COVER PAGE

US EPA HPV Challenge Program

Test Plan Submission

1H-1,2,4-Triazole

CAS No. 2888-88-0

July 2009

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Test Plan Submission

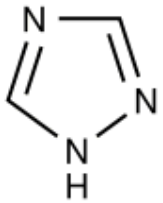
1H-1,2,4-Triazole

CAS No. 288-88-0

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 288-88-0
IUPAC Name: 1H-1,2,4-triazole
Molecular Formula: C2H3N3
Structural Formula:



Molecular Weight: 69
Synonyms: 1H-1,2,4-Triazole
s-Triazole TA
1,2,4-Triazole
4H-1,2,4-Triazole
S-Triazole
TA

1.2 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference
Physical state	solid	
Melting point	120.4 °C	Ciba-Geigy, 1983
Boiling point	260 °C	Lide, 2007-2008
Relative density	No data	
Vapour pressure	0.0022 hPa at 20 °C	Bayer, 2001a
Water solubility	700 g/l at 20 °C	Bayer, 2001b
Partition coefficient n-octanol/water (log value)	0.71 at 25 °C	RCC, 2005
Henry's law constant	2 x 10E-05 at 20 °C (calculated)	Bayer, 2001b

2 GENERAL INFORMATION ON EXPOSURE

2.1 Environmental Fate

2.1.1 Photodegradation

The hydroxyl radical reaction was calculated using AOPWIN® ver. 1.92. The overall reaction half-life in air is estimated to be 107 days for 1H-1,2,4-triazole (TA) (USEPA, 2008). A study was conducted to determine the sunlight photoreactivity of TA in distilled water and in humic acid solutions, in order to estimate its photoreactivity in actual environmental situations (Miller, 1983). TA does not undergo appreciable direct photolysis in sunlight nor does humic acid have a major effect on increasing the rate of loss by indirect photochemical reactions.

2.1.2 Stability in Water

A study was conducted to determine hydrolysis rate constants and half-lives for TA in aqueous buffered solutions of pH 5, 7, and 9 at 25 ^{o}C (Biospherics, 1983). The test method was not specified. Throughout the study, the parent molecule accounted for 89.6 to 97.9% of all spotted radioactivity. At all three tested pH values 5, 7, and 9, the test material was found to be stable for 30 days at 25°C. Therefore, the half-life was concluded to be in excess of 30 days.

2.1.3 Transport between Environmental Compartments

The EQC Level III Fugacity model (EPISuite, v.3.20) was used to evaluate the fate, transport and distribution of TA between environmental matrices (USEPA, 2008). Level III fugacity modelling, using loading rates of 1000 kg/h each for air, soil and water, shows the following percent distribution TA is released simultaneously to all three compartments: Air <1%, Water = 38.9%, Soil = 61% and Sediment <1%.

2.1.4 Biodegradation

In an OECD Guideline 302B "Inherent biodegradability: Modified Zahn-Wellens Test", TA exhibited minimal degradation (1%) after 28 days, indicating that it is not readily biodegradable (Institute, 1990).

The degradation kinetics of TA were investigated in three different soils under aerobic conditions. [3,5-14C] TA was aerobically incubated in three soils (Laacher Hof AXXa, sandy loam; BBA 2.2, loamy sand; Laacher Hof A III, silt loam) at 20°C in the dark at a moisture content of approximately 40% of the water holding capacity (NOTOX, 2000). TA was applied at a concentration of about 0.06 mg/kg dry soil. This was equivalent to an application rate of triazole-releasing fungicides of 750 g a.i./ha, reaching the soil for 50%, incorporation in 5 cm of soil and assuming a soil bulk density of 1500 kg/m3, a maximum metabolite formation of 50% and a molar mass ratio of TA to parent of 0.25. The DT50 values assuming non-linear first order kinetics in the three soils investigated were: 6.32 days for Laacher Hof AXXa sandy loam soil, 9.91 days for BBA 2.2 loamy sand soil (only first phase was used) and 12.27 days for Laacher Hof A III silt loam soil.

