Ausgabe: September 2001

1,2,5,6,9,10-Hexabromocylodecan (HBCD)

(CAS-Nr.: 3194-55-6/25637-99-4)

Proposal made by the "advisory group on toxicology" on the hazardous substances committee with regard to the classification of Hexabromocyclododecane (HBCD) (CAS Nos. 3194-55-6; 25637-99-4 (mixed stereo isomers))

Genotoxicity:

In vitro:

Hexabromocyclododecane (HBCD) has been tested in a Salmonella typhimurium assay in presence and absence of metabolic activation systems with the tester strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 in doses ranging from 1 up to 5,000 µg/plate (TSCATS, 1990e). HBCD was not mutagenic nor toxic. The same results were obtained in several other respective bacterial tests (Oesch, 1978; Zeiger et al., 1987; Ogaswara and Hanafusa, 1993, EPA, 1990; TSCATS, 1990a; TSCATS, 1990d).

An in vitro gene mutation assay in Saccharomyces cerevisiae with doses from 0.5–50 µg/ plate with and without metabolic activation was also negative (TSCATS, 1990a).

An in vitro mammalian cytogenetic test using human peripheral blood lymphocytes with doses from 10 up to 600 µg/ml in activated and non-activated systems showed no significant increase in structural chromosome aberrations nor in numerical aberrations; toxicity more than 50% was observed in the 2 upper doses (300 and 600 µg/ml) [Gudi and Schadly, 1996].

In an in vitro test with respect to recombination HBCD has been examined at an endogenous locus in mammalian test cells (V79). Treatment was 24 hours with 5 doses ranging from 2 up to 20 µg/ml. HBCD caused a low (2-fold) but statistically significant increase in recombination frequency in this system. This assay cannot be fully assessed since relevant information is missing in the publication, e.g.: purity of the test substance, details of culture conditions (e.g. pH or osmolality may influence the results), observed cytotoxicity. Furthermore, no information is given whether a two-fold increase is convincing as compared to historical control data. Information on the reproducibility of the results, validation of the test system and dose effect response are also missing. The result shows that the recombination activity is rather low and its relevance is questionable (Helleday et at., 1999).

A UDS-assay (TSCATS, 1990b) in primary rat hepatocytes without metabolic activation (dose range 0.05–1,000 µg/well) performed in 1978 according to Williams' method (no further details given) with positive outcome cannot be assessed. The test substance “FM residue” was only described as "brittle, black solid" without any chemical characterization, whereas HBCD is a white crystalline solid. This result will not be regarded in the final assessment. Remark: With this test substance also a questionable positive result in an Ames test was found by the same laboratory.

In vivo:

HBCD (test substance: 85.1% γ-, 8.8% α- and 6.1% β-isomer) was tested according to OECD No. 474 and 92/69/EEC,B12 under GLP conditions for clastogenicity (small micronuclei) and for the ability to induce spindle poison effects (large micronuclei) in NMRI mice using the micronucleus test method. For this purpose, the test substance was administered twice intraperitoneally, with a 24-hour interval between administrations, to male animals at dose levels of 500 mg/kg, 1,000 mg/kg and 2,000 mg/kg body weight. The administration of the test substance led to evident signs of toxicity. According to the results of this study, the intraperitoneal administration of HBCD did not lead to any increase in the number of polychromatic erythrocytes containing either small or large micronuclei. The rate of micronuclei was always in the same range as that of the concurrent negative control in all dose groups and within the range of the historical control data. Thus, HBCD did not have any chromosome-damaging (clastogenic) effect, and there were no indications of any impairment of chromosome distribution in the course of mitosis [BASF, 2000].

