Comments of Troy Chemical Company B.V. on the CLH report of Isoproturon (25 January 2016)

Background

In the CLH Report for Isoproturon (November 2015), harmonised classification with Repr. 2; H361f according to Regulation (EC) No 1272/2008 (CLP) has been proposed for consideration by the Committee for Risk Assessments (RAC) on the basis of the outcomes of two key generational dietary reproduction toxicity studies in rats (Becker *et al*. (1989) TOX9551913, Bhide (1991) TOX9651099[1]) which fulfill relevant regulatory requirements, and additionally, under consideration of a published exploratory study in rats by Sarkar *et al*. ((1997) ASB2012-14739). The suggested classification is based on potentially reduced male fertility.

Comparative assessment of regulatory key study results (Becker *et al*. (1989), Bhide (1991))

Referring to the results of the key generational reproductive toxicity studies, the only histopathological finding pointing towards impaired male fertility is "retarded spermatogenesis" noted in the study by Bhide (1991) at low incidence in F_1 generation males only. In this study, retarded spermatogenesis was noted at low incidence in 1/15 mid dose F_1 males (200 ppm, corresponding to 20 mg/kg bw/d[2]) and 3/15 high dose F_1 males (400 ppm, 40 mg/kg bw/d) only. There were no corroborative macroscopical or histopathological findings, or changes in male sex organ weights in this study.

Of note, in the second regulatory key two-generation reproductive toxicity study by Becker *et al*. (1989), there were no Isoproturon-related histopathological findings in reproductive organs of high dose male rats of the same strain (25 F_0 and 25 F_1 males) at the considerably higher maximum dietary concentration of 2000 ppm (at least 129 and 113 mg/kg bw/d for 25 F_0 and 25 F_1 males, respectively).

Almost identical no-observed-effect-levels (NOELs) of 80 ppm (5 - 10 mg/kg bw/d, Becker *et al*. (1989)) and 100 ppm (10 mg/kg bw/d, Bhide (1991)) were established at the low dose level in both

[1] Two additional two-generation reproduction toxicity studies in rats were provided by the same author (Bhide (1990) TOX9300293, Bhide (1991) TOX9500349). These supplementary studies with methodological deficiencies support the findings of the key study including retarded spermatogenesis (lower or similar incidence as compared to the key study) at parentally toxic dietary concentrations, and are thus not further referenced.

[2] Converted by means of factor of 0.1, in line with the CLH Report for Isoproturon (November 2015).

studies based on parental toxicity, as evidenced by decreased body weight gain in conjunction with decreased food consumption. At the parentally toxic higher doses, endpoints which are indicative of reproductive toxicity were altered. As compared to concurrent controls, there were reductions in mating index, pregnancy rate, number of implantations, litter size, and pup body weight (Becker *et al*. (1989)) as well as lower mating index and decreased pup body weight gain (Bhide (1991)).

Taking together the outcomes of both key studies which compare favorably in most endpoints assessed, the weight of evidence is considered to indicate that the finding of retarded spermatogenesis in few F_1 animals noted in one of these studies only (Bhide (1991)) can be regarded either as a finding of negligible toxicological significance which should be re-assessed in a pathology peer review, or as spontaneous in nature (incidental finding).

Assessment of a published exploratory study (Sarkar et al. (1997))

The study by Sarkar *et al*. (1997) has relevant reporting and methodological deficiencies[3] and is therefore considered to be scientifically valid with limitation. Due to these deficiencies, the results of this study do not represent an adequate basis for decisions on classification of Isoproturon. With regard to repeated dose oral exposure of rats in a sub-chronic time frame, very high dose levels of 200, 400 and 800 mg/kg bw/d were administered *per os* (presumably by oral gavage) on 6 days/week for 10 weeks. This corresponds to 20, 40 and 80 times the current EU-agreed lowest relevant NOAEL of 10 mg/kg bw/d for reproductive effects in diet exposed rats, and to 36, 71 and 143 times the lowest sub-chronic repeated dose NOAEL of 5.6 mg/kg bw/d in diet exposed rats. Non-dietary oral (gavage) administration presumably resulted in different toxicokinetics characterized by higher peak plasma concentrations (C_{max}) as compared with the key dietary rat studies, and may thus have further contributed to toxicologically significant signs of systemic toxicity. The absence of information on guideline recommended in-life observations and (post)necropsy examinations prevents a reliable assessment of the toxicological significance of the reported adverse effects on the male reproductive system seen at very high dose levels.

For comparison, in the key sub-chronic dietary repeated dose toxicity study in Wistar rats (Wragg *et al*. (1991) TOX9300281) the NOAEL was established at 80 ppm (5.6 mg/kg bw/d) based on reduced food consumption and body weight, a toxicologically significant reduction in red blood cells (anaemia), the presence of methemoglobinemia, deposits of haemosiderin pigment and foci of basophilic hepatocytes in the liver, and extramedullary haemopoiesis noted at 800 ppm and above.

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[3] The test item purity is not reported and the rat strain not specified.

In this explorative study, no mortality incidences, clinical signs, food consumption data, body weight changes, endpoints of clinical laboratory investigations (haematology, clinical biochemistry), macroscopic findings, and only few of the recommended organ weight determinations and histopathological examinations were assessed/reported. The assessment of these endpoints represents a basic guideline requirement for toxicological studies of this type (*i.e.* repeated dose 90-day oral toxicity in rats, OECD guideline 408), which are specifically designed to assess and quantify hazards under consideration of concomitant corroborative findings across endpoints in order to provide a reliable scientific basis for any classification.

The mean values of toxicological endpoints should have been presented with standard deviations (S.D.), not with the standard error of the mean (SEM).

Conclusions

With special regard to the outcomes of the scientifically valid and reliable two-generation reproduction toxicity studies, the weight of evidence is not conclusive and not sufficient for the suggested classification of Isoproturon for reproductive toxicity/effects on fertility in category 2 (H361f: Suspected of damaging fertility) according to the CLP criteria.

There was no indication for effects on male fertility in the key study by Becker *et al*. (1989); and retarded spermatogenesis was the only finding (at low incidence, in F_1 males only) directly associated with effects on male fertility in the key study by Bhide (1991), which was confined to dose levels associated with clear parental systemic toxicity.

In both key studies, other slight reproductive effects were noted in conjunction with parental toxicity, and similarly not considered to trigger classification for reproductive effects in previous assessments. As outlined in the CLP Regulation, classification may not necessarily be the outcome if the only effects recorded in experimental animals are of low toxicological significance, which includes small changes in semen parameters (Annex I to CLP, point 3.7.2.3.3 under the headline *weight-of-evidence*).

There is evidence to conclude that the observed isolated effect on male fertility, *i.e.* retarded spermatogenesis, either represents a finding of negligible toxicological significance which was solely produced as a non-specific secondary consequence of considerable parental toxicity, or represents an incidental finding.

Due to the low incidence of the single finding of retarded spermatogenesis noted in the absence of corroborative findings in only one of the key regulatory two-generation reproductive toxicity studies and in view of the lack of respective adverse Isoproturon-related effects in the relevant regulatory dietary short-term toxicity studies in rats (no relevant histopathological findings and/or organ weight changes in organs of the reproductive system), there is no sound indication for the presence of any primary direct effect of Isoproturon on male fertility.

Against this background, the suggested classification of Isoproturon in Repr. 2 (H361f: Suspected of damaging fertility) is not adequate.