2.1.5 Bioaccumulation

The estimated BCF of TA (EPISuite, v.3.20), is = 3.16, suggesting this compound does not bioaccumulate (USEPA, 2008).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Acute Toxicity

Acute oral and dermal toxicity studies are available for TA.

Studies in Animals

Dermal

Groups of at least five Wistar rats/sex/group were exposed to TA by the dermal route for 24 hours under occlusive cover (Bayer, 1976; RCC, 2003). Doses were 1000, 2000, 2500, 3500, 4000, and 5000 mg/kg bw. The protocol followed was EPA OPP 81-2 and EPA FIFRA, Subdivision F, 81-2 (equivalent to 92/69/EEC B.3). The animals exhibited reduction in general well-being, sedation and breathing disorders. At higher doses, the animals were lying in the abdominal or side position. The symptoms appeared within an hour of administration and were observed for a maximum of up to 13 days after administration. Gross pathology examination of the animals revealed no major changes. The LD50s were = 4200 mg/kg bw (males) and = 3129 mg/kg bw (females).

Groups of two male New Zealand White rabbits were exposed to TA by the dermal route for 24 hours under occlusive cover (Rohm and Haas, 1992; RCC, 2003). Doses were 200, 2000 and 5000 mg/kg bw. The protocol followed was EPA OPP 81-2 and EPA FIFRA, Subdivision F, 81-2 (equivalent to 92/69/EEC B.3). In the 5000 and 2000 mg/kg groups, both rabbits in each dose group died by day 4 of the study. Clinical signs observed in the 2000 and/or 5000 mg/kg bw groups included abdominal breathing, ataxia, clear nasal discharge, gasping, iritis, moribundity, salivation, scant droppings, soft feces, tremors, and yellow nasal discharge. In the 200 mg/kg bw group, no deaths occurred and no clinical signs were observed. There was no effect on body weight. Decedents in the 2000 and 5000 mg/kg bw group exhibited numerous gross findings related to the

test substance; there were no findings in survivors at 200 mg/kg bw. No erythema to well-defined erythema and no edema to very slight edema were observed during the study. The LD50 was > 200 – 2000 mg/kg bw.

Oral

Groups of at least 15 Wistar rats/sex/group were administered TA by oral gavage at doses of 100, 250, 500, 1000, 1250, 1500, 1750, 1850, 2000, and 2500 mg/kg bw (Bayer, 1976; RCC, 2003). The test substance was emulsified in distilled water and Cremophor EL. The study was conducted in accordance with EPA OPP 81-1 and EPA FIFRA, Subdivision F, §81-1 (equivalent to 92/69 B.I). Clinical signs included reduction in general well-being, sedation and breathing disorders. At higher doses, the animals were lying in the abdominal or side position. The symptoms appeared within an hour of administration and were observed for a maximum of up to 13 days after administration. Gross pathology examination of the animals revealed no major changes. The LD50s were = 1650 mg/kg bw (males) and = 1648 mg/kg bw (females).

TA was dispersed in 0.5% methylcellulose and administered by gavage to two groups of three male rats at 500 or 5000 mg/kg bw (Rohm and Haas, 1992; RCC, 2003). The study was conducted in accordance with EPA OPP 81-1 and EPA FIFRA, Subdivision F, §81-1 (equivalent to 92/69 B.I). All rats died in the 5000 mg/kg bw group within ten minutes after dosing; no clinical signs were observed prior to death. No deaths occurred and no clinical signs were observed in the 500 mg/kg bw group. There were no effects on body weight. Necropsy of decedents (5000 mg/kg) revealed reddened duodenum and reddened glandular portion of stomach. There were no visible lesions in animals that survived to study termination. The LD50 is between 500 and 5000 mg/kg in male rats.

Conclusion

The acute dermal LD50s in Wistar rats were = 4200 mg/kg bw (males) and = 3129 mg/kg bw (females). The animals exhibited reduction in general well-being, sedation and breathing disorders. At higher doses, the animals were lying in the abdominal or side position. Gross pathology examination of the animals revealed no major changes. The acute dermal LD50 in male New Zealand White rabbits was > 200 – 2000 mg/kg bw. Clinical signs included abdominal breathing, ataxia, clear nasal discharge, gasping, iritis, moribundity, salivation, scant droppings, soft feces, tremors, and yellow nasal discharge. There were no gross necropsy findings in survivors. No erythema to well-defined erythema and no edema to very slight edema were observed during the study.