Carcinogenicity:

There is one long-term study in mice:
In a 18 months study groups of 50 male and 50 female mice (B6C3F1) received diets containing 0, 100, 1,000 or 10,000 ppm of HBCD (equivalent to about 0, 13, 130 or 1,300 mg/kg bw-d; no information on test substance characterization given) (Kurokawam et al., 1996). Survival and growth rates for the treated groups were similar to controls. Remarkable liver changes were observed in the 1,000- and 10,000-ppm groups. The results of the histopathological examinations are given in the table:

Table:

Summary of histopathological findings in mice fed HBCD for 18 months

Organs	**Male**								**Female**							
- Findings	**Killed**				**Died**				**Killed**				**Died**			
No. of animals examined	**45**[1]	**47**[2]	**45**[3]	**45**[4]	**5**[1]	**3**[2]	**5**[3]	**5**[4]	**48**[1]	**49**[2]	**49**[3]	**49**[4]	**2**[1]	**1**[2]	**1**[3]	**1**[4]
Lung																
- adenocarcinoma	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
- adenoma	5	9	8	5	0	1	0	1	1	5	3	2	0	0	0	0
Liver (gross pathology)																
- nodule	12	21	30	24	2	2	2	2	2	1	5	6	0	1	0	0
- small ponctate on surface	5	7	12	5	0	0	0	0	2	0	0	0	0	0	0	0
Liver																
- hepatocellular carcinoma	12	17	25	13	2	2	2	2	0	0	1	5	0	1	0	0
- hemangioma	2	2	2	0	0	0	0	0	1	0	0	1	0	0	0	0
- altered foci	20	20	41	25	3	1	2	1	12	6	10	10	0	0	0	0
- liver cell swelling	29	31	43	39	3	3	3	2	37	39	41	42	2	1	1	1
- vacuolization	8	19	4	4	2	1	2	1	17	18	18	9	1	0	0	1
- fatty change	0	3	9	4	0	0	1	0	0	0	0	0	0	0	0	0
- mixed/vacuoli. + fatty change	8	9	31	20	0	0	0	0	17	18	20	28	0	1	0	0
- necrosis	14	19	27	15	0	0	1	0	12	6	10	8	1	0	0	0
- cyst	3	5	7	3	0	0	2	2	0	0	0	0	0	0	0	0
Kidney																
- adenocarcinoma	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Spleen/Lmyph node																
- leukemia/lymphoma	2	1	2	0	0	0	0	1	0	1	2	0	1	0	1	0
Ovary																
- granulosa cell tumor	-	-	-	-	-	-	-	-	0	0	0	1	0	0	0	0
Uterus																
- cystic change	-	-	-	-	-	-	-	-	31	27	21	22	0	0	0	0
Skin																
- adenocarcinoma	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0
- malignant lymphoma	0	0	1	0	0	0	0	2	0	1	2	0	0	0	0	0
- adenoma	0	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0
- fibroma	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
No. of effective (tumor) mouse	14	19	27	13	2	3	3	3	2	8	6	8	1	0	1	0

[1] = 0 ppm [3] = 1,000 ppm

[2] = 100 ppm [4] = 10,000 ppm

There was no correlation between the dosage and the incidence of neoplastic changes in the liver. The number of altered foci (preneoplastic lesions) was comparable in all groups. The higher number of hepatocellular tumors in the females in the high dose group (10%) is considered to be by chance since the percentage is clearly within the range of historical control data. Maronpot et al. (1999) give a range from 0 – 20% for females, the corresponding control data for males were 6–29%. With respect to lung tumors, all the tumors were benign except 1 case of adenocarcinoma in the 100 ppm group. These benign tumors are often observed with advancing age in this strain of mice, and there was no correlation with the dosage. Spontaneously induced tumors were also reported by J.K. Haseman (1985) in the liver of the B6C3F1 strain of mice with a range of 0–15% for females and of 8–32% for males and by Maekawa (1981) as cited by the authors.

Various types of tumors were observed in different organs, but the incidences were sporadic and these were not due to HBCD administration.

The authors concluded that there is no carcinogenicity by HBCD in mice after oral administration mixed in diet for 18 months at the dosage of 100, 1,000 and 10,000 ppm as the experimental condition of this study.

The study cannot be validated since no details with respect to methods and results are given in the paper by Kurokawam.

