AZOCYCLOTIN

First draft prepared by
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Explanation

Azocyclotin (tri(cyclohexyl)-1*H*-1,2,4-triazole-1-yltin) and cyhexatin (tricyclohexyltin hydroxide) are chemically-related organotin compounds that are used as agricultural acaricides. Azocyclotin breaks down to cyhexatin and 1,2,4-triazole. The systemic toxicological properties of azocyclotin are similar to those of cyhexatin and azocyclotin may also have additional properties attributable to the 1,2,4-triazole that is formed.

Toxicological data on cyhexatin were reviewed by the JMPR in 1970, 1973, 1977, 1978, 1980, 1981, 1988, 1989, 1991 and 1994. Azocyclotin was evaluated by the JMPR in 1974, 1981, 1989 and 1991. The Meeting in 1991 considered that the acceptable daily intake (ADI) for cyhexatin should also cover exposure to azocyclotin. In 1994, an ADI of 0–0.007 mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 0.7 mg/kg bw per day for reduced pup survival and decreased pup body-weight gain during lactation in a multigeneration study in rats.

Azocyclotin and cyhexatin were considered by the present Meeting as part of the Codex Committee on Pesticide Residues periodic review programme.

For cyhexatin, several new studies that complied with good laboratory practice (GLP) were evaluated that had not been previously available, including investigations of absorption, distribution, metabolism and excretion, short-term studies of toxicity, tests for genotoxicity, and a long-term study of combined toxicity/carcinogenicity incorporating a neurotoxicity phase.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats

Groups of three male Sprague-Dawley rats were given [^{113}Sn]azocyclotin in Cremophor as a single oral dose at 8 mg/kg bw by gavage and killed at intervals of 4, 24, 48, 72, 96, 120, 168 or 240 h after treatment. The radiolabel (radioactive tin, ^{113}Sn) appeared to be poorly absorbed, as estimated from excretion plus residues left in the body. Most of the radioactivity (94%) was recovered in the faeces, almost entirely within the first 120 h after dosing and 1% was recovered in the urine. Of the administered radiolabel, 3% was left in the body (including the gastrointestinal tract) after 3 days, and 1% after 10 days. At 4 h after dosing, ^{113}Sn was found mainly in the gastrointestinal tract, with lesser amounts in the lungs and liver. Blood concentrations peaked between 24 and 48 h after dosing, with concentrations of azocyclotin equivalents of between 0.065 and 0.070 ppm. From 72 h after dosing onwards, the kidneys contained the highest concentration of residues, with azocyclotin equivalents reaching 0.67 ppm at 72 h and 0.18 ppm at 240 h (Grubenbecher & Figge, 1979a).

Groups of two male Sprague-Dawley rats were given [^{14}C]azocyclotin (radiolabelled on the 1-carbon of the cyclohexyl rings) as a single dose at 8 mg/kg bw by oral gavage and were killed at 4, 24, 48, 72, 96, 120, 168 or 240 h after treatment. An additional group received [^{14}C]azocyclotin at 1 mg/kg bw and was killed 48 h after dosing. One rat was given an oral dose of unlabelled azocyclotin and acted as a control. Concentrations of ^{14}C were measured in the faeces, urine, blood, gastrointestinal tract, liver, kidneys, muscle, fat, brain, lungs and testes.

After 48 h, 77–80% of the administered radioactivity was recovered in the faeces, 9–12% in the urine, 4% in the gastrointestinal tract and 3.4% in the rest of the body. Blood concentrations of radioactivity were highest at the first time-point (4 h), with the concentration of azocyclotin equivalents being 0.22 ppm. The liver was the organ containing the highest amount of radioactivity at all time-points (azocyclotin equivalents, 1.2 ppm at 4 h), apart from 24 h when the kidney contained the highest concentrations (azocyclotin equivalents, 1.14 ppm). At 240 h, concentrations had depleted to 0.22, 0.11 and 0.22 ppm in blood, liver and kidneys, respectively. Levels in other tissues were low at all times (Grubenbecher & Figge, 1979b)

In a study using whole-body autoradiography, five Sprague-Dawley rats were given [^{14}C]azocyclotin as a single dose at 8 mg/kg bw by oral gavage. Two rats were killed at 4 and 24 h after dosing and the remaining rat was killed at 48 h. At 4 h after treatment, most of the radioactivity was found in the gastrointestinal tract, with small amounts in the liver. At 24 and 48 h, radioactivity was seen evenly in most parts of the body, with higher concentrations seen only in the gastrointestinal tract, liver and kidneys (Glubenbecher & Figge, 1979b).

Three Sprague-Dawley rats were given [1-^{14}C]cyclohexyl-labelled azocyclotin as an oral dose at 10 mg/kg bw. The carbon dioxide (CO_2) produced by these animals over the following 48 h was trapped and examined for radioactivity. No $^{14}CO_2$ was detected during the first 40 h. However, in the following 8 h, 0.39–0.48% of the administered radioactivity was detected in the

trapped CO_2. The authors noted that $^{14}CO_2$ could have been derived either from exhaled air or from bacterial decomposition of the faeces. However, the levels found were low, so it was clear that there was little elimination of radioactivity in exhaled air (Grubenbecher & Figge, 1979b).

A series of experiments were performed on groups of Sprague-Dawley rats given [cyclohexyl-^{14}C]-labelled azocyclotin dissolved in Cremophor by oral gavage. Two male rats were given a dose of 10 mg/kg bw per day. Only 0.12% and 0.04% of the administered dose of radiolabel given to each rat was detected in exhaled air within the 24 h after dosing. Other groups of four male and four female rats were given [^{14}C]azocyclotin as a single dose at either 0.7 or 10 mg/kg bw, or 14 doses of unlabelled azocyclotin followed by [^{14}C]azocyclotin as a single dose at 0.7 mg/kg bw. The absorption and excretion of [^{14}C]azocyclotin was similar in all groups and in both sexes. At 144 h after dosing, 84–97% of the administered dose was recovered in the faeces, urine and body tissues. Of the administered radioactivity, 7.3–10.89% was in the urine, 71.79–82.98% in the faeces and 1.80–3.04% in the body tissues. Most of the residual radioactivity was in the carcass (1.26–2.78%), gastrointestinal tract (0.14–0.34%) and liver (0.06–0.22%). The authors suspected that the large amount of radiolabel in the faeces was attributable to poor absorption from the gut (Grubenbecher et al., 1985).

1.2 Biotransformation

A study of hydrolysis was performed at 20 °C using [1,2,4-triazole-3,5-^{14}C]azocyclotin and [cyclohexyl-UL-^{14}C]azocyclotin at a concentration of 25 or 32 mg/l in buffered aqueous solutions (pH 4, pH 7 and pH 9) or drinking-water (pH 7.6). Analyses were performed by liquid scintillation counting, high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and mass spectroscopy (MS) at sampling times of 10, 30 and 60 min. It was shown that azocyclotin was rapidly and completely hydrolysed to cyhexatin and 1,2,4-triazole. Complete hydrolysis was achieved within 10 min. Similar results were obtained at all pHs tested (Scholz, 1998).

