Minority opinion on the classification of tribenuron-methyl (ISO), CAS 101200-48-0 for STOT RE 2 hazard class and category

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General considerations

According to the CLP regulation (1907/2006) target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. Classification for target organ toxicity (repeated exposure) identifies the substance as being a specific target organ toxicant and, as such, it may present a potential for adverse health effects in people who are exposed to it. These adverse health effects include consistent and identifiable toxic effects in humans or in experimental animals, which can be considered relevant for human health.

The CLP regulation states that "Substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement, on the basis of the weight of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s)." The use of expert judgement and weight of all evidence and relevance to human health is further emphasized later in the CLP regulation.

The guidance values given in the CLP regulation for STOT RE classification are intended only for guidance purposes, i.e. to be used as part of the weight of evidence approach. The guidance values refer to effects seen in a standard 90-day toxicity study conducted in rats. Even though they can be extrapolated for toxicity studies of greater or lesser duration using Haber's rule, this assessment shall be done on a case-by-case basis. As mentioned in the CLP regulation, Haber's rule assumes that the effective dose is directly proportional to the exposure concentration and the duration of exposure.

Morbidity or death resulting from repeated or long-term exposure can be taken into account in STOT RE classification. Morbidity may be relevant at relatively low doses when it is due to bioaccumulation of the substance or its metabolites, and/or due to the overwhelming of the de-toxification process by repeated exposure to the substance or its metabolites. When morbidity is caused by bioaccumulation, Haber's law can be considered to apply. These cases need, naturally, to be distinguished from the morbidity resulting from short term exposure to high, excessive doses.

Tribenuron-methyl

RAC has proposed to classify tribenuron-methyl as STOT RE 2 on the basis of two pilot developmental study in the rabbit, made for dose-finding purposes. In the first of these studies, all maternal animals died at the doses of 500 mg/kg and 750 mg/kg. No further information on the cause of death, signs and symptoms, or on the exact day of death were available. Thus, it is unknow if animals died already after a couple of doses, reflecting rather an acute toxicity in rabbits than specific target organ toxicity after repeated exposure. In this study, severe toxicity was observed also with a dose of 250 mg/kg, which was lowered to 125 mg/kg after five doses. In the second rabbit pilot study, two deaths were observed with a dose of 150 mg/kg. Furthermore, these high dose rabbits (dosed with 150 mg/kg bw) in this second pilot study were reported to suffer from severe weight loss, but no further information on the cause of death, other signs or symptoms or exact day of the deaths were provided. Overall, there is no information available on several key aspects that would have allowed proper evaluation of the reliability and relevance of the two pilot rabbit studies, including the purity of the test compound, the day(s) on which the mortalities occurred and the clinical observations and/or causes of deaths.

In the full developmental toxicity study at the highest dose (80 mg/kg bw) two animals were found dead. Findings in the first dead high-dose animal included reduced food consumption, body weight loss, stomach distended with food, lack of formed faeces and widespread hepatisation of lungs. It should be noted that the typical cause for hepatization of the lungs is local pneumonia. The second rabbit suffered from reduced food consumption, body weight loss and alopecia. Its tail was stained and red discharge was found in the cageboard, in addition, one paw appeared injured. This rabbit also aborted its fetuses at GD29. The signs and symptoms in these two rabbits that died in the high dose group were rather unspecific and differing and did not suggest a common, substance specific mechanism. Food consumption in the whole high dose group was reduced 48%. Only reduced food consumption and body weight loss seem to be a common finding in these two animals. There was one additional dead rabbit dosed with the mid-dose (20 mg/kg). This animal was found to have multiple mucosal haemorrhages in the stomach associated with a hairball. It was considered by the RAC that this death is not likely to be treatment-related and therefore not relevant for classification.

Tribenuron-methyl has been widely tested also in other animal species, including rat, mouse and dog. There are altogether 10 repeated dose or long term studies in these three species reported in CLH report. All of them show no or very little toxicity after repeated or long term exposure. In these species, no deaths have been observed even at the highest doses tested, at ≥300 mg/kg in 90 day studies or at ~50-250 mg/kg in 1-2 years studies. RAC considered that effects on liver (rats/mice), spleen (rats), blood parameters and thyroid (dogs) seen in some of these studies were not severe enough to warrant classification.

In addition, there was a single 28-day dermal toxicity study in the rabbit, in which adverse effects and mortality were seen at a very high dose of 1000 mg/kg bw. The clinical effects seen were mainly local effects on the skin. Due to the high dose, the human relevance of these effects is questionable.

Conclusion

As described earlier, STOT RE classification should be based on the use of expert judgement and the weight of all available evidence. In this case, RAC decided to base its STOT RE classification in two extremely poorly reported pilot rabbit developmental toxicity studies, in which deaths were seen at doses ≥150 mg/kg and with exposure up to 13 days. These limited quality pilot rabbit studies were used even though there were no information on the symptoms or causes of deaths or on whether the animals were found dead soon after the start of the dosing (suggesting rather an acute toxicity) or only after the whole dosing period. Guidance values were extrapolated to 13 days study by using Haber's law without further consideration on the applicability of Haber's law in this case. This was done regardless of considerable negative evidence from rats, mice and dogs showing no significant toxicity or deaths at higher doses in longer term studies. Neither did the main developmental toxicity study in rabbits at the doses up to 80 mg/kg provide support for any substance related effects, which can be considered relevant for humans. This approach selected by RAC for the classification of tribenuron-methyl for STOT RE 2 does not seem to follow the weight of evidence approach which should be used for the classification of STOT RE.

Furthermore, rabbits are known to be very sensitive to gastrointestinal (GI) imbalances due to substances which disturb the gut microflora and cause diarrhoea and reduced food consumption. These substances may include e.g. bactericidal/fungicidal substances, poorly soluble and irritant/corrosive substances. These GI imbalances may result in death of rabbits. These effects are species-specific and therefore not relevant for humans and should not be used for regulatory purposes (Reuter et al., 2018, 46th Annual Meeting of the European Teratology Society 10.-13.09.2018, Berlin, Germany). In the case of tribenuron-methyl, there is limited information

available on the GI symptoms of the rabbits at the doses causing rabbit deaths due to the very poor reporting of the pilot studies. However, in the main developmental study, the first dead animal at the dose of 80 mg/kg showed stomach distended with food and lack of formed faeces together with body weight loss and reduced food consumption. Body weight loss and reduced food consumption was reported also in the other dead animal in the same study and severe weight loss was reported in the second pilot study at the dose of 150 mg/kg bw. When taking into account that other three animal species (rats, mice and dogs) did not show any mortality at relevant dose ranges, rabbit seems to be extremely sensitive to tribenuron-methyl when compared to other species. Although due to the poor reporting of the pilot studies there is no further information on the possible GI symptoms, this might provide a plausible mechanism explaining the deaths seen in rabbits.

Overall, using the weight of all evidence as proposed in CLP regulation, tribenuron-methyl should not to be classified as STOT RE 2 on the basis of these rabbit findings.