Reproductive Toxicity:

Effects on fertility:

In the absence of information from multigeneration studies, results from repeated dose assays and from a pre-, peri-, postnatal study are used for assessment:

In a 28 day feeding study with doses of 0, 10,000, 25,000 and 50,000 ppm (ca. 830, 2,080 and 4,170 mg/kg bw-d) the animals in the extreme high dose group (> 4,000 mg/kg bw-d) showed a significant, but questionable inhibition of oogenesis with distinctly reduced numbers of mature and developing follicles present in ovaries. The male sex organs (testes and epididymes) showed normal differentiation and no inhibition of spermiogenesis (Zeller and Kirsch; 1969). – In another 28 day study (WIL Research Lab., 1997) according to OECD guideline No. 407 in the highest dose group (1,000 mg/kg bw-d) no effect on oogenesis or other function/organs of the reproductive system was discovered.

In a 90 day feeding study no histological or other changes in the sex organs (testes, epididymes and ovaries) were seen (Zeller and Kirsch; 1970) up to the highest dose group (12,800 ppm ≙ 1,070 mg/kg bw-d).

In the pre-, peri- and postnatal developmental toxicity study described below (Murai et al. 1985) no influence on female fertility was observed in animals treated from day 0 to day 20 of pregnancy. The numbers of corpora lutea, of implants and of resorbed, dead and live fetuses were not affected.

Developmental toxicity:

There are two developmental studies in rats:

In a pre-, peri-, postnatal developmental toxicity study rats were given 0, 100, 1,000 and 10,000 ppm HBCD in the diet (≙ 5; 50; 500 mg/kg bw/day) during day 0–20 of gestation (Murai et al., 1985). In the highest dose group food intake was slightly suppressed and liver weights (absolute and relative) were significantly increased. No significant change in the number of corpora lutea, number of implants, number of resorbed, dead or live fetuses was reported nor were there any effects on the body weight of live fetuses, the incidence of external, visceral or skeletal anomalies in the offspring or on delivery, nursing, lactation, or neonatal development. No abnormality in parturition, weaning status or growth of newborns was observed at the highest dose of 500 mg/kg. Though there were only 14 of 20 animals were used for the teratology assessment and only 1/3 of the fetuses have been examined for visceral anomalies the study results can be regarded as valid with restriction.

In a recent prenatal toxicity study according to OECD No. 414 (WIL Research Lab., 1999) rats were exposed from day 6 – 19 of gestation in dosage levels of 250, 500 and 1,000 mg/kg body weight/per day in corn oil by gavage. No treatment related clinical signs were observed at any dose level. Body weight gain and food consumption were not adversely affected at any dose level. At necropsy no treatment related findings were observed. Intrauterine growth and survival were not affected by the test substance and no treatment-related fetal malformations and developmental variations were observed in any of the treated groups. The NOAEL for maternal developmental toxicity was found to be 1,000 mg/kg bw.

Conclusion:

Genotoxicity:

In conclusion HBCD did not show a genotoxic potential in in vitro point mutation assays (Ames test), in an in vitro gene mutation assay in Saccharomyces cerevisiae, in an in vitro chromosome aberration test in mammalian cells nor in an in vivo micronucleus test in mice. The weakly positive outcome of an recombination assay is of questionable relevance. Thus, HBCD can be regarded as non-genotoxic; according to EU criteria the substance has not to be classified (M: -).

Carcinogenicity:

In a 18 month feeding study in mice with restricted validity no evidence of carcinogenicity was found in doses up to 1,300 mg/kg bw-d. Since this study cannot be validated, classification according to EU criteria with respect to carcinogenicity is not possible (C: -).

Reproductive Toxicity/Fertility:

In repeated dose studies (28- and 90 d) up to 1,000 mg/kg bw there was no impairment of sex organs. Only in an 28-day study with the extreme dosage of > 4,000 mg/kg bw an inhibition of oogenesis was observed. In a pre-, peri-, postnatal development study no impairment of female fertility was observed. According to EU-criteria the substance has not to be classified (R_F: -).