Samples of urine and faeces that had been collected in the study in rats (Grubenbecher et al., 1985; described in section 1.1) were extracted in methanol and analysed by one- and two-dimensional thin-layer chromatography using a variety of solvent systems. The chromatographic behaviour of the radiolabelled compounds in the samples was compared with that of a small selection of reference compounds (some postulated metabolites of azocyclotin: tricyclohexyltin hydroxide (cyhexatin), dicyclohexyltin oxide and monocyclohexylstannonic acid). About 50% of the faecal radioactivity was extracted into methanol. Two major metabolites were separated out from the methanol extracts of faecal samples, representing 12–25% of the total radioactivity in the faecal extract. One of the peaks appeared to be azocyclotin and/or cyhexatin (these two compounds were indistinguishable). The other major metabolite was less polar, but was not identified. A small amount (5–9%) of dicyclohexyltin oxide was detected in faeces from rats given [^{14}C]azocyclotin at 0.7 mg/kg bw per day, but not in those at 10 mg/kg bw per day. Monocyclohexylstannonic acid was detected (11–14% of total radioactivity) only in faeces from the rats at 10 mg/kg bw per day. At least five other unidentified polar metabolites were separated from faecal samples, one of which constituted 12% of the radioactivity in the faecal extracts from the group at 10 mg/kg bw per day, but was not found in the faeces from the group at 0.7 mg/kg bw per day. Urine from rats at 0.7 mg/kg bw per day contained only a trace amount of azocylotin and/or cyhexatin. However, in female rats at 10 mg/kg bw per day, these materials made up about 23% of the radioactivity in the urine extracts. Monocyclohexylstannonic acid was a major metabolite in the urine, making up 18–32% of the total radioactivity. Various other unidentified metabolites separated out, but none represented more than 3% of the recovered radioactivity (Holloway et al., 1986).

Biotransformation data from studies performed on cyhexatin (see chapter on cyhexatin, this volume) are relevant to azocyclotin as cyhexatin is a major metabolite of azocyclotin. A proposed metabolic pathway for azocyclotin is given in Figure 1.

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity are summarized in Table 1. The more recent studies were performed in accordance with good laboratory practice (GLP). The conduct of the experiments by van Huygevoot (2002a and 2002b) was in line with OECD guideline 423 (OECD, 2001), that by Bomann (1991) conformed to OECD guideline 401 (OECD, 1987), and those by Thyssen & Kimmerle (1974) were performed to in-house protocols.

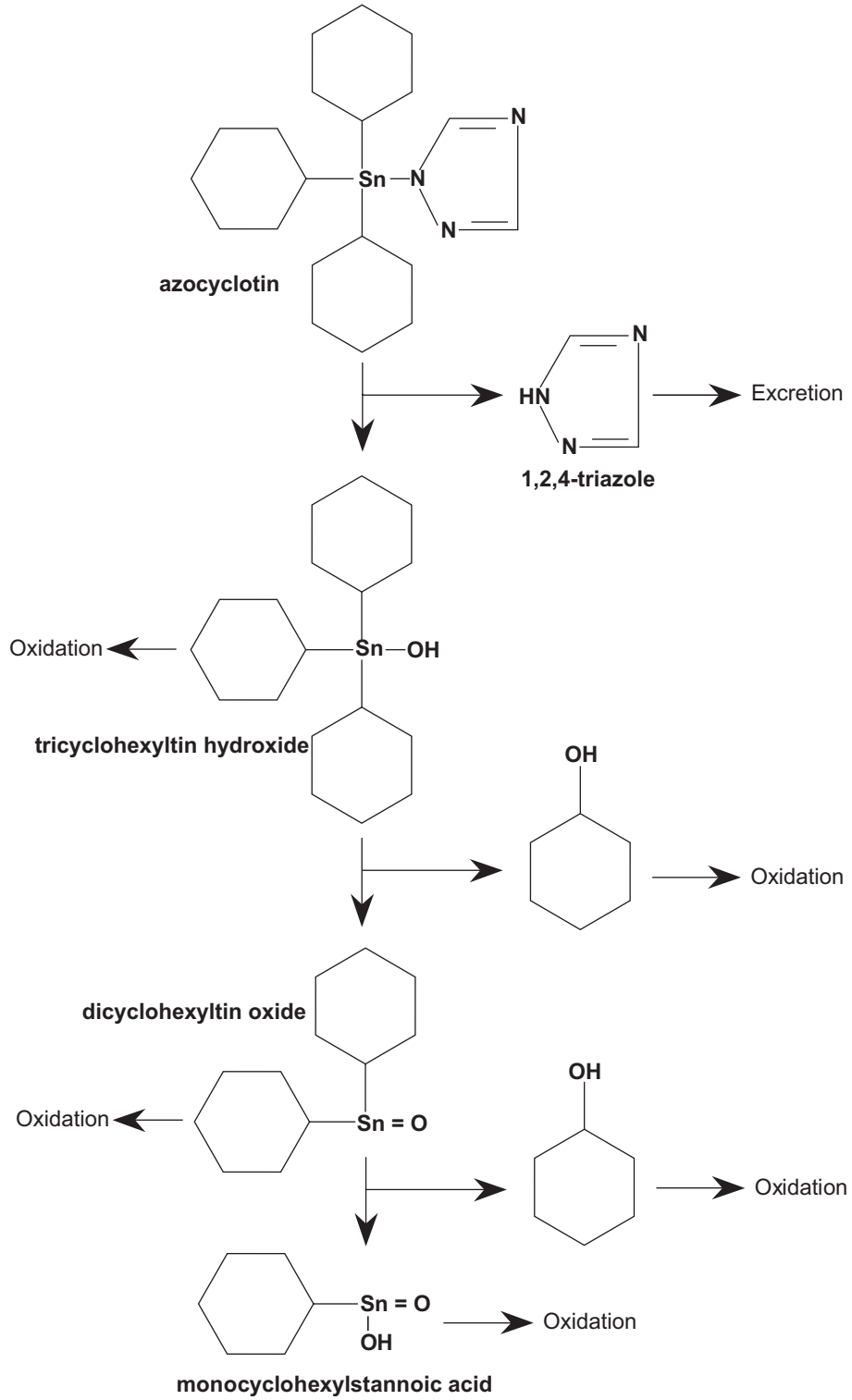
Azocyclotin was of moderate toxicity when administered by the oral route, low toxicity by the dermal route and of high toxicity when given by inhalation. In studies using administration by the oral route and by inhalation, the acute toxicity of azocyclotin was characterized by diarrhoea, apathy, spastic gait, laboured breathing, piloerection, increased salivation, emaciation, increased water intake, increased urinary output, reduced motility, staggering gait and a bloody muzzle. Postmortem examination of rats that were killed by oral doses of azocyclotin showed red discolouration of the stomach, thymus and caecum, and haemorrhaging in the eyes (van Huygevoort, 2002a).

Table 1. Acute toxicity of azocyclotin

Species	Strain	Route	Purity (%)	LD_{50} (mg/kg bw) or LC_{50} (mg/l)	Reference
Rat	Sprague-Dawley	Oral	94.5	Males, 209 Females, 363	Bomann (1991)
Rat	Wistar	Oral	NS	200–2000 (males & females)	van Huygevoort (2002a)
Rat	Wistar	Dermal	NS	> 2000 (males & females)	van Huygevoort (2002b)
Mouse (male)	NMRI	Inhalation (4-h exposure)	Approx. 95	0.035	Thyssen & Kimmerle (1974)
Rat	Wistar	Inhalation (4-h exposure)	Approx. 95	Males, 0.017 Females, 0.029	Thyssen & Kimmerle (1974)
Golden hamster (male)	NS	Inhalation (4-h exposure)	Approx. 95	0.0055	Thyssen & Kimmerle (1974)

Approx., approximately; NS, not specified

Figure 1. Proposed metabolic pathway of azocyclotin in rats



(b) Dermal and ocular irritation and dermal sensitization

Azocyclotin of unspecified purity was tested for irritancy to the skin of New Zealand White rabbits. The study conformed to GLP and was performed in accordance with OECD guideline 404 (OECD, 1992). Three rabbits were each given 0.5 g of azocyclotin moistened with 50% solution of ethanol in water, which was applied to the clipped dorsal skin and kept under occlusive dressing for 4 h. Skin reactions were assessed at 1, 24, 48 and 72 h and 7, 14 and 21 days after the removal of the dressing.