The acute oral (gavage) LD50s in Wistar rats were = 1650 mg/kg bw (males) and = 1648 mg/kg bw (females). Clinical signs included reduction in general well-being, sedation and breathing disorders. At higher doses, the animals were lying in the abdominal or side position. Gross pathology examination of the animals revealed no major changes.

3.1.2 Irritation

Eye irritation data are available for TA.

Skin Irritation

Studies in Animals

No data available.

Eye Irritation

Studies in Animals

TA was instilled into the conjunctival sac of the left eye of each of two rabbits at a dose of 50 mg/animal (Bayer, 1976; RCC, 2003). The study was conducted in accordance with EPA OPP 81-4 and EPA FIFRA, Subdivision F, 81-4 (equivalent to 92/69/EEC B.5). One hour after application, intense reddening and a very intense swelling of the conjunctivae of the treated eyes had developed. Intense reddening and moderate swelling of the conjunctivae could still be observed 24 hours after application. In one animal there was still a slight redness and swelling of the conjunctivae 5 days after application. In the other animal, the conjunctivae were normal. The conjunctivae of both animals were normal 7 days after application. During the first and second days after application, a slight, dispersed, diffuse opacity of the cornea was observed. The iris was slightly reddened and swollen. TA is considered highly irritating to the rabbit eye.

0.1 g of TA was applied into the conjunctival sac of the left eye of two male rabbits (Rohm and Haas, 1992; RCC, 2003). The study was conducted in accordance with EPA OPP 81-4 and EPA FIFRA, Subdivision F, 81-4 (equivalent to 92/69/EEC B.5). Corneal, iridal and conjunctival effects were observed at 4 hours. Corneal and conjunctival effects were no longer evident on day 7; iridal effects were no longer evident on day 14. TA was considered moderately irritating to the rabbit eye.

Conclusion

Skin irritation data were not located for TA. TA is considered a moderate to severe eye irritant.

3.1.3 Sensitisation

Skin sensitization data are available for TA.

Studies in Animals

Skin

In an OECD Guide-line 406 "Skin Sensitization" study, groups of Dunkin Hartley Crl (HA) guinea pigs were exposed to TA as follows: Induction 10% TA by intracutaneous injection; Induction 75 % TA under semiocclusive application and Challenge 75% TA under semiocclusive application (Tox Labs, 1998; RCC, 2003). TA caused slight skin irritation with and without Freund's complete adjuvant after intradermal injection. No skin reaction was recorded after dermal induction. At 48 and 72 hours after the start of epidermal challenge no signs of allergic skin reactions were noted in test or control animals. TA was not a skin sensitizer in this study.

Conclusion

TA is not considered a skin sensitizer.

3.1.4 Repeated Dose Toxicity

Repeated dose dietary studies in rats and mice were located for TA.

Studies in Animals

Oral

Groups of 15 Wistar rats sex received TA for three months in the following concentrations in their food: 0 (control), 100, 500, and 2500 ppm (males/females: 7.79/10.27 mg/kg bw, 37.85/54.20 mg/kg bw and 212.30/266.69 mg/kg bw, respectively)mg/kg bw/day) (Bayer, 1979; RCC, 2003).

