baua:
Bundesanstalt für Arbeitsschutz
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Federal Institute for Occupational
Safety and Health

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DE-CA Comment on Carcinogenicity

Substance name:	**hexythiazox** (ISO); trans-5-(4-chlorophenyl)-N-cyclohexyl-4-methyl-2-oxo-3-thiazolidine-carboxamide
CAS Number:	78587-05-0
EC Number:	-

Carcinogenicity: In his analysis, the DS does "not consider the causality between hexythiazox treatment and the slight increases in the incidences of mammary gland fibroadenoma and parafollicular cell adenoma (benign tumours) in high dose F344 male rats credible" thus proposing no classification for carcinogenicity. Upon assessment of the available data from long term animal studies, we found also arguments that Category 2 (suspected human carcinogen) classification might be applicable. It is acknowledged that consideration can be made in support of both, classification in Cat. 2 as well as non-classification.

Argumentation:
In the 2-year rat study there was a dose dependent increase ((p<0.01, p.37 of CLH report) in incidences of fibroadenoma of mammary gland in treated males, with the following frequencies: 0/50 at 0 ppm, 1/56 at 3.2 mg/kg, 1/59 at 23 mg/kg and 6/53 at 163 mg/kg bw/d at terminal sacrifice.
Fibroadenomas are the most common benign neoplasm of the mammary gland in female laboratory rats. In general, this tumour type has not been described in literature as a common tumour in male Fisher 344 rats. Nevertheless, according to a recent review, background incidence is 1–2% in male rats, regardless of strain, although incidences as high as 11% in Sprague-Dawley and Wistar or 13.4% in F344 were reported (*Russo (2015) Significance of Rodent Mammary Tumors for Human Risk Assessment. Toxicol Pathol. 2015 Feb; 43(2): 145–170. doi: 10.1177/0192623314532036*). Occurrence of fibroadenomas at the top-dose male rats (11.3% incidences in terminally sacrificed and 9% for all animals) falls within the upper range of the above reported background incidences. The rate also corresponds to the higher end of the distribution observed for this type of tumour in the National Toxicology Program, USA (0-12%) (Haseman et al., 1985 and 1998) and exceeds average rate (4.3%) observed in the NTP laboratories (Haseman et al 1998). When compared to historical data originating from the study performing laboratory for the period between 1986 and 1995 with a narrower incidences distribution, the incidence rate observed in the current study is, however, exceeding the range of 0-6.0% and the average incidence rate of 2% observed in control male rats (See table 1 below).

Comparison to the appropriate concurrent historical control data is not possible as submitted historical control data do not meet the EU data requirements.

The relevance of fibroadenomas as a single neoplastic finding in the mammary gland of rats has been subject to debate since it is common in the rat but rather rare in humans. Progression to malignancy was not observed (only

1 of 67 at the top dose with adenocarcinoma (1.5%)). In addition, data on mutagenicity included in the report did not show any evidence of genotoxic potential for hexythiazox. It should be noted, however, that pathogenesis of fibroadenoma has been linked to an endocrine (estrogenic) mode of action (Russo, 2015). Notably, absolute and relative testis weight was dose dependently increased and frequency of benign interstitial cell tumour in testis was 0/0/10/30 % at interim sacrifice but close to 100% for all dose groups at study termination. No mechanistic in vivo endpoints (e.g. reproductive hormones measurements) were investigated in the study.

Table 1. Incidences of fibroadenoma of mammary gland in male Fischer 344 rats (the CLH Report and open literature).

Tumour type	Concurrent control, 1981-1983, incidence rate, %	Data from performing laboratory (1986-1995), 10 studies, rate(%)/ range (%)	NTP, Haseman 1998 (7 years, 3 laboratories, 27 NTP feeding studies), rate(%)/ range (%)	NTP Haseman 1985 (5 years, 37 feeding studies, (?)laboratories), rate(%)/ range (%)	NTP, Haseman 1983 (4 laboratories, 25 NTP feeding studies), rate(%)/ range (%)
Mammary gland fibroadenoma	0	2/0-6.0	4.3/0-12.0	3/0-12.0	Not described