Immediately after treatment there was necrosis, severe erythyema and severe oedema to the skin at the application sites. The reactions healed over the 7 days following treatment. A bald area and scaliness of the skin were noted at 14 and 21 days and one animal showed scarring. The presence of scar tissue was taken as evidence of full thickness destruction of the skin (i.e. corrosion). It was concluded that azocyclotin was corrosive to rabbit skin (van Huygevoort, 2002c).

As azocyclotin had been shown to be corrosive to skin, it was not necessary to perform a study of irritancy in eyes. It is reasonable to assume that azocyclotin will also be corrosive to eyes.

The potential for skin sensitization in a Magnusson and Kligman maximization test was investigated in 20 female Dunkin-Hartley guinea-pigs. A control group of 10 animals was kept for comparison. The study conformed to GLP and was performed in accordance with OECD guideline 406 (OECD, 2002). The results of the study indicated that azocyclotin did not produce skin sensitization (van Huygevoort, 2002d).

(c) Studies of toxicity with repeated doses

Rats

In a 30-day study of oral toxicity, groups of 15 male and 15 female Wistar rats were given azocyclotin of "pure technical grade" (purity not specified) at a dose of 0, 0.2, 2 or 20 mg/kg bw per day. A quality assurance certificate was not provided. Samples of blood and urine were taken for analysis only at the end of the study. All animals were necropsied and a wide range of tissues and organs were examined microscopically. One male and two females at the highest dose died during the study. The general condition of animals in this group at the highest dose was poor from the second week of treatment and several of the rats in this group suffered breathing difficulties. Urine analysis revealed no adverse effects. Body-weight gain was decreased in the males at the highest dose. Total leukocyte counts were decreased in both sexes given the highest dose. Serum alkaline phosphatase activity was increased in both sexes at the highest dose. Several organ weights were changed at the highest dose: thymus weight was decreased and liver weight was increased in both sexes and heart, lung and kidney weights were decreased in males only. Relative weights of kidneys and brain were decreased in females at 2 mg/kg bw per day. All of the effects mentioned above were statistically significant ($p < 0.01$) when compared with concurrent control values. Necropsies revealed no treatment-related gross pathology and light microscopy revealed no histopathology.

The Meeting agreed that the NOAEL for this study was 2 mg/kg bw per day on the basis of miscellaneous adverse effects that were seen at a dose of 20 mg/kg bw per day. The statistically significant decreases in relative organ weights that were seen in females at this dose were not considered to be treatment-related effects (Thyssen & Luckhaus, 1974b).

In a 90-day feeding study, groups of 15 male and 15 female rats were given diets containing azocyclotin (purity, 95.4%) at a concentration of 5, 15, 50 or 150 mg/kg. From these diets, the

male rats received average doses of 0.41, 1.24, 3.99 and 12.67 mg/kg bw per day and the females received doses of 0, 0.48, 1.40, 4.62 and 14.06 mg/kg bw per day. Thirty rats of each sex were used in a control group that was given diet without any azocyclotin. The report of the study did not include a quality assurance certificate. Blood and urine were taken for analysis at 6 weeks and at the end of the study. All animals were necropsied and a wide range of tissues and organs were examined microscopically. One male rat in the group at the highest dose died prematurely, but apart from this there was not mortality. The only clinical sign seen was mild drowsiness of the rats at the highest dose. Mean body weights were significantly lower ($p < 0.05$) in rats of each sex in the groups at 50 or 150 ppm than in concurrent controls, and food consumption was also lower. There were no treatment-related effects on haematology, clinical chemistry, urine analysis, gross pathology or histopathology. The mean absolute weights of several organs (thymus, heart, liver, spleen, kidneys, adrenal, pancreas and brain) of animals at 50 and 150 ppm were lower than those of control animals, but the relative weights were not affected. This appeared to be a consequence of the lower final body weights of the animals in these treatment groups.

The NOAEL was 15 ppm (equal to 1.24 mg/kg bw per day) on the basis of decreased body-weight gain at 50 ppm (3.99 mg/kg bw per day) (Löser & Luckhaus, 1975).

In a 90-day feeding study that complied with GLP, groups of 20 male and 20 female Wistar rats were fed diets containing azocyclotin (purity, 94%) at a concentration of 0, 15, 50 or 150 ppm. These dietary concentrations provided mean doses of 0, 0.85, 2.86 and 8.73 mg/kg bw per day for males and 0, 0.94, 3.11 and 8.29 mg/kg bw per day for females. Blood and urine were collected for analysis at the end of the study. All animals were necropsied and a wide range of tissues and organs were examined microscopically. One female at 15 ppm died prematurely, but apart from this there was no mortality. At intermittent times early in the study and consistently from week 7 onwards, mean body weights were lower in groups at 50 or 150 ppm than in concurrent controls. Food and water intakes were reduced in animals at the highest dose. Haematology showed decreased counts of total leukocytes and of lymphocytes in males at 150 ppm and in females at 50 or 150 ppm. There was also a small decrease in mean corpuscular volume in the males at 150 ppm. There were statistically significant changes in at least some clinical chemistry parameters at all doses, but in most cases the absence of a dose–response relationship or unusually low control values indicated that the effects at doses of up to 50 ppm were not treatment-related. Some of the clinical chemistry changes did, however, appear to be treatment-related: serum alkaline phosphatase activity and blood urea nitrogen were elevated in both sexes at the highest dose; serum glutamic oxalic transferase activity was elevated in highest dose males and in females at 50 or 150 ppm; glutamic pyruvic transaminase activity was elevated in females at the highest dose. Serum gamma-glutamyl transferase activity was decreased at 50 and 150 ppm in males and at 15 and 50 ppm in females, but it was increased at 150 ppm in females. The toxicological relevance of these findings is not known (gamma-glutamyl transferase is not normally measured in studies in rats). There were no treatment-related effects on urine analysis, gross pathology, organ weights or histopathology. The elevated serum activities of alkaline phosphatase, glutamic oxalic transferase activity and glutamic pyruvic transaminase activity suggested that some hepatotoxicity might have occurred, but this was not confirmed by gross pathology or histopathology results.

The NOAEL was 15 ppm (0.85 mg/kg bw per day) on the basis of the decreased body-weight gain and clinical chemistry changes seen at 50 ppm (2.86 mg/kg bw per day) (Hirano et al., 1981).

In a non-GLP study, groups of five male and five female Wistar rats were exposed by nose only to concentrations of 0, 0.0901, 0.275 or 0.961 mg/m^3 of a mist of azocyclotin (purity, about 95%) dissolved in a 1 : 1 mixture of ethanol and polyethylene glycol. The ethanol/polyethanol glycol contained 0.05% “oil red” (the precise identity of this colourant was not clear) as a marker. The rats were exposed for 6 h per day, 5 days per week, for 3 weeks. The aerosol droplets were all

of diameter < 5 μm and 90% of the droplets were between 0.5 and 1.5 μm. At the end of the exposure period, blood and urine were taken from each animal, which were all killed for necropsy.

