Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

to the Opinion proposing harmonised classification and labelling at EU level of

isoproturon (ISO); 3-(4-isopropylphenyl)-1,1-dimethylurea

EC Number: 251-835-4
CAS Number: 34123-59-6

CLH-O-0000001412-86-115/F

Adopted

3 June 2016

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ISOPROTURON (ISO); 3-(4-ISOPROPYLPHENYL)-1,1-DIMETHYLUREA

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

Comments provided during public consultation are made available in the table below as submitted through the web form. Any attachments received are referred to in this table and listed underneath, or have been copied directly into the table.

All comments and attachments including confidential information received during the public consultation have been provided in full to the dossier submitter (Member State Competent Authority), the Committees and to the European Commission. Non-confidential attachments that have not been copied into the table directly are published after the public consultation and are also published together with the opinion (after adoption) on ECHA's website. Dossier submitters who are manufacturers, importers or downstream users, will only receive the comments and non-confidential attachments, and not the confidential information received from other parties.

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Substance name: isoproturon (ISO); 3-(4-isopropylphenyl)-1,1-dimethylurea
EC number: 251-835-4
CAS number: 34123-59-6
Dossier submitter: Germany

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Spain		MemberState	1
Comment received				
The Spanish CA supports the proposed classification regarding human health.				
Dossier Submitter's Response				
The comment is registered.				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Germany	Adama Agriculture B.V. on behalf of Adama Agan Ltd.	Industry or Trade Association	2
Comment received				
Adama does not consider classification as Repro Cat. 2 warranted				
Dossier Submitter's Response				
The comment is registered. See our comments below.				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Germany	Adama Agriculture B.V. on behalf of Adama Agan Ltd.	Industry or Trade Association	3

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON ISOPROTURON (ISO); 3-(4-ISOPROPYLPHENYL)-1,1-DIMETHYLUREA

Comment received
Adama considers the current classification with R40 not warrented based on irrelevance for humans.
Dossier Submitter's Response
The comment is registered. See our comments about new data below.
RAC's response
Noted

Date	Country	Organisation	Type of Organisation	Comment number
20.01.2016	France		MemberState	4
Comment received				
We support the proposed classification as Carc. 2 H351; Repr. 2, H361f; STOT RE 2, H373 (blood). We agree with the classification proposal regarding environmental hazard. For the acute and chronic M factors, we also agree with the proposed values. Page 4, table 1 and page 11, table 5 The minimum purity of the active substance specify in table 1 is different from what is specified in the table 5. Purity of isoproturon should be clarified.				
Dossier Submitter's Response				
The comment is registered. The Adama Agriculture B.V. has submitted new data on a receptor-mediated mode of action of tumorigenicity involving the sustained activation of the hepatic constitutive androstane receptor (CAR). Based on these data it can be assumed that this mechanism contributes to the increases of the incidence of hepatocellular tumours in rats. According to the recent scientific discussion this mode of a action is considered to be qualitatively not plausible for humans (Elcombe et al., Crit Rev Toxicol. 44, 64-82, 2014). The degree of purity at page 4 in table 1 should be ≥970 g/kg. In the review process of isoproturon it was concluded that the active substance shall comply with the FAO specification (AGP: CP/250) (Isoproturon SANCO/3045/99-final 12 March 2002). According to the FAO specification the isoproturon content shall be declared not less than 970 g/kg.				
RAC's response				
Noted				

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Germany	Troy Chemical Company B.V.	Industry or Trade Association	5
Comment received				
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) 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.
Troy holds the opinion that the suggested classification of Isoproturon in Repr.2 (H361f: Suspected of damaging fertility) is not adequate.

ECHA note - The following attachments were submitted with the comment above:
Troy Chemical Company BV_Comment CLH report_Isoproturon_Public Cons_16-01-25.pdf

Dossier Submitter's Response

No new toxicological data have been submitted by Troy Chemical Company B.V. for the evaluation of reproductive toxicity.
According to the conclusions of the above mentioned comments submitted by Troy Chemical Company B.V. " … retarded spermatogenesis was the only finding (at low incidence, in F1 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." " …, other slight reproductive effects were noted in conjunction with parental toxicity, and similarly not considered to trigger classification for reproductive effects in previous assessments."