The study was conducted in accordance with EPA OPP 82-1, EPA-FIFRA, Subdivision F, § 82-1, OECD 409, and 87/302/EEC (B.27). Appearance, behaviour, growth, food consumption and mortality were unaffected up to 33 mg/kg bw. Food consumption was reduced at 167 mg/kg bw during the first two weeks of treatment. Body weight gains were reduced at 167 mg/kg bw. Temporary slight convulsions were observed in two males and two females at 167 mg/kg bw. The blood was not affected up to 33 mg/kg bw. There were statistically significant changes in red blood cell parameters at 167 mg/kg bw (reduced hemoglobin, hematocrit, MCV and MCH) that indicated a slight microcytic hypochromic anemia. Clinical chemistry, gross pathology and histopathology did not detect any liver damage in the male and female rats receiving up to 33 mg/kg bw. Slight to moderate fat accumulation in liver parenchyma cells was found in three of 15 males examined at 167 mg/kg bw. There were deviations in absolute organ weights (thymus, heart, lung, spleen, testes) at 167 mg/kg bw, particularly in males, that were attributed to lower terminal body weights. There were no findings at necropsies or histopathological examinations with the exception of the liver findings mentioned. The No Observed Adverse Effect Level (NOAEL) was = 37.9 mg/kg bw (males) and 54.2 mg/kg bw (females) based on retarded body weight development, temporary slight CNS effects, lower red blood cell parameters (males, only) and hepatocellular fat accumulation (males only).

Twenty Wistar rats/sex/group received TA in the diet for approximately 14 weeks, at nominal dietary concentrations of 0, 250, 500, 3,000 or 1,000/4,000 ppm (1,000 ppm for four weeks and 4,000 ppm thereafter; equivalent to 16, 33, 183, and 210 mg/kg bw for males, and 19, 41, 234, and 275 mg/kg bw for females) (Bayer, 2004a; RCC, 2003). The study followed OECD Guideline No. 424 Neurotoxicity Study in Rodents. Body weight was unaffected in both sexes at doses up to and including 33/41 mg/kg bw; and decreased in the higher dose groups. The FOB revealed effects in both sexes including ungroomed appearance, muscle fasciculations, tremor, gait in-coordination, decreased activity in the open field, and decreased rearing in the two highest dose groups. Slightly increased hepatic enzyme activities were observed in both sexes at the two highest dose levels; the limited magnitude of the alterations as well as the lack of corresponding morphological evidence indicate an adaptive response by the liver. There were no treatment related effects on serum chemistry, food consumption/utilization, ophthalmology, hematology, and urinalysis. There was a slight decrease in both sexes in absolute brain weight in the two highest dose groups; the relative brain weights were not affected. Other apparent variations in organ weights relative to controls were best characterized as a secondary response to alterations in body weight gain. Gross pathological evidence of toxicity was not observed. A non-statistical tendency was noted toward a greater number of total and recent-cycle corpora lutea in the two highest dose groups. The increase was likely due to an increase in the number of present cycle corpora lutea or those undergoing morphological regression, the extent of which, in conjunction with normal ovarian weights, is interpreted to be within normal physiological limits. Microscopic findings were observed in both sexes in the brain and nerve tissue in the two highest dose groups. In the brain, the lesions were limited to the more anterior dorsal cerebellum (vermis) and were coded overall as a degeneration/necrosis, which tended to be slightly more prominent in males. Findings were characterized by extensive loss of Purkinje cells, variable white matter degeneration and gliosis. Subtle atrophy of the molecular layer or loss of granule cells was occasionally present. Individual nerve fiber degeneration, which is a common background change in the peripheral nerves (sciatic, tibial, sural), was increased in incidence and severity over control levels in the two highest dose groups. Additionally, minimal to mild neuronal chromatolysis and vacuolation of the lumbar dorsal root ganglia was observed at the highest dose, but no similar change was seen in the cervical dorsal root ganglia. Based on the lack of adverse compound-related effects at 500 ppm in males and females, the NOAEL was = 33 mg/kg bw/day (males) and 41 mg/kg bw (females).

Twenty CD-1 mice/sex/group (with an additional 15 mice/sex assigned to control, 3,000, and 6,000 ppm groups received TA for approximately 13 weeks at nominal dietary concentrations of 0, 500,