One animal in the group receiving the highest dose died after the seventh exposure. Rats in the group receiving the highest dose had impaired breathing after the second week and appeared to have impaired general health. Body-weight gain, haematology and clinical chemistry were not affected by the treatments. No treatment-related gross pathology or histopathology was noted, but some organ weights were statistically significantly ($p < 0.05$) affected at the highest dose, with increased lung weight (absolute and relative) in both sexes and decreased thymus weight (absolute and relative) in females.

The no-observed-adverse-effect concentration (NOAEC) was 0.275 mg/m^3 (Kimmerle, 1974).

In a non-GLP study, groups of three male and three female New Zealand White rabbits had azocyclotin (purity, 86.2%) at concentrations equivalent to doses of 0, 5 or 25 mg/kg bw per day in Cremophor applied to clipped and abraded skin for 7 h per day, 5 days per week, for 3 weeks. Separate groups of three rabbits of each sex had the same doses applied to clipped but unabraded skin for the same times. At the end of the exposure period, blood and urine were taken from each animal, which were all killed for necropsy. There was no treatment-related mortality. At the highest dose, the rabbits with intact skin had retarded body-weight gain and the body weights of those with abraded skin decreased over the treatment period. Severe skin damage was seen at the application sites for all treated animals. There were no treatment-related effects on haematology, clinical chemistry, urine analysis or organ weights and gross pathology and histopathology showed only local effects to the skin. It was concluded that azocyclotin was corrosive to skin at all doses tested, but that no systemic toxicity was caused by doses of up to 25 mg/kg bw per day applied to the skin (Thyssen & Kaliner, 1978).

Dog

In a 90-day feeding study, groups of four male and four female beagle dogs were fed diets containing azocyclotin (purity, 95.4%) at a concentration of 0, 5, 50 or 500 ppm. These dietary concentrations provided mean doses of 0, 0.16, 1.76 and 18.30 mg/kg bw per day to males and 0, 0.18, 1.73 and 16.95 mg/kg bw per day to females. Ophthalmoscopy was performed at the start and finish of the treatment period. Blood and urine samples were taken for analysis at week 7 and at terminal kill. All animals were necropsied and a wide range of tissues and organs were examined microscopically. At 50 and 500 ppm, males and females showed reduced food intake, diarrhoea and vomiting with a dose-related severity. Body-weight gain was significantly decreased in a dose-related manner in both sexes. Some males at 500 ppm had mild anaemia at the end of the study (decreases in erythrocyte count, erythrocyte volume fraction and haemoglobin), but otherwise there was no effect on haematological parameters. The only organ weight affected by treatment was the absolute and relative weight of the adrenals in the females at 50 or 500 ppm, which was higher than controls. There were no treatment-related effects on clinical chemistry parameters, ophthalmoscopy, gross pathology or histopathology.

The NOAEL was 5 ppm (0.16 mg/kg bw per day) on the basis of clinical signs and reduced body-weight gain at 50 ppm (1.73 mg/kg bw per day) (Hoffmann & Schilde, 1975).

In a 2-year feeding study, groups of four male and four female beagle dogs were fed diets containing azocyclotin at a concentration of 0, 10, 30 or 100/300 ppm. The group receiving the highest dose initially received azocyclotin at 100 ppm, but this was raised to 200 ppm for weeks 52–82 and to 400 ppm for weeks 83–104. The report of the study did not include a quality assurance certificate. Blood and urine were collected at 3-month intervals. All animals were killed for necropsy at the end of the study and a wide selection of organs was examined by light microscopy.

Diarrhoea was seen in all of the animals at dietary concentrations of 30 ppm or more. Throughout the second year of the study, the mean body weights of the males and females in the group receiving the highest dose were lower than those of concurrent controls. No treatment-related effects on intakes of feed and water, ophthalmoscopy, haematology, clinical chemistry, urine analysis or organ weights. At necropsy, a yellow colouring of the gastrointestinal serosa, pericardium and perirenal fat was noted in both sexes of animals at the highest dose. Microscopy showed small amounts of yellowish brown pigment in the tunica propria of the gall bladder. The pigment was phagocytosed within cells and it stained negative with Turnbull's blue, oil red O and Gmelin. The pigment and tissue staining were thought to be treatment-related, but were not regarded as adverse effects.

The NOAEL was 10 ppm (equal to 0.36 mg/kg bw per day in females and 0.38 mg/kg bw per day in males) on the basis of the diarrhoea observed at 30 ppm (equal to 1.09 mg/kg bw per day in both sexes) (Hoffmann & Schilde, 1979).

The Meeting considered that it was likely that the adverse effects seen in dogs were secondary to the corrosive effects of the azocyclotin on the gastrointestinal tract, rather than being attributable to any systemic toxicity.

2.2 Long-term studies of toxicity and carcinogenicity

Mice

In a long-term study of toxicity/carcinogenicity, groups of 60 male and 60 female CF_1 mice were fed diets containing azocyclotin (purity, 86.2%) at a concentration of 0, 5, 15 or 50 ppm for 2 years. These dietary concentrations provided average doses of 0, 0.71, 2.12 and 7.58 mg/kg bw per day for males and 0, 0.83, 2.72 and 9.04 mg/kg bw per day for females. The report of the study did not include a quality assurance certificate. Blood samples were taken after 3, 6, 12 and 24 months of exposure. Five animals of each sex from each group were killed after 6 and 12 months and were necropsied. After 24 months of treatment, all the surviving animals were killed for necropsy. Necropsies were also performed on animals dying at unscheduled times during the study. A wide range of organs and tissues from each animal was examined microscopically.

There were no treatment-related effects on mortality, food consumption or clinical signs. During the first 24 weeks of the study, body weight was slightly, but often significantly ($p < 0.05$), lower in the males at the highest dose than in concurrent controls. There was no effect on body weight later in the study. Several haematological and clinical chemistry parameters were significantly different to control values at various times during the study, but the effects were inconsistent and/or within the normal physiological range and were therefore not considered to be treatment-related. There was no effect on organ weights and no treatment-related gross pathology in the animals killed at 6, 12 or 24 months or in those dying prematurely. No treatment-related histopathology was seen, and there was no difference between treated groups and controls in the incidence of any type of tumour.

The NOAEL was 15 ppm (equal to 2.12 mg/kg bw per day) on the basis of depressed body-weight gain in males at 50 ppm (7.58 mg/kg bw per day) (Krötlinger et al., 1981).

Rats

In a long-term study of toxicity/carcinogenicity, groups of 50 male and 50 female Wistar rats were fed diets containing azocyclotin (purity, 85.5%) at a concentration of 0, 5, 15 or 50 ppm for 2 years. These dietary concentrations provided average doses of 0, 0.26, 0.79 and 1.08 mg/kg bw per day for males and 0, 0.35, 1.08 and 3.67 mg/kg bw per day for females. The report of the study did not include a quality assurance certificate. Blood samples were taken after 3, 6, 12 months of exposure from five rats of each sex from each group and necropsies were performed on these rats. Five rats of each sex from each group were killed after 12 months for measurement of hepatic microsomal enzyme activities (*N*- and *O*-demethylases), and another 10 rats of each sex

per group were used for this purpose at the end of the experiment. All rats that died during the study and all those killed the end of the 24-month treatment period were necropsied. Histopathological examinations of selected tissues were performed only on the animals from the control group and the groups receiving the highest dose.