Considering the two-generation reproduction toxicity studies in rats using dietary dose levels up to 2000 ppm, there were seen clear signs of parental toxicity (reduced body weight gain and feed consumption) and reproductive toxicity (reduced mating index, pregnancy rate, number of implantations, litter size, and pup weight) at dose levels of 400 ppm or above. Furthermore, there is evidence of impaired male fertility from results in appropriate two-generation reproduction toxicity studies including histopathological changes in the testes revealing retarded spermatogenesis. In a supplementary published study there was evidence of an affected spermatogenesis in rats possibly based upon impaired androgen biosynthesis at high doses. Reproductive toxicity was observed at clear parental toxicity. However, there is no clear evidence to conclude that the observed reproductive toxicity is solely produced as a non-specific secondary consequence of parental toxicity. Therefore, classification is proposed.

The reproductive toxicity potential was discussed during the pesticide peer review expert meeting. Reliability of the database was considered, too. The European Food Safety Authority concluded that: "the majority of the experts considered that reproductive toxicity seen in rats can be due to reduced male fertility suggesting that classification as 'Reproductive toxicity Cat. 2 (H361f: Suspected of damaging fertility)' would be required for isoproturon, as proposed by the RMS." (Conclusion on the peer review of the pesticide risk assessment of the active substance isoproturon, EFSA Journal 2015;13(8):4206)

Considering the fact that effects are only observed at dose levels also inducing parental toxicity, and the fact that effects are not consistently observed in all generations, we agree that there is only some evidence for effects on fertility and thus with classification in Cat 2.

RAC's response

Thank you for the comments. Your position and the clarification provided by the DS have been noted.

Date	Country	Organisation	Type of Organisation	Comment number
20.01.2016	Netherlands	RIVM	National Authority	6
Comment received				

P5, table 3: The proposed classification for carcinogenicity is 'none', whereas this should be 'Carc. 2; H351' (same as current classification) since this (existing) classification was considered appropriate by the dossier submitters, or 'not addressed by this proposal'.
Dossier Submitter's Response
Agreed. This endpoint is not addressed in the dossier and therefore both the current and the proposed classification should be "Carc. 2; H351" - in accordance with table 2.
RAC's response
Noted

CARCINOGENICITY

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Germany	Adama Agriculture B.V. on behalf of Adama Agan Ltd.	Industry or Trade Association	7
Comment received				
The current classification with R40 is based on rat liver tumors. New mode of action data was generated that challenges human relevance of these tumors for humans. Despite being summarized in the RAR, this data was not found by the applicant in the CLH Report. Therefore the data is submitted again. Apologies if the data was already available to RAC. ECHA note - The following attachments were submitted with the comment above: *03_IPU_MOA_rat liver tumors.pdf* *04_IPU_cell proliferation in vitro rat (wt and KO) and human hepatocytes.pdf*				
Dossier Submitter's Response				
Carcinogenicity was not addressed in the dossier. However, Adama Agriculture B.V. has submitted new data on a receptor-mediated mode of action of tumorigenicity involving the sustained activation of the hepatic constitutive androstane receptor (CAR). Based on these data it can be assumed that this mechanism contributes to the increases of the incidence of hepatocellular tumours in rats. According to the recent scientific discussion this mode of a action is considered to be qualitatively not plausible for humans (Elcombe et al., Crit Rev Toxicol. 44, 64-82, 2014).				
RAC's response				
The hazard class carcinogenicity was not included or addressed by the DS, and not opened for public consultation. Therefore, the new data on carcinogenicity submitted by Industry will not be evaluated by RAC, as also summarised in the ECHA general comment of the opinion.				

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Germany	Adama Agriculture B.V. on behalf of Adama Agan Ltd.	Industry or Trade Association	8
Comment received				
Expert Analysis of the reproductive properties of Isoproturon is provided in the 3 attachments. It is concluded that no classification for reproductive toxicity is warranted for Isoproturon.				

ECHA note - The following attachments were submitted with the comment above:
00_IPU_Classification_Adama position.pdf
01_IPU_analysis of Becker 1989 and Bhide 1990.pdf
02_IPU_analysis of Sakar et al. 1997.pdf

Dossier Submitter's Response

No new toxicological data have been submitted by Adama Agriculture B.V. for the evaluation of reproductive toxicity.

According to the "Expert Statement" submitted by Adama Agriculture B.V. (Isoproturon Position on Classification for Reproductive Toxicity, Project No. 90019522, 25 January 2016)
" … significant methodological deficiencies were identified in both the two-generation study by Bhide and the publication by Sakar." … "A reliable two-generation study in rats is available (Becker et al., 1989)." " … all effects seen in this study on reproductive performance are secondary to maternal toxicity."

