide products that contain azinphos-methyl. Worker Health and Safety Branch, Department of Pesticide Regulation, California Environmental Protection Agency, 02 Jan 2001. https://www.cdpr.ca.gov/docs/whs/pdf/hs1650.pdf, accessed 16 Aug 2017, document no longer available

Cal EPA (California Environmental Protection Agency) (2004) Azinphos-methyl (Guthion) risk characterization document (revision no. 1). Medical Toxicology and Worker Health and Safety Branches, Department of Pesticide Regulation, California Environmental Protection Agency, 26 Feb 2004. https://www.cdpr.ca.gov/docs/risk/rcd/azmrcdre.pdf, accessed 14 Jul 2016

Feldmann RJ, Maibach HI (1974) Percutaneous penetration of some pesticides and herbicides in man. Toxicol Appl Pharmacol 28: 126–132. DOI: 10.1016/0041-008x(74)90137-9

Franklin CA, Muir NI, Moody RP (1986) The use of biological monitoring in the estimation of exposure during the application of pesticides. Toxicol Letters 33: 127–136. DOI: 10.1016/0378-4274(86)90077-9

Greim H (ed) (2002) Azinphos-methyl. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 34. Lieferung. Wiley-VCH, Weinheim. Also available from DOI: 10.1002/3527600418.mb8650d0034

Hartwig A, MAK Commission (2017) Diazinon. MAK Value Documentation, 2015. MAK Collect Occup Health Saf 2: 1473–1544. DOI: 10.1002/3527600418.mb33341e5917

Henschler D (ed) (1977) Azinphos-methyl. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten, 5. Lieferung. VCH, Weinheim. Also available from DOI: 10.1002/3527600418.mb8650d0005

Kimmerle G (1976) Subchronic inhalation toxicity of azinphos-methyl in rats. Arch Toxicol 35: 83–89. DOI: 10.1007/bf00372761

Kraus JF, Richards DM, Borhani DM, Mull R, Kilgore WW, Winterlein W (1977) Physiological response to organophosphate residues in field workers. Arch Environ Contam Toxicol 5: 471–485. DOI: 10.1007/bf02220926

Lewalter J (1995) Acetylcholinesterase inhibitors. BAT Value Documentation, 1986. In: Lehnert G, Greim H (eds) Biological Exposure Values for Occupational Toxicants and Carcinogens, vol 2. VCH, Weinheim, 15–24. Also available from DOI: 10.1002/3527600418.bb0astrinhe0002

Lisi P, Caraffini S, Assalve D (1987) Irritation and sensitization potential of pesticides. Contact Dermatitis 17: 212–218. DOI: 10.1111/j.1600-0536.1987.tb02715.x

MCW Ltd (Makhteshim Chemical Works Limited) (1987) Cotnion technical: toxicity study by oral (gavage) administration to CD rats for 13 weeks. LSR Report No. 86/MAK057/342, 23 Jan 1987, Makhteshim Chemical Works Limited, Beer-Sheva, unpublished

MCW Ltd (Makhteshim Chemical Works Limited) (1988 a) Cotnion-M, teratogenicity study in the rat. LSR Report No. MAK/124/AZM, 10 Feb 1988, Makhteshim Chemical Works Limited, Beer-Sheva, unpublished

MCW Ltd (Makhteshim Chemical Works Limited) (1988 b) Cotnion-M, teratogenicity study in the rabbit. LSR Report No. MAK/126/AZM, 15 Jun 1988, Makhteshim Chemical Works Limited, Beer-Sheva, unpublished

Miles Inc (1995) A subchronic dietary neurotoxicity screening study with technical grade azinphos-methyl in Fischer 344 rats. Study number 93-472-VJ, 14 Feb 1995, Miles Inc, Kansas City, MO, unpublished

Mobay Chemical Corp (1985) Oncogenicity study of azinphos-methyl in mice. Toxicology report no. 612, Study number 60-271-02, 10 Apr 1985, Mobay Chemical Corporation, Kansas City, MO, unpublished

NCI (National Cancer Institute) (1978) Bioassay of azinphos-methyl for possible carcinogenicity, CAS No. 86-50-0. TR 69. NCI, Bethesda, MD. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr069.pdf, accessed 26 Oct 2016

Pevny I (1980) Pestizid-Allergie. Allergisches Kontaktekzem bei einer Winzerin. Derm Beruf Umwelt 28: 186–189

Rider JA, Swader JI, Puletti EJ (1971) Anticholinesterase toxicity studies with methyl parathion, guthion and phosdrin in human subjects. Fed Proc 30: 443

Sheets LP, Hamilton BF, Sangha GK, Thyssen JH (1997) Subchronic neurotoxicity screening studies with six organophosphate insecticides: an assessment of behavior and morphology relative to cholinesterase inhibition. Fundam Appl Toxicol 35: 101–119. DOI: 10.1006/faat.1996.2269

Short RD, Minor JL, Lee C-C, Chernoff N, Baron RL (1980) Developmental toxicity of Guthion in rats and mice. Arch Toxicol 43: 177–186. DOI: 10.1007/bf00297583

Simpson GR (1965) Exposure to guthion during formulation. Arch Environ Health 10: 53–54. DOI: 10.1080/00039896.1965.10663952

US EPA (US Environmental Protection Agency) (1978) Teratology of Guthion. Midwest Research Institute, Contract No. 68-02-2746; EPA-600/1-78-056, Aug 1978. US EPA, Research Triangle Park, NC. https://nepis.epa.gov/Exe/ZyPDF.cgi/2000ZPD3.PDF?Dockey=2000ZPD3.PDF, accessed 26 Oct 2016

Waters MD, Sandhu SS, Simon VF, Mortelmans KE, Mitchell AA, Jorgenson TA, Jones DCL, Valencia R, Garrett NE (1982) Study of pesticide genotoxicity. In: Fleck RA, Hollaender A (eds) Genetic toxicology – an agricultural perspective. Plenum Press, New York, NY, 275–326. DOI: 10.1007/978-1-4684-4352-3_23

WHO (World Health Organization) (1986) Organophosphorus insecticides: a general introduction. IPCS – Environmental health criteria 63. WHO, Geneva. http://www.inchem.org/documents/ehc/ehc/ehc63.htm, accessed 26 Oct 2016

WHO (World Health Organization) (2009) Azinphos-methyl. In: Pesticide residues in food 2007: evaluations: part 2, toxicological. WHO, Geneva, 139–172. http://apps.who.int/iris/bitstream/10665/44064/1/9789241665230_eng.pdf, accessed 14 Jul 2016