Mortality was statistically increased ($p < 0.05$) in males at the lowest dose and in females at the intermediate dose, but not in either sex in the group receiving the highest dose. The body weights of the male and female rats in the groups at 15 and 50 ppm were seen to be slightly (< 10%) although usually significantly ($p < 0.05$) lower than in the control group, in the group at 15 ppm mainly in the second half of the study and in the group at 50 ppm during almost the whole of the feeding experiment. The only haematological changes were in the group receiving the highest dose at 3 months. There were significant decreases in reticulocyte counts in the males and in total leukocyte count in the females. No such effects were detected at the other sampling times. Total protein was significantly decreased in females at the highest dose at all sampling times. Serum alkaline phosphatase activity was decreased in males at the highest dose at 24 months and in females at the highest dose at 6 and 12 months. Urine analysis showed significant increases in urea concentration in rats of each sex at the highest dose at 24 months and decreases in creatinine concentrations in males at the highest dose at 3 and 12 months. Necropsies revealed no treatment-related gross pathology. In the rats killed after 24 months, there were significant decreases in the absolute weights of various organs (thyroid, heart, lungs, kidneys, adrenals and pancreas) at the intermediate and highest doses. The effects on organ weight were caused by the decreased body weights of these animals. The microscopic examination of tissues from rats in the control group and groups receiving the highest dose revealed no treatment-related histopathology. There was no evidence to suggest that the treatment had caused an increased incidence of any type of tumour or had caused an earlier onset of tumours.

The NOAEL was 5 ppm (equal to 0.26 mg/kg bw per day) on the basis of low body weight at 15 ppm (0.79 mg/kg bw per day) (Bomhard et al., 1979).

2.3 Genotoxicity

The results of tests for genotoxicity of azocyclotin are summarized in Table 2. The tests cover an appropriate range of end-points, including gene mutation, clastogenicity, mutagenicity in vivo and germ cell mutation. Azocyclotin gave negative results in all the tests for genotoxicity.

The tests appeared to have been well-conducted by the standards of the time, but some of the protocols did not conform to the recommendations of current OECD testing guidelines. One of the assays for reverse mutation in bacteria (Herbold, 1979) used only four bacterial strains rather than the five strains currently recommended in guideline 471 (OECD, 1997a) for this test. In addition there were no independent repeats of the tests bacterial reverse mutation (Herbold, 1979a; Shirasu et al., 1981) to confirm the negative results obtained. The report of the assay in mouse lymphoma cells (Cifone, 1982) did not give methodological details, so it was not clear whether it conformed to internationally agreed guidelines. In both of the tests for micronucleus formation (Herbold, 1979b; Siou, 1979), bone marrow was harvested only at 6 h after the final dose, while guideline 474 (OECD, 1997b) recommends the use of two harvest times, at 18–24 h and 36–48 h after the last treatment.

Certificates of compliance with GLP were provided with the more recent studies (Sasaki, 1987; Brendler-Schwaab, 1994), but no certificates of quality assurance were provided with the other studies.

The Meeting concluded that azocyclotin is unlikely to be genotoxic.

Table 2. Results of studies of genotoxicity with azocyclotin

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA98 and TA100	4–2500 µg/plate in DMSO +S9; and 2500 µg/plate –S9	86.2	Negative	Herbold (1979a)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA98 and TA100 and *E. coli* WP2 hcr	0.1–5000 µg/plate in DMSO ±S9	94	Negative	Shirasu et al. (1981)
DNA damage in bacteria (rec assay)	*Bacillus subtilis* H17 rec+ and M45 rec-	5–5000 µg/disc in DMSO ±S9	94	Negative	Shirasu et al.(1981)
Forward mutation	L5178Y $Tk^{+/-}$ mouse lymphoma cells	125–2000 ng/l, in DMSO or acetone +S9; and 3.13–300 ng/l –S9	95.1	Negative	Cifone (1982)
Chromosomal abberration	Chinese hamster lung (CHL) cells	3.3×10^{-7} to 1.5×10^{-5} mol/l in DMSO +S9; and 3.3×10^{-9} to 3.3×10^{-7} mol/l –S9	95.4	Negative	Sasaki (1987)
Unscheduled DNA synthesis	Primary culture of hepatocytes from male Sprague-Dawley rats	0.0195–5 µg/ml, solvent unspecified	94.1–94.7	Negative	Brendler-Schwaab (1994)
In vivo					
Micronucleus formation	Bone-marrow polychromatic erythrocytes of male and female NMRI mice	Two daily intraperitoneal injections at 50 or 100 mg/kg bw per day, in Cremophor	86.2	Negative	Herbold (1979b)
Micronucleus formation	Bone-marrow polychromatic erythrocytes of male Swiss mice	Two daily oral doses at 50–150 mg/kg bw per day in peanut oil	Not stated	Negative	Siou (1979)
Dominant lethal mutation	Germ cells of male NMRI mice	Twelve oral doses at 2.5 mg/kg bw, in Cremophor, during 48 days	86.2	Negative	Machemer (1977a)

DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant fraction from the livers of male Sprague-Dawley rats pretreated by intraperitoneal injection with Aroclor 1254.

2.4 Reproductive toxicity

(a) Multigeneration study

Groups of 10 male and 20 female Wistar rats were given diets containing azocyclotin (purity, 85.5%) at a concentration of 0, 5, 15 or 50 ppm for two generations. A certificate of quality assurance was not provided. The rats of the F_0 generation were treated for 10 weeks before mating. There were two matings per generation, with the second litter being used to produce the next generation. The parent animals (male and female) were killed by exsanguination under ether anaesthesia along with any mated females that failed to litter. These animals were necropsied. Dead, culled and non-selected offspring were subjected to a macroscopic examination. A wide

selection of organs from pups of the F_3 generation in the control group and the group receiving the highest dose were examined histopathologically.

There was no treatment-related effect on mortality in the parent animals. Body weight was significantly less ($p < 0.05$) at 50 ppm in the parental animals of the F_0 generation (females only) and F_{2b} generation (both sexes) than in the concurrent controls. Although the rate of lactation was decreased at 15 and 50 ppm in the F_{1b} generation and at 5 and 50 ppm in the F_{2b} generation, there was no clear dose–response relationship and the authors considered the effect to be unrelated to treatment. There were no treatment-related effects on other reproductive parameters, including fertility. Among pups of the F_3 generation, there was no difference in histopathology between the control group and the group receiving the highest dose.

The Meeting concluded that the NOAEL was the highest dose tested, 50 ppm (equivalent to 5 mg/kg bw per day) (Löser, 1980).

(b) Developmental toxicity

Rats

In two experiments, groups of approximately 20 pregnant Long-Evans rats were given azocyclotin suspended in a 0.5% aqueous solution of Cremophor as daily oral doses administered by gavage on days 6–15 of gestation. The daily doses given in the first experiment were 0, 3, 10 and 30 mg/kg bw and those given in the second experiment were 0, 0.3, 1 and 3 mg/kg bw. The purity of the azocyclotin was not stated in the report, but the purity of the same batch was reported elsewhere to be 85.5%. The report of the study did not include a quality assurance certificate.