1,000, 3,000 or 6,000 ppm (80, 161, 487, and 988 for males and 105, 215, 663, and 1346 for females) (Bayer, 2004b; RCC, 2003). The additional 30 animals per group in the control, 3,000 and 6,000 ppm levels were sacrificed following 28 days on study. The study was conducted in general accordance with OPPTS 870.3100, OECD 409, 87/302/EEC (B.27). Alterations in body weight were measured in males at 487 mg/kg bw and in both sexes 988/1346 mg/kg bw male and females. Clinical observations included increased incidence of tremors, yellow staining (likely urine stains), and rough coat for 988 mg/kg bw males. Analysis of the activity of selected hepatic enzymes indicated slight increases in both sexes in the two highest dose groups following 28 days and the high dose group following 90 days. However, the magnitude of the alterations as well as the lack of corresponding morphological evidence indicates an adaptive response by the liver. There were no effects on food consumption/utilization, mortality, hematology, or clinical chemistry. Gross lesions attributable were observed in 988 mg/kg bw males and included an increased incidence of rough coat and wet/stained ventrum. Organ weight changes included decreased testicular weights in 988 mg/kg bw males and decreased brain weights (absolute only) in 487 mg/kg bw males and 988/1346 mg/kg bw males and females. Histopathological findings included an increased incidence of lesions noted in the brain in high dose males and females, and in the testes of the two highest dose group males. The finding in the testes in the highest dose group was accompanied by a secondary, indirect effect in the epididymis. The lesion identified in the brain consisted of a loss of Purkinje cells in the cerebellum, accompanied by occasional degeneration of the Purkinje cell axons located in the white matter of the cerebellar folia (minimal to slight severity). Although the lack of neurons is considered to be a late-stage lesion, no earlier stages preceding this effect were observed.

In the testes increased incidences of apoptotic-like bodies, tubular atrophy, and abnormalities in spermatid development (spermiogenesis and spermiation) were seen at >= 487 mg/kg bw. The finding in the epididymis, observed in high dose males only, was considered to be a reflection of the testicular effects which were most pronounced at the highest dose level and consisted of exfoliated germ cells and debris within the lumen of the epididymal duct (the epididymal parenchyma was unchanged compared to controls). Additionally, one of the mice showed an overall decrease in the number of spermatozoa in the epididymal ducts at the highest dose. The NOAEL was = 161 mg/kg bw/day (males) (1000 ppm) based on decreased body weights, decreased absolute brain weight and micropathological finding in the testes and = 663 mg/kg bw/day (females) (3000 ppm) based on decreased body weights, decreased absolute brain weight and micropathological finding in the brain.

Conclusion

In a 90 day dietary study with male and female Wistar rats, the NOAEL was = 37.9 mg/kg bw (males) and54.2 mg/kg bw (females) based on retarded body weight development, temporary slight CNS effects, lower red blood cell parameters (males, only) and hepatocellular fat accumulation (males only). In a 90 day dietary study with male and female Wistar rats, based on the lack of adverse compound-related effects at 500 ppm in males and females, the NOAEL was = 33 mg/kg bw/day (males) and 41 mg/kg bw (females). In a 90 day dietary study in mice the NOAEL was = 161 mg/kg bw/day (males) based on decreased body weights, decreased absolute brain weight and micropathological finding in the testes and = 663 mg/kg bw/day (females) based on decreased body weights, decreased absolute brain weight and micropathological finding in the brain.

3.1.5 Mutagenicity

In vitro bacterial and mammalian genotoxicity studies have been conducted with TA.

In vivo Studies

No data available.

In vitro Studies

TA was negative for mutagenicity in two bacterial reverse mutation assays with *Salmonella typhimurium* TA98, TA100, TA1535, and TA1537, in the presence and absence of metabolic activation (RCC, 2003; Rohm and Haas, 1981). The study protocol followed OECD TG 471.

TA was negative for the induction of structural and numerical chromosome aberrations in CHO cells in the presence and absence of metabolic activation in an OECD TG 473 study (Toxicology & Environmental Research and Consulting, 2007a).

TA was not mutagenic in the CHO/HGPRT gene mutation assay in the presence and absence of metabolic activation in an OECD TG 476 study (Toxicology & Environmental Research and Consulting, 2007b).

Conclusion

TA is not mutagenic in *in vitro* or mammalian genotoxicity studies.

3.1.6 Toxicity for Reproduction

Reproductive toxicity studies are available for TA.