The reproductive toxicity potential was discussed during the pesticide peer review expert meeting. Reliability of the database was considered, too. The European Food Safety Authority concluded that: "the majority of the experts considered that reproductive toxicity seen in rats can be due to reduced male fertility suggesting that classification as 'Reproductive toxicity Cat. 2 (H361f: Suspected of damaging fertility)' would be required for isoproturon, as proposed by the RMS." (Conclusion on the peer review of the pesticide risk assessment of the active substance isoproturon, EFSA Journal 2015;13(8):4206)

Considering the two-generation reproduction toxicity studies in rats using dietary dose levels up to 2000 ppm, there were seen clear signs of parental toxicity (reduced body weight gain and feed consumption) and reproductive toxicity (reduced mating index, pregnancy rate, number of implantations, litter size, and pup weight) at dose levels of 400 ppm or above. Furthermore, there is evidence of impaired male fertility from results in appropriate two-generation reproduction toxicity studies including histopathological changes in the testes revealing retarded spermatogenesis. In a supplementary published study there was evidence of an affected spermatogenesis in rats possibly based upon impaired androgen biosynthesis at high doses. Reproductive toxicity was observed at clear parental toxicity. However, there is no clear evidence to conclude that the observed reproductive toxicity is solely produced as a non-specific secondary consequence of parental toxicity.

Considering the fact that effects are only observed at dose levels also inducing parental toxicity, and the fact that effects are not consistently observed in all generations, we agree that there is only some evidence for effects on fertility and thus with classification in Cat 2.

RAC's response

Thank you for the comment. Your position and the clarification provided by the DS have been noted.

Date	Country	Organisation	Type of Organisation	Comment number
22.01.2016	United Kingdom	BCS-CGNS Isoproturon Task Force	Industry or Trade Association	9

Comment received

Isoproturon should not be classified for reproductive toxicity. The CLH report, 4.11.6 proposes classification on the basis:
"There is evidence that reproduction toxicity seen in rats is due to reduced male fertility."
However, apparent evidence for testicular toxicity is a flawed interpretation and should be entirely dismissed.

"Retarded spermatogenesis" in 2 generation studies by Bhide (1990, 1991) is an unreliable histological diagnosis;

Apparent testicular toxicity reported by Sarkar et al (1997) was seen only at the single, very high dose tested (800 mg/kg bw/day), where other toxicity (not described in the publication) would have been severe. The toxicity was not specific to the testis, and should be attributed as secondary to marked general toxicity;

The CLH report fails to assess or give weight to the absence of testicular pathology in at least 5 repeat-dose studies adequate for the purpose and conducted at higher doses;

Apparent reduced fertility seen in females was clearly not a function of male infertility. Similar reductions in female reproductive parameters were evident in the 2 generation study by Becker et al (1989), with all males being clearly fertile at a higher dose; an effect on male fertility can be dismissed on the basis of Becker. The pregnancy deficits are secondary to non-specific maternal toxicity, and not cause for classification.

1. "Retarded spermatogenesis"

"Retarded spermatogenesis" in the 2 generation studies by Bhide (1990, 1991) is an unreliable histological diagnosis and does not comply with any standard pathology terminology. The diagnosis is unreliable since H&E sections are not an appropriate sample to determine rate of spermatogenesis, because the tissue section represents only a moment in time; the rate at which sperm mature is not seen. In the absence of an appropriate description it is not known what the pathologist may have been reporting. Only very few rats were purported to have been affected (a maximum of three in any group); statistical significance is not claimed.

The animal colony ("IIT Animal House") is not one frequently encountered in CLH discussions, and the normal background of testicular findings at the test facility (particularly under conditions of stress) is uncertain. The strain is reported as Wistar. From the CLH report, the bodyweight of the IIT rats was extremely low (starting weight of F1 males ca. 35g, vs 143 g in Becker (1989); terminal bodyweight of top dose F1 males 121g, vs 390g in Becker); and must be considered as abnormal for Wistar rats. These profoundly low bodyweights may imply interpretation of testicular findings of top-dose animals to be confounded by immaturity as well as bodyweight stress. Further, a "retardation" in spermatogenesis does not suggest serious testicular toxicity or imply any loss of fertility. It is feasible the terminology describes atrophy of occasional testicular tubules, a known background lesion (when of mild severity) even in young male rats. The finding may also be attributable to defects of fixation during tissue sampling, or of staining. Toxicity in the testes frequently results in a secondary downstream effect in the epididymides; however, epididimydes in the Bhide studies (presumed to have been examined as part of an OECD 416 guideline-compliant study design, and included in the one study available to this commentator) offer no corroboration in all three studies.