There were no effects on mortality of the dams. Maternal body weight was significantly ($p < 0.01$) decreased during treatment at 10 or 30 mg/kg bw per day, and 8 out of 22 dams at the highest dose appeared emaciated with a ruffled coat and a lack of responsiveness. Pregnancy rate was significantly decreased ($p < 0.05$) and the mean number of resorptions was significantly increased in the group at 30 mg/kg bw per day. The sex ratio was significantly altered at 10 and 30 mg/kg bw, with more females at 10 mg/kg bw and more males at 30 mg/kg bw, but the authors considered this observation to be unrelated to treatment. Fetal weight, placental weight and implantation rate were unaffected by treatment. There was no effect of treatment on visceral abnormalities or skeletal abnormalities in the fetuses. The Meeting concluded that azocyclotin was not fetotoxic or teratogenic, but it did cause embryotoxicity at 30 mg/kg bw per day. The embryotoxicity was associated with maternal toxicity.

The NOAEL was 3 mg/kg bw per day on the basis of maternal toxicity, as indicated by reduced body-weight gain during the dosing period (Machemer, 1977b).

In a GLP-compliant study of developmental toxicity, groups of 25 pregnant Wistar rats were given azocyclotin (purity, 94.8%) at a dose of 0, 1, 3 or 10 mg/kg bw per day by oral gavage suspended in carboxmethylcellulose solution. The doses were given daily on days 6–15 of gestation. Animals that aborted were killed and necropsied. The rest of the animals were killed with CO_2 on day 21 of gestation and necropsied.

There was no mortality and no clinical signs in the dams. Body-weight gain and food consumption were significantly decreased ($p < 0.05$) in the dams at the highest dose throughout the treatment period. There were no treatment-related effects on the weights of maternal liver and gravid uterus, the number and position of fetuses in utero, the numbers of live and dead fetuses, number and position of absorption sites, numbers of corpera lutea, sex ratio of fetuses and fetal weight. There were also no treatment-related effects on the incidences of soft tissue and skeletal abnormalities.

The NOAEL was 3 mg/kg bw per day on the basis of decreased body-weight gain and food consumption by the dams. There was no embryotoxicity, fetotoxicity or teratogenicity at any of the doses tested (Becker, 1981).

Rabbits

In a GLP-compliant study, groups of four artificially inseminated Charles River New Zealand White rabbits were given azocyclotin (purity, 93.1%) at a dose of 0, 1, 3 or 10 mg/kg bw per day by oral gavage on days 7–18 of gestation. The administered azocyclotin was suspended in an aqueous solution of Cremophor. The does were killed on day 29 of gestation and autopsied. Two of the does in each of the groups at 3 and 10 mg/kg bw per day died or were killed in extremis. All had ulceration of the stomach and one also had a low kidney weight. The does in the groups at 3 and 10 mg/kg bw per day had reduced food intakes, appeared thin and lost body weight. None of the does in these groups successfully completed their pregnancies and one doe in the group at 1 mg/kg bw per day also aborted. Reduced mean fetal weight was seen at 1 mg/kg bw per day, but there were no effects on other litter parameters (uterus weight, number and position of fetuses, number and position of resorption sites, numbers of corpora lutea and sex ratio of fetuses) the numbers of abnormal fetuses (by soft tissue, brain and skeletal examinations) or on gross pathology of does in this group. As adverse effects were seen at all doses tested, it was not possible to identify a NOAEL in this study (Tesh & Ross, 1981).

In a GLP-compliant study, groups of 14 artificially inseminated Charles River New Zealand White rabbits were given azocyclotin (purity, 93.1%) at a dose of 0, 0.1, 0.3 or 1.0 mg/kg bw per day by oral gavage on days 6–18 of gestation. The administered azocyclotin was suspended in an aqueous solution of Cremophor. The does were killed on day 29 of gestation and autopsied.

There was an initial reduction in maternal body-weight gain in the animals at 1 mg/kg bw per day at the start of treatment, but from day 10 of gestation onwards there was no treatment-related effect on body-weight gain. Two does at 1 mg/kg bw per day aborted and died during the study. In both animals, gastrointestinal damage was seen at autopsy. This may have been caused by irritancy of the test material and/or physical trauma during intubation. No gross pathology was seen in the does killed on schedule on day 29 of gestation. There were no treatment-related effects on uterus weight, number and position of fetuses, number and position of resorption sites, numbers of corpora lutea, fetal weight, sex ratio of fetuses or on the numbers of abnormal fetuses (by soft tissue, brain and skeletal examinations). The Meeting concluded that azocyclotin was not embrytoxic, fetotoxic or teratogenic in the rabbit at doses of up to 1 mg/kg bw per day.

The NOAEL was 0.3 mg/kg bw per day on the basis of maternal toxicity as indicated by reduced body-weight gain at 1 mg/kg bw per day (Tesh et al., 1981).

In two separate experiments that complied with GLP, groups of 15 artificially inseminated Himalayan CHBB:HM New Zealand white rabbits were given azocyclotin (purity, 94.9%) by daily percutaneous application to shaved dorsal skin. Doses of 0, 30, 100 or 300 mg/kg bw per day and 0 or 10 mg/kg bw per day were given on days 6–18 of gestation. The azocyclotin was given at a volume of 1 ml as a suspension in aqueous Cremophor. The application site was cover with an occlusive dressing and left for 6 h at each application.

There was embyotoxicity, as indicated by an increased number of resorptions, at doses of 30 mg/kg bw per day or more. At 300 mg/kg bw per day, the rate of pregnancy was reduced. No fetotoxicity or teratogenicity was seen at any dose. There was decreased body-weight gain in the does at all doses.

It was not possible to identify a NOAEL for these experiments, as maternal toxicity (as indicated by reduced body-weight gain) was seen at all doses tested (Renhof, 1989a and 1989b).

3. Observations in humans

No health problems were reported in workers manufacturing the product Peropal, (containing 25% azocyclotin), except in one case where working regulations were not followed. The product is not readily soluble in water, but can form a dust. The affected worker was exposed

to azocyclotin while performing cleaning operations with a water jet without wearing the recommended personal protective equipment (full body protection and a full face mask). The exposure led to "an irritating toxic, spastic bronchitis". Recovery was complete within 3 days. X-ray examination of the lungs and "laboratory analysis" did not reveal any signs of damage. No further details were available (Bayer AG, 1982).

Comments

Biochemical aspects

Azocyclotin was shown to completely break down in aqueous solution to form cyhexatin and 1,2,4-triazole. There were no investigations available on the extent to which 1,2,4-triazole is absorbed from the gastrointestinal tract or on whether it undergoes any metabolism in the body. Oral doses of azocyclotin and cyhexatin were absorbed to a limited extent in rats. About 12% of azocyclotin or its breakdown products were absorbed from the gut lumen in rats and 1.6–10% in the case of cyhexatin. In rabbits given oral doses of cyhexatin, less than 10% of the administered dose was absorbed from the gut.

Cyhexatin is metabolized by hydroxylation, which splits off cyclohexyl rings to produce dicyclohexyltin and monocyclohexylstannoic acid. The products of the initial reactions can undergo oxidation to produce unidentified polar metabolites. In addition, hydroxylated and destannylated derivatives have been identified in faeces of animals treated with cyhexatin, but it is not clear whether these were the products of bacterial and chemical breakdown in the gut lumen or the products of metabolism of absorbed material that had been excreted in bile. There was extensive distribution of metabolites of azocyclotin and cyhexatin to various organs and tissues of the body, with the highest amounts being found in the liver and the kidneys. Elevated concentrations of tin and ^{14}C radiolabel were detected in fetuses, amniotic fluid and placenta in pregnant rabbits given oral doses of ^{14}C-labelled cyhexatin.