Effects on Fertility

In an OECD Guide-line 416 "Two-generation Reproduction Toxicity Study", TA was administered continuously via the feed to Wistar Hannover rats (30/sex/dietary level) at nominal dietary concentrations of 0, 250, 500, and 3000 ppm (Bayer, 2005; RCC, 2003; TDMG, 2006). To maintain a more constant dosage (on basis of mg/kg bw/day) throughout the entirety of the study the dietary levels were reduced for females during lactation. In addition to fulfilling standard guideline requirements, this two-generation reproduction study contained the following additional investigations: (1) in-depth examinations of brain tissue from the P- and F1-generation adults, as well as the F1- and F2-generation 21-day-old pups, which included qualitative microscopic evaluations and gross and microscopic morphometric analyses; (2) the inclusion of additional microscopic investigative work on the ovarian tissue from the P-generation rats as it related to the identification of infertility in the 3000 ppm group during the conduct of this study; (3) a more in-depth study of the corpora lutea, based on findings from the P-generation ovarian data; and (4) determination of the onset of preputial separation and vaginal patency for F2 pups. There were no effects on food consumption or clinical signs in either generation at any dietary level. Compound-related declines in body weight and body weight gain were evident in the P-generation adult males and females of the 3000-ppm dose group. A slight decrease in body weight and weight gain that was attributed to treatment was also evident in F1-generation adult males in both the 250 and 500ppm dose groups. A marked reduction in fertility was evident at the 3000 ppm dietary level of the P-generation, with only two females delivering viable offspring (one each) and only three implantation sites (compared to 265 for controls). All high-dose (3000 ppm) P-generation females and pups were sacrificed before weaning, since there were too few pups to provide a second generation. There were no test substance-related effects on the mating, gestation, or fertility indices, number of days to insemination, or gestation length at any dietary level in either F1- or F2-generation except for decreased fertility in 3000 ppm P-generation females. No effects were observed on the oestrous cycle length or the number of cycles at any dose in either generation, including the 3000 ppm group of the P-generation. Other than the reduced fertility evident in the 3000 ppm dose group, no other effects were observed on any litter parameter in any dose group in either generation. There was also no effect on any sperm parameter that was attributed to the test substance. At termination, treatment-related findings were evident only in the P-generation at the 3000 ppm dietary level and included: 1) decreased absolute brain weights in males and females; 2)

increased incidence of cerebellar degeneration/necrosis in both genders; 3) statistically increased number of total Corpora lutea measured by quantitative ovarian analysis and increased ovary weights; and 4) increased incidence of uterine horn dilatation. No similar findings were evident in P-generation animals at lower dietary levels or in the offspring from either generation. Based on these results, the reproductive (fertility) NOAEL is 34.4 mg/kg bw/day (500 ppm) and the LOAEL is 231.7 mg/kg bw/day (3000 ppm), based on findings in P-generation animals. No definitive parental NOAEL was established, based on a slight decrease in body weight in the F1-generation males at 16.0 mg/kg bw/day (250 ppm). However, for females, the parental NOAEL was demonstrated to be 36.2 mg/kg bw/day (500 ppm).

Developmental Toxicity

In an OECD Guide-line 414 "Teratogenicity" study, TA was administered by oral gavage to groups of 25 successfully mated and presumably pregnant Bor: WISW (SPF Cpb) rats from day 6 to 15 of pregnancy at daily dose levels of 0, 10, 30, or 100 mg/kg bw/day (Bayer, 1989a; RCC, 2003). There were no mortalities. No treatment-related clinical signs were observed. Mean body weight gain was significantly reduced at 100 mg/kg bw. Fetal weight was reduced at 100 mg/kg bw/day. The incidence of runts was higher at 100 mg/kg bw/day. There were no treatment related malformations. The maternal NOAEL was =30 mg/kg bw/day based on retarded weight gain at 100 mg/kg bw and the developmental NOAEL was= 30 mg/kg bw/day based on an increased incidence of runts and lower fetal weights at 100 mg/kg bw/day.