The other type relevant for classification (in the same species and the same sex) was thyroid gland with an increase in incidences of parafollicular cell adenomas (C-cell adenoma) in males (11.3% in terminally sacrificed and 10.3% for all animals) in top dose (3000 ppm). The thyroid parafollicular cell adenomas are benign tumours which show no tendency to malignity in the present study: parafollicular cell carcinoma was observed only on a single occasion, in the mid-dose treated group (1.4%). According to the CLH report, the incidence of thyroid parafollicular cell adenoma was slightly and statistically not significant increased in males of the high dose group at termination of the study (10.3% vs 4.3% in control). Although no clear dose response was found by the authors of the report (incidences 4.3%, 4.3%, 2.9%, 10.3% at 0, 3.2, 23 and 163 mg/kg bw/d), a trend testing (Cochrane-Armitage) for incidences of parafollicular cell adenomas in males at sacrifice demonstrates a significant dose-dependent increase ($p=0.0334$). While according to the CLH report, the increase was not statistically significant in comparison to control group, we noticed in a confirmatory Chi-square test that the test had low power (0.34 vs desired 0.8), thus negative result should be interpreted with caution. Comparison to the appropriate concurrent historical control data is not possible as submitted historical control data do not meet the EU data requirements.
Observed rate (10.3%) was above the rate reported for in the National Toxicology Program, USA (8.2%) (Haseman et al 1983) but within the range (0-12%) of other historical control data (Haseman et al., 1985). When compared to the incidence range of parafollicular cell adenoma (0-12.0%) observed in the performing laboratory between 1986 and 1998, comprising 10 studies, the values of the current study correspond to the top end of the range. It is noted that this range has a median incidence rate of 0.0%. Further available historical control ranges are presented in the table 2 below.
Worth to note that male rats receiving 3000 ppm (top dose) did not show signs of excessive toxicity as indicated by absence of clinical signs, MTD-defined 10% body weight reduction and increase in food consumption, thus carcinogenic response in top dose group was observed in the range of but not above the MTD.

Table 2. Incidences of parafollicular cell adenoma for Fischer 344 male rats (CLH report and open literature)

Tumour type	Concurrent control, 1981-1983, incidence rate, %	Data from performing laboratory (1986-1998), 10 studies, rate(%)/ range (%)	NTP, Haseman 1998 (7 years, 3 laboratories, 27 feeding studies), rate(%)/ range (%)	NTP Haseman 1985 (5 years, 37 feeding studies, (?)laboratories), rate(%)/ range (%)	NTP, Haseman 1983 (4 laboratories, 25 feeding studies), rate(%)/ range (%)
C-cell adenoma	4.3%	2.7/0-12.0 Mean: 2.67 SD: 3.21 Median: 0.0	13/2-35.0	5.7/0-18.0	8.2/ Not reported
C-cell-carcinoma	0	0.8/0-6.0 Mean: 0.77 SD: 1.914 Median: 0.0	1.8/0-6.0	3.5/0-12.0	

Notably, the occurrence of both tumour types discussed above was limited to the male sex and to one species. Furthermore, both tumour types are benign tumours which showed no tendency to malignancy in the study.

Specific comments:

4.9.1.1 Carcinogenicity: oral, Table 24, page 37. Could the DS state in the legend to the table what used abbreviations (DOS and SAC) exactly mean? Would it be correct to conclude that "SAC" states for sacrificed and "DOS" for animals that died spontaneously (death and unscheduled sacrifice)? Why in the group of male rats treated with 3000 ppm hexythiazox, number of animals analysed for certain tissues varies (16 for adrenal and 14 for mammary gland)?

4.9.1.1 Carcinogenicity: oral, Table 24, page 37. Which type of statistical trend test was applied for analysis of incidences of fibroadenoma of mammary gland in male rats?

4.9.1.1 Carcinogenicity: oral, Table 24, page 41. Which type of statistical test was applied for analysis of parafollicular cell adenomas in male rats?

We agree with DS argumentation against classification based on an increase in the interstitial cell tumours observed in male rats after 12 month. Leydig cell tumours is a common histological finding in F344 rats (Guidance for the application of CLP criteria, 2017). Due to a high background rate in the control group by the end of the study, no effect related to treatment could be distinguished.

In a 2-year study in mice, a statistically significant increase in incidences of hepatic adenoma in female mice (22.9% in 1500 ppm group vs 10% in Ctrl group) was observed. Comparison to the appropriate concurrent historical control data is not possible as submitted historical control data do not meet the data requirements. In this study a B6C3F1/Slc strain of mice was used. The background strain B6C3F1 is characterised by high background rate of this hepatic adenomas (2-50 % range and average of 17.3%), but available data were collected from different facilities under the National Toxicology Program, USA (Haseman et al 1998), thus represent only relative reference point. Data provided by a notifier, collected in a different time-frame than the study period but

in the same facility, indicate that observed incidences are exceeding average rate (14.7%), but stays within the HCD range of available studies (8-32%).

However, an increase in incidences of hepatoblastoma observed in male (4.3%) and female (1.4%) mice in top dose group are exceeding data collected from NTP, USA (range 0-2%, occurrence rate 0.1% for males and females) and from in-house facility (range 0-2%, occurrence rate 0.4% in males and 0% in females) collected in non-representative time frame. Comparison to the appropriate concurrent historical control data is not possible as submitted historical control data do not meet the data requirements.