In all species investigated (rat, rabbit and guinea-pig), excretion of the metabolites of azocyclotin and cyhexatin was mostly in the urine and to a lesser extent in the bile. As a result of poor absorption, large proportions of orally administered doses of azocyclotin and cyhexatin were found in the faeces. Minimal amounts were exhaled as carbon dioxide.

Toxicological data

Azocyclotin has moderate acute toxicity by the oral route. The LD_{50} value for azocyclotin in rats was 209 mg/kg bw when administered by the oral route. Azocyclotin has very low acute systemic toxicity when applied dermally, with LD_{50} value for rats of 3600 mg/kg bw, but high acute toxicity after exposure by inhalation, with an LC_{50} in rats of approximately 0.02 mg/l.

Azocyclotin is more irritating than cyhexatin, being corrosive to rabbit skin. Azocyclotin did not cause skin sensitization in tests in guinea-pigs.

Exposure to azocyclotin by inhalation at a dose of 0.96 µg/l caused poorly groomed appearance, impaired breathing and increased lung weight in rats exposed for 6 h per day, 5 days per week, for 3 weeks. The NOAEC for the study was 0.28 µg/l.

No systemic toxicity was seen in rats given azocyclotin at doses of up to 25 mg/kg bw per day applied dermally for 7 h per day, 5 days per week, for 3 weeks.

In short-term studies with azocyclotin, the main toxicological effects seen in rats were local effects on the gastric mucosa, haematological changes and hepatotoxicity.

Three short-term studies of oral toxicity with azocyclotin were performed in rats, one using gavage dosing and the others using dietary administration. Low body weights were reported in treated animals in all the studies at dietary concentrations of 50 ppm (equal to 2.86 mg/kg bw per day) or more. Decreased total leukocyte counts were reported in two of the studies, with decreased lymphocyte counts in one of these. The NOAEL was 2 mg/kg bw per day. Increased liver weight and increased activities of serum enzymes, such as alkaline phosphatase, alanine

aminotransferase and aspartate aminotransferase, were reported in two of the studies. The NOAEL for these effects was 15 ppm (equal to 0.85 mg/kg bw per day).

Feeding studies in dogs given azocyclotin for 90 days or 24 months both showed diarrhoea to be a critical end-point, with a NOAEL of 0.36 mg/kg bw per day. In the 24-month study, diarrhoea was seen in all dogs given azocyclotin at a dose of 1.09 mg/kg bw per day or more. In the 90-day study, there was also a decrease in body-weight gain at 1.73 mg/kg bw per day or more, although this effect was not seen at doses of up to 1.09 mg/kg bw per day in the 24-month study. Thus the NOAEL for decreased body-weight gain was 1.09 mg/kg bw per day. Haematological effects (decreases in erythrocyte count, erythrocyte volume fraction and haemoglobin) were seen in some of the males at 18.3 mg/kg bw per day in the 90-day study. It was considered to be likely that the decreased body weight and diarrhoea were related to the corrosiveness of azocyclotin.

The most sensitive effect seen in long-term studies of toxicity/carcinogenicity with azocyclotin in mice and rats was decreased body weight compared with that of the controls, with NOAELs of 2.12 mg/kg bw per day in mice and 50 ppm (equal to 0.26 mg/kg bw per day) in Wistar rats.

Azocyclotin did not produce any tumours in combined long-term studies of toxicity/carcinogenicity in mice and rats. The Meeting concluded that azocyclotin is unlikely to be carcinogenic in rodents.

Azocyclotin was not genotoxic in an extensive range of tests for genotoxicity in vitro and in vivo. The Meeting concluded that azocyclotin is unlikely to be genotoxic in vivo.

In the absence of genotoxicity in vivo and with the finding of an equivocal increase in the incidence of benign liver tumours at the highest dose in female rats in only one out of four studies of carcinogenicity in rodents, the Meeting concluded that use of azocyclotin as a pesticide is unlikely to pose a carcinogenic risk to humans.

In a multigeneration study in rats given diets containing azocyclotin at concentrations of up to 50 ppm (equivalent to 3.7 mg/kg bw per day), there were no treatment-related adverse effects. Two studies of developmental toxicity with azocyclotin in rats treated orally by gavage found no fetotoxicity or teratogenicity at any dose tested up to 30 mg/kg bw per day and no effects on embryotoxicity at doses that were not maternally toxic. The NOAELs for maternal toxicity of azocyclotin administered by gavage, as indicated by effects on body weight, were 3 and 0.3 mg/kg bw per day in rats and rabbits, respectively. Embryotoxicity (increased number of resorptions) was seen in rats given azocyclotin a dose of 30 mg/kg bw per day by gavage.

Two studies of developmental toxicity in rabbits have been performed with azocyclotin. There was no embryotoxicity, fetotoxicity or teratogenicity in rabbits given azocyclotin at doses of up to 1 mg/kg bw per day by gavage. The NOAEL for maternal toxicity was 0.3 mg/kg bw per day. The Meeting concluded that azocyclotin is not teratogenic or fetotoxic.

As cyhexatin is a major metabolite of azocyclotin, the results of toxicological studies with cyhexatin (see monograph on cyhexatin, this volume) are also relevant to azocyclotin.

No health problems were reported in most workers at a factory producing a product that contained 25% azocyclotin. However, one worker who had not worn the recommended personal protective equipment had an exposure that led to “an irritating toxic spastic bronchitis”. Recovery was complete within 3 days.

The Meeting concluded that the existing database on azocyclotin was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

In dogs, azocyclotin caused reduced body weight and clinical signs, including diarrhoea, at dietary concentrations of 30 ppm (equal to1.09 mg/kg bw per day) or more. The NOAEL was 5 ppm (0.16 mg/kg bw per day). These effects were not used in the establishment of an ADI or an

ARfD. The Meeting recognized that some of the reported adverse effects of azocyclotin and cyhexatin were a secondary consequence of an irritating effect on the gastrointestinal mucosa and therefore were not relevant for establishing reference values.

The Meeting established a group ADI for azocyclotin and cyhexatin of 0–0.003 mg/kg bw based on the NOAEL of 0.34 mg/kg bw per day for retinal atrophy in a long-term study of toxicity/carcinogenicity with cyhexatin in rats and using a safety factor of 100.

The Meeting established a group ARfD for azocyclotin and cyhexatin of 0.02 mg/kg bw based on the NOAEL of 1.5 mg/kg bw per day for embryotoxicity in studies of developmental toxicity with cyhexatin in rabbits, and using a safety factor of 100. The ARfD is applicable to women of childbearing age. No ARfD is necessary for the rest of the population, as the only other acute responses were related to dietary refusal and/or local irritation of the gut.

The Meeting recognized that the ARfD might be conservative, but it was not possible to determine whether the embryotoxicity was the result of systemic toxicity to the conceptus or the result of reduced nutrition caused by reduced maternal food intake and local adverse effects to the maternal gastrointestinal mucosa as a result of the irritant nature of the cyhexatin.