OECD Guide-line 414 "Teratogenicity" study, TA was administered by gavage to groups of twenty-five time-mated New Zealand White rabbits [Hra: NZW:SPF] in 0.5% aqueous CMC at dose levels of 0, 5, 15, 30 and 45 mg/kg bw/day on days 6 though 28 (Argus, 2004). Five does in the 45 mg/kg bw/day dosage group were sacrificed due to their moribund condition. All other does survived to day 29 of gestation (GD 29). Clinical observations were noted in the 45 mg/kg bw/day group and included decreased motor activity, clear perinasal substance, ptosis, excess salivation and hyperpnoea. Most of these observations occurred in the does that were sacrificed moribund. Additional observations included scant feces, ungroomed coat, head tilt, lacrimation, flared nostrils and cold to touch. Body weight gains were reduced and gravid uterine weights were significantly reduced in the 45 mg/kg bw/day group. Fetal weights were significantly reduced in the 45 mg/kg bw/day group. There were a few alterations of the urogenital system (low set, small, absent kidneys and/or an absent ureter) which occurred in several fetuses of the maternally toxic 45 mg/kg/day dosage group. There were no other dosage-dependent and/or significant differences in the litter or fetal incidences of any gross external, soft tissues or skeletal alterations. Skeletal ossification averages per foetus per litter did not differ among the groups. The maternal NOAEL was = 30 mg/kg bw bw/day based on mortality, reduction in body weight gain, and clinical symptoms at 45 mg/kg bw/day and the developmental NOAEL was = 30 mg/kg bw/day based on lower fetal weights and a slight increase in urogenital alterations for several fetuses at 45 mg/kg bw/day.

In an OECD Guide-line 414 "Teratogenicity" study, TA administered by oral gavage to groups of 25 successfully mated and presumably pregnant Bor: WISW (SPF Cpb) rats from day 6 to 15 of pregnancy at daily dose levels of 0, 100 or 200 mg/kg bw/day in the vehicle (0.5% aqueous Cremophor-EL) (Bayer, 1989b; RCC, 2003). There were no mortalities. No treatment-related clinical signs were observed. Food consumption was not affected. Mean body weight gain was slightly reduced at 100 mg/kg bw/day and markedly lower at 200 mg/kg bw/day. Fetal weight and placental weight were reduced at 100 and 200 mg/kg bw/day. The incidence of runts was higher at 100 and 200 mg/kg bw/day. The incidence of fetuses with minor skeletal deviations was higher at 100 mg/kg bw/day. The number of surviving fetuses per dam was reduced at 200 mg/kg bw/day. The incidence of fetuses with malformations was higher at 200 mg/kg bw/day. The maternal NOAEL was < 100 mg/kg bw/day based on retarded weight gain at 100 mg/kg bw and the

developmental NOAEL was < 100 mg/kg bw/day based on an increased incidence of runts, lower fetal and placental weights, and a higher incidence of minor skeletal deviations at 100 mg/kg bw.

In an OECD 416 “Two Generation Reproductive Toxicity” study, TA was administered continuously via the feed to Wistar Hannover rats (30/sex/dietary level) at nominal dietary concentrations of 0, 250, 500, and 3000 ppm (Bayer, 2005; RCC, 2003; TDMG, 2006). The exposure period was from 10 weeks premating P-generation through 61 days old for F2-pups. The histopathological lesions in the cerebellum were identical to that observed in the sub-chronic and neurotoxicity study in rats and are characterized by variable loss of Purkinje cells, white matter degeneration and gliosis. The white matter tract degeneration presented as nerve fiber (axonal) swelling or fragmentation often with digestion chambers containing debris and macrophage. No similar changes were noted in the 500 ppm animals. The statistical differences associated with certain parameters of F2-pups (pup weights, preputial separation, brain- and spleen weights) are considered to be attributed to an incidental difference in the time of delivery for both the 250- and 500-ppm dietary groups, relative to concurrent controls, rather than exposure to the test substance. The control pups were heavier than the treated group pups and above the range of historic controls because more control dams delivered on gestation day 23 and fewer on day 21 in comparison to the treatment groups. These results, including an extensive investigation of brain morphology, provided no evidence of developmental neurotoxicity at a dietary level of 500-ppm. The developmental NOAEL was > 500 ppm (equivalent to > 35.8 mg/kg bw/day) based on lack of treatment-related effects in F1 and F2 pups at 250 and 500 ppm.