Reproductive Toxicity/Developmental Effects:

From two developmental toxicity studies in rats it can be concluded that HBCD showed no adverse effects on prenatal development. According to EU-criteria the substance has not to be classified (R_E: -).

References:

[1] BASF, 2000: Cytogenetic Study in vivo with Hexabromcyclododecane in the Mouse Micronucleus Test after two Intraperitoneal Administrations; report November 2000; Experimental Toxicology and Ecology, BASF Aktiengesellschaft, 67056 Ludwigshafen, Germany; Project No. 26M0100/004018

[2] EPA (US Environmental Protection Agency) (1990): Hexabromocyclododecane – CAS No.: 3194-55-6. Letter from Ameribrom Inc. EPA/OTS Doc #86-900000433

[3] Gudi, R. and Schadly, E.H., 1996: Chromosome Aberrations in Human Peripheral Blood Lymphocytes. Microbiological Associates, Inc. Rockville, MD. Study No. G96A061.342. Sponsor: Chemical Manufacturers Association, Arlington, VA

[4] Haseman, J.K. et al., 1985: JNCI, Vol. 75, November 1985

[5] Helleday, T., Tuominen, K.L., Begman, A. and Jenssen, D., 1999: Brominated flame retardants induce intragenic recombination in mammalian cells. Mutation Research 439, 137 - 147

[6] Kurokawam, Y., Inoue, T., Uchida, Y. and Momma, J. (1996): Carcinogenesis test of flame retarder hexabromocyclododecane in mice. Department of Toxicology, National Public Health Research Institute, Biological Safety Test and Research Centre (not published, translated into english)

[7] Maekawa, A. et al., 1981: Science forum. Mutagens and Toxicity 4, No. 4, 22 (cited in “Kurokawam”)

[8] Maronpot, R.R., 1999: Pathology of the Mouse. Reference and Atlas. National Institute of Environmental Health Sciences, Research Triangle Park, NC; Cache River Press, USA

[9] Murai, T., Kawasaki, H., Kanoh, S., (1985): Studies on the toxicity of insecticides and food additives in pregnant rats (7). Fetal toxicity of hexabromocyclododekane. [Satchuzai oyobi shokuhin tenkabutsu nadono ratto ni okeru taishi dokusei ni kansuru kenkyu (Dai 7-ho). Hexabromocyclododecane no taishi dokusei.] Pharmacometrics 29, 981 – 986 (in Japanese; English translation available)

[10] Oesch, F., 1978: Ames Test for Hexabromid S. Unpublished report, Sponsor BASF

[11] Ogaswara, S. and Hanafusa, T. (1993): Report on mutagenicity test on pyroguard SR-103 using microorganisms (not published; cited in “Risk Assessment”)

[12] Risk Assessment (EU), Draft of March 5, 1999 (Kemi, Sweden)

[13] TSCATS, 1990a: NTIS/OTS 05-23257

[14] TSCATS, 1990b: NTIS/OTS 05-22234

[15] TSCATS, 1990c: NTIS/OTS 05-23259

[16] TSCATS, 1990d: NTIS/OTS 05-22948

[17] TSCATS, 1990e: NTIS/OTS 05-23254

[18] WIL Research Laboratories (1997): A 28-day repeated dose oral toxicity study of HBCD in rats.

[19] WIL Research Laboratories (1999): A prenatal developmental toxicity study of Hexabromocyclo-dodecane (HBCD) in rats.

[20] Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K. and Speck, W., 1987: Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environmental Molecular Mutagenesis 9 (Suppl. 9): 1 - 10

[21] Zeller, H. and Kirsch, P. 1969: Hexabromocyclododecane: 28-day Feeding Trials With Rats. BASF Institute for Industrial Hygiene and Pharmacology. Federal Republic of Germany. EPA/OTS Doc. #86-900000376

[22] Zeller, H. and Kirsch, P., 1970: Hexabromocyclododecane: 90-day Feeding Trials With Rats. Pharamkologisches Institut, BASF Institute for Industrial Hygiene and Pharmacology, Federal Republic of Germany. EPA/OTS Doc #86-900000380.

Stand: Mai 2001