Levels relevant to risk assessment

(i) Studies with azocyclotin

Species	Study	Effect	NOAEL	LOAEL
Mouse	Long-term study of toxicity[c]	Toxicity	15 ppm (equal to 2.12 mg/kg bw per day)	7.58 mg/kg bw per day
		Carcinogenicity	50 ppm (equal to 7.58 mg/kg bw per day)[a]	—
Rat	Long-term study of toxicity[c]	Toxicity	5 ppm (equal to 0.26 mg/kg bw per day)	15 ppm (equal to 0.79 mg/kg bw per day)
		Carcinogenicity	50 ppm (equal to 1.08 mg/kg bw per day)[a]	—
	Multigeneration study[c]	Reproductive toxicity	50 ppm (3.7 mg/kg bw per day)[a]	—
	Developmental toxicity[b]	Maternal toxicity	3 mg/kg bw per day	10 mg/kg bw per day
		Embryotoxicity	10 mg/kg bw per day	30 mg/kg bw per day
		Teratogenicity and fetotoxicity	30 mg/kg bw per day[a]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity (reduced body-weight gain)	0.3 mg/kg bw per day	1.0 mg/kg bw per day
		Developmental effects	1.0 mg/kg bw per day[a]	—

[a] Highest dose tested

[b] Gavage administration

[c] Dietary administration

(ii) Studies with cyhexatin

Species	Study	Effect	NOAEL	LOAEL
Mouse	Long-term study of toxicity/carcinogenicity[d]	Toxicity	3 mg/kg bw per day[b]	6 mg/kg bw per day[b]
		Carcinogenicity	6 mg/kg bw per day[a,b]	—

Rat	Long-term study of toxicity/carcinogenicity [d]	Toxicity (retinal atrophy)	7.5 ppm (equal to 0.34 mg/kg bw per day)	30 ppm (equal to 1.39 mg/kg bw per day)
	Multigeneration study[d]	Toxicity	0.5 mg/kg bw per day[b]	6.0 mg/kg bw per day[b]
		Toxicity	0.5 mg/kg bw per day[b]	6.0 mg/kg bw per day[b]
		Developmental toxicity	7.0 mg/kg bw per day[a, b]	—
	Developmental toxicity[c]	Maternal toxicity	1 mg/kg bw per day	5 mg/kg bw per day
		Developmental toxicity	10 mg/kg bw per day[a]	—
	Neurotoxicity[d]	Toxicity	30 ppm (equal to 1.99 mg/kg bw per day)	180 ppm (equal to 10.94 mg/kg bw per day)
Dog	2-year study[d]	Toxicity	3 mg/kg bw per day	6 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	1 mg/kg bw per day	3 mg/kg bw per day
		Developmental toxicity	1.5 mg/kg bw per day	3 mg/kg bw per day

[a] Highest dose tested

[b] Dietary concentrations were regularly adjusted to achieve set doses

[c] Gavage administration

[d] Dietary administration

Estimate of acceptable daily intake for humans

0–0.003 mg/kg bw

Estimate of acute reference dose

0.02 mg/kg bw for women of childbearing age

Unnecessary for the rest of the population

Studies that would provide information useful to the continued evaluation of the compound

The metabolic fate of the 1,2,4-triazole that splits off from azocyclotin when it breaks down to form cyhexatin is unknown.

Critical end-points for setting guidance values for exposure to azocyclotin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Limited absorption in rats: about 12% absorption of azocyclotin or its breakdown products
Distribution	Extensive, with the largest amounts being found in the liver and kidneys
Potential for accumulation	Accumulation is unlikely
Rate and extent of excretion	Excreted in urine (1% of the administered ^{113}Sn and about 10% of the administered ^{14}C from radiolabelled azocyclotin) and probably also in bile. Minimal amounts were exhaled as carbon dioxide.
Metabolism in mammals	Hydrolyses rapidly in aqueous solution to cyhexatin and 1,2,4-triazole.

Toxicologically significant compounds (animals, plants and environment)	Azocyclotin and cyhexatin
Acute toxicity	
Rat LD_{50} oral	209 mg/kg bw for males; 363 mg/kg bw for females
Rat LD_{50} dermal	> 2000 mg/kg bw
Rat LC_{50} inhalation	0.017 mg/l for males; 0.029 mg/l for females
Mouse LC_{50} inhalation	0.035 mg/l
Golden hamster LC_{50} inhalation	0.0055 mg/l for males
Rabbit, skin irritation	Corrosive
Rabbit, eye irritation	Not tested but taken to be corrosive
Skin sensitization (test method used)	No skin sensitization potential in guinea-pigs (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effects	Reduced body-weight gain (rats, rabbits, dogs)
Lowest relevant oral NOAEL	0.3 mg/kg bw per day (rabbits)
Genotoxicity	
	Not genotoxic in vitro or in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effects	Low body weight
Lowest relevant oral NOAEL	0.26 mg/kg bw per day (rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproduction target/critical effect	No adverse effects on reproduction at any dose in the multigeneration study
Lowest relevant reproductive NOAEL	3.7 mg/kg bw per day (highest dose tested)
Developmental target/critical effect	Embryotoxicity
NOAEL for maternal toxicity	0.3 mg/kg bw per day (reduced maternal body-weight gain in rabbits)
Lowest relevant developmental NOAEL	10 mg/kg bw per day (embryotoxicity in rats)
Medical data	
Health monitoring of workers	An “irritating toxic spastic bronchitis” reported in one exposed worker in a factory

Critical end-points for setting guidance values for exposure to cyhexatin

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Limited absorption in rats (1.6–10%) and rabbits (10%)
Distribution	Extensive, with the largest amounts being found in the liver and kidneys
Potential for accumulation	Accumulation is unlikely
Rate and extent of excretion	Excretion was mainly in the urine and to a lesser extent in bile

Metabolism in mammals	Splitting off of cyclohexyl rings and oxidation to produce a variety of substances (most of which were unidentified)
Toxicologically significant compounds (animals, plants and environment)	Cyhexatin
Acute toxicity	
Rat LD_{50} oral	407 mg/kg bw for males; 265 mg/kg bw for females
Rat LD_{50} dermal	7600 mg/kg bw for males; 3600 mg/kg bw for females
Rabbit LD_{50} dermal	> 2000 mg/kg bw
Rat LC_{50} inhalation	0.016 mg/l
Rabbit, skin irritation	Irritant
Rabbit, eye irritation	Severely irritant
Skin sensitization (test method used)	No skin sensitization potential in guinea-pigs (Buehler test)
Short-term studies of toxicity	
Target/critical effect	Hepatotoxicity (rats); low body weight (dogs)
Lowest relevant oral NOAEL	0.68 mg/kg bw per day (rats)
Genotoxicity	
	Not genotoxic in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effects	Mortality and body weight (mice); retinal atrophy (rats)
Lowest relevant oral NOAEL	0.34 mg/kg bw per day for retinal atrophy (rats)
Carcinogenicity	Unlikely pose a carcinogenic. risk to humans
Reproductive toxicity	
Reproduction target/critical effect	Decreased pup weight at weaning and decreased survival to weaning at parentally toxic doses
Lowest relevant reproductive NOAEL	Parents and offspring: 0.5 mg/kg bw per day Reproductive toxicity: 7.5 mg/kg bw per day, highest dose tested (rats)
Developmental target/critical effect	Embryotoxicity (postimplantation loss) in rabbits
NOAEL for maternal toxicity	1.5 mg/kg bw per day in studies of developmental toxicity in rabbits (low body-weight gain). 0.5 mg/kg bw per day in a two-generation study in rats (hepatotoxicity)
Lowest relevant developmental NOAEL	1.5 mg/kg bw per day for embryotoxicity in rabbits.
Medical data	
Health monitoring of workers	No adverse effects seen

Summary for azocyclotin and cyhexatin

	Value	Study	Safety factor
Group ADI	0–0.003 mg/kg bw	Rat, 2-year study, NOAEL	100
Group ARfD*	0.02 mg/kg bw	Rabbit, developmental toxicity, NOAEL	100

*For women of childbearing age, unnecessary for the rest of the population

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