Application of criteria in 3.9.1.1 and 3.9.1.6 showing that a substance can be classified into both specific target organ toxicity (repeated exposure) hazard class, for non-lethal effects, and into the acute toxicity hazard class, for lethal effects

Case 2

Information on substance

Data

Acute toxicity animal data

Route	Species	LD_{50} /LC_{50} Value	Observations
Oral	Rat	In two OECD 401 studies values were 160 and 273 mg/kg	• Effects were observed in the lungs (haemorragies) and stomach
Dermal	Rat	404 mg/kg	• No observations reported
Inhalation (Dust/Mist)	Rat	In two 4-hour studies the LC_{50} values were 0.115 and 0.139 mg/L	• No observations reported

Specific target organ toxicity – repeated exposure

(a) Study 1: Oral route of exposure

90-day OECD TG 408 equivalent study

30 Sprague Dawley rats (15 male/15 female); Oral dose levels: 0, 1.9, 6, 17.5 mg/kg bw/day

Dose level (mg/kg bw/day)	Observations
17.5	• Death (28 animals died or were sacrificed between days 18 and 34 and remaining animals by day 36) with severe clinical signs of toxicity including o Tremors, convulsions and aggression/hypersensitivity/difficulty when handling o Appeared to be weak, thin and dehydrated o Cold to touch o Lying on their sides and decreased home-age activity levels • Reduced body weight, • Decreased food and water intake, • Blood biochemical changes, • Behavioural effects, • Neuropathological lesions (mild ventricular dilation and moderate neuronal necrosis) • Decrease in absolute and relative thymus weight was observed at the interim sacrifice (week 4) evaluation for the males. • Several preterminal non-perfused animals with small thymus and/or spleen
6	• Death in 1 animal, with abnormal clinical signs limited to: o Tremors, hypersensitivity (difficulty when handled), o Thin dehydrated for the animal that died, o Transitory dehydrated appearance in another animal, and o Hypersensitivity, convulsions and reduced activity for another animal • Reduced body weight (males only) • Decreased food and water intake • Motor activity was reduced (females only), • Neuropathological lesions were observed • Gross pathological findings include smaller absolute and relative thymus and/or spleen weight at the terminal sacrifice • Terminal evaluations indicated possible treatment-related lymphoid atrophy of the thymus
1.9	• No mortality • Findings were limited to: o Reduced food (males only) and water intake o Neuropathological lesions (slight to moderate vacuolization in the brain and spinal cord tissue) • The NOAEL is considered to be less than this dose level.

(b) Study 2: Oral route of exposure

90-day Oral OECD TG 408 Study

16 Wistar rats (8 male/8 female); Oral dose levels: 0, 0.07, 0.4, 1.0 and 17 mg/kg bw/day

Dose Level (mg/kg bw)	Result
17	• Death in 3 animals during the first month and most remaining animals showed severe neurological and neurobehavioral signs, including tremors, convulsions and increased foots play. All remaining animals of this group were sacrificed. • Mean body weights for males were significantly lower • Upon microscopic examination, changes were observed in the brain, the kidneys and thymus. o Females had increased incidence of corticomedullary haemorrhage in the thymus and most showed cortical lymphoid depletion in the thymus o All animals showed decreased accumulation of brown pigment in the spleen. o Pronounced neuronal death in a number of areas of the cerebellum was more pronounced in females. The areas with predominant lesions were the hippocampal region, the piriform, entorhinal and perirhinal cortices and amygdala, the olfactory nuclei and the tenia tecta. Also, a slight increase in swollen axons in the spinal cord was observed.
1.0	• No clinical signs • Microscopic examination revealed no changes compared to the control group • The NOAEL is placed at this level.
0.4	• No clinical signs
0.07	• No clinical signs

Answer

Acute oral toxicity, Category 3

Acute dermal toxicity, Category 3

Acute inhalation toxicity, category 2

Specific target organ toxicity – Repeated exposure, Category 1

(Target organs: Nervous system, thymus)

Rationale

(a) Acute oral toxicity

Classification via application of criteria in GHS Table 3.1.1 is possible. The study used the preferred test species (i.e., rat) as noted in paragraph 3.1.2.3 and the 2 Oral (rat) LD_{50} between 160 and 273 mg/kg are both within the Category 3 range of $50 < ATE \leq 300$ resulting in a Category 3 classification via the oral route.

(b) Acute dermal toxicity

Classification via application of criteria in GHS Table 3.1.1 is possible. The study used a preferred test species (i.e., rat or rabbit) as noted in paragraph 3.1.2.3 and the Dermal (Rat) LD_{50} of 404 mg/kg is within the Category 3 range of $200 < ATE \leq 1000$ resulting in a Category 3 classification via the dermal route.

(c) Acute inhalation toxicity

Classification via application of criteria in GHS Table 3.1.1 is possible. The study used the preferred test species (i.e., rat) as noted in paragraph 3.1.2.3 and the two 4-hour inhalation (dust/mist) (Rat) LC_{50} values of 0.115 and 0.139 mg/L are within the Category 2 range of $0.05 < ATE \leq 0.5$ resulting in a Category 2 classification via inhalation.

(d) Specific target organ toxicity – Repeated exposure

Oral route

Together, these two oral 90-day studies indicate that the main target organ is the nervous system. Deaths and severe neurological signs occurred from 6 mg/kg bw/day in Study 1 and at 17 mg/kg bw/day in Study 2. In Study 1, neuropathological lesions were observed from the lowest dose at 1.9 mg/kg bw/day as evidenced by moderate vacuolization in the brain and spinal cord tissue as well as ventricular dilation and neuronal necrosis at the highest dose (17.5 mg/kg bw/day).

The absolute and relative weights of the thymus were reduced in the 6 mg/kg bw/day dose level in Study 1 and there were indications of possible treatment-related lymphoid atrophy of the thymus. In Study 2, females had an increased incidence of corticomedullary haemorrhage in the thymus and most of these high-dose females also showed cortical lymphoid depletion in the thymus at the 17.5 mg/kg bw/day dose level. Thus, both studies support including the thymus as a target organ.

Classification via application of criteria using the guidance values provided in GHS Tables 3.9.1 and 3.9.2 is possible for the nervous system and thymus effects. The guidance dose value for a Category 1 classification in an oral rat 90 day study is ≤ 10 mg/kg bw/day. The effects seen in the thymus (and to a lesser extent in the spleen) as well as in the nervous system in the two rat 90-day studies (i.e., 6 mg/kg bw/day) justify classification in STOT RE Category 1 according to the GHS criteria with the central nervous system and the thymus as the specific target organs/systems.

(Reference document: ST/SG/AC.10/C.4/2020/14, example 4, as amended by informal document INF.32 (39th session))