Conclusion

In a two-generation study, TA produced evidence of toxicity in P-generation animals at a dietary level of 3,000 ppm (188.6 and 217.9 mg/kg/day for males and females, respectively), including reduced fertility and neuropathology. By comparison, the only evidence of toxicity at lower dietary levels of 250 and 500 ppm was slightly reduced body weight in F1-generation males. Based on these results, the reproductive (fertility) NOAEL is 34.4 mg/kg bw/day (500 ppm) and the LOAEL is 231.7 mg/kg bw/day (3000 ppm), based on findings in P-generation animals. No definitive parental NOAEL was established, based on a slight decrease in body weight in the F1-generation males at 16.0 mg/kg bw/day (250 ppm). However, for females, the parental NOAEL was demonstrated to be 36.2 mg/kg bw/day (500 ppm).

In an OECD Guide-line 414 "Teratogenicity" study with pregnant Bor: WISW (SPF Cpb) rats the maternal NOAEL was = 30 mg/kg bw/day based on retarded weight gain at 100 mg/kg bw and the developmental NOAEL was = 30 mg/kg bw/day based on an increased incidence of runts and lower fetal weights at 100 mg/kg bw/day.

In an OECD Guide-line 414 "Teratogenicity" study with pregnant New Zealand White rabbits [Hra: NZW:SPF], the maternal NOAEL was = 30 mg/kg bw bw/day based on mortality, reduction in body weight gain, and clinical symptoms at 45 mg/kg bw/day and the developmental NOAEL was = 30 mg/kg bw/day based on lower fetal weights and a slight increase in urogenital alterations for several fetuses at 45 mg/kg bw/day.

In an OECD Guide-line 414 "Teratogenicity" study with pregnant Bor: WISW (SPF Cpb) rats, the maternal NOAEL was < 100 mg/kg bw/day based on retarded weight gain at 100 mg/kg bw and the developmental NOAEL was < 100 mg/kg bw/day based on an increased incidence of runts, lower fetal and placental weights, and a higher incidence of minor skeletal deviations at 100 mg/kg bw.

In an OECD 416 “Two Generation Reproductive Toxicity” study with Wistar Hannover rats, the developmental NOAEL was > 500 ppm (equivalent to > 35.8 mg/kg bw/day) based on lack of treatment-related effects in F1 and F2 pups at 250 and 500 ppm.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute aquatic toxicity test are available for TA.

Acute Toxicity Test Results

Fish

A static study with *Oncorhynchus mykiss,* conducted following OECD Guide-line 203 "Fish, Acute Toxicity Test" resulted in a 96 hour LC_{50} of >100 mg/L (measured) (Wildlife, 2002). A static study with *Salmo gairdneri,* conducted following OECD Guide-line 203 "Fish, Acute Toxicity Test" resulted in an 96 hour $LC5_0$ of >760 mg/L (nominal) (Ciba-Geigy, 1981).

Aquatic Invertebrates

The 48 hour EC_{50} of TA was > 100 mg/L (nominal) for the water flea (*Daphnia magna*) under static conditions following OECD TG 202 (Huntingdon, 1995).

Algae
An OECD TG 201 study was conducted for *Selenastrum capricornutum* exposed to TA (Wildlife, 2001). The 72-hour ErC_{50} and EbC_{50} values were 12 and 13 mg/L, respectively (measured). The 72-hour NOAEC was 3.1 mg a.i./L, based on cell density, biomass and growth rate: In a study conducted comparable to OECD TG 201, *Scenedesmus subspicatus* was exposed to TA (Ciba-Geigy, 1982). The 5-day EC_{50} was= 6.3 mg/L (nominal).

Chronic Toxicity Test Results

In an OECD 215 “Fish, Juvenile Growth test”, based on nominal concentrations and growth rate calculations, the 28-day NOErC for 1,2,4-triazole in rainbow trout is 100 mg/L, the highest concentration tested (Bayer, 2002).

5 RECOMMENDATIONS FOR THE TEST PLAN

All physical/chemical, environmental fate and toxicity and human health endpoints have been met for TA.

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