The "retardedspermatogenesis" observation is unlikely to represent a true toxic finding since no testicular lesion was seen in a comparable 2-generation study (Becker 1989) in a

reputable laboratory, testing dose levels up to 5 times higher (2000 ppm vs 400 ppm).

The CLH report fails to consider absence of testicular pathology in numerous subchronic and chronic toxicity studies in which testes were examined by the same methodology (H&E sectioning), usually at higher doses. Tables 9 and 10 of the CLH report list eighteen repeat-dose studies considered relevant for classification; from Tables 9 and 10 testes are implicated as target tissue in none of the eighteen. The following studies are at least equally valid as multigeneration studies for determination of testicular pathology:

- Leuschner et al (1973), rats dosed up to 20,000 ppm in diet for 13 weeks without effects on the testes. Although considered supplementary in the CLH report, the deviations noted for this study do not detract from the histopathological examination of testes;

- Bhide (1984), rats dosed by gavage at up to 750 mg/kg bw/day for 13 weeks without effects on the testes. Although considered supplementary in the CLH report, the deviations noted for this study do not detract from the histopathological examination of testes;

- Dickhaus and Heisler (1987), rats dosed at up to 5,000 ppm in diet for 13 weeks without effects on the testes. Although considered supplementary in the CLH report, the deviations noted for this study do not detract from the histopathological examination of testes;

- Bhide (1990), rats dosed at up to 2,400 ppm in diet for 13 weeks without effects on the testes. Although considered supplementary in the CLH report, the deviations noted for this study do not detract from the histopathological examination of testes;

- Wragg et al (1991), rats dosed at up to 8,000 ppm in diet for 13 weeks without effects on the testes. The CLH report concludes this study to be acceptable.

In addition, and not described in the CLH report, the following chronic toxicity/carcinogenicity study of isoproturon by Hunter et al (1981) (described in the 1999 DAR) also provides examination of testes following prolonged exposure at a higher dose than the questionable Bhide studies:

- Hunter et al (1981), rats dosed at up to 2000 ppm in diet for up to 115 weeks without effects on the testes. An interim sacrifice was conducted at 104 weeks; the DAR concludes the study to be acceptable. This study examined testes in 80 male rats per group (50 main group plus 30 interim) so is statistically powerful; although most animals were sacrificed in old age, so testicular findings may have been affected to some extent by geriatric change. The large number of animals, substantially higher dosage, and extended exposure period would however be expected to significantly outweigh the influence of geriatric change.

The vague observation of "retarded spermatogenesis" reported by Bhide (1990, 1991) at doses around 400 ppm is therefore toxicologically implausible, may be a quirk of the test facility, and is not a sufficiently reliable endpoint to support classification.

2. "Testicular toxicity" reported by Sarkar et al (1997)

Undue weight is given to a non-GLP publication, using small numbers of animals (6/group), which in essence duplicates investigations reported in numerous GLP- and

guideline-compliant repeat-dose studies. The top dose used by Sarkar et al would appear to be ethically challenging. Sarkar et al gavaged isoproturon to six rats/group at doses of up to 800 mg/kg bw/day, considerably higher than was tolerable in any of the multigeneration studies. By contrast, the multigeneration study of Becker et al (1989) found a top dose of 2000 ppm (approximately 170 mg/kg bw/day) as the highest tolerable due to systemic toxicity; and the studies of Bhide (1990, 1991) used a top dose of 400 ppm (approximately 40 mg/kg bw/day, according to the DAR) also showing marked systemic toxicity (as shown by bodyweight). Sarkar et al 1997 did not take systemic toxicity into account; a companion paper (Sarkar et al, 1995) shows a 75% impairment of weight gain at the top dose.

The age of rats is not stated in either paper by Sarkar (1995, 1997), but the bodyweights at start (ca. 100g) implies very young rats. Male rats are generally considered to reach sexual maturity at about 10-12 weeks of age, and maturity may be retarded if growth is impaired. The duration of the Sarkar study (10 weeks) therefore closely matches a "window of vulnerability" for potential confounding by growth-related maturational delay, where control rats have matured but the testes of treated, smaller rats remain immature; the difference in appearance is sometimes erroneously interpreted as toxicity.

Sarkar did not measure haematology. By comparison, the 13-week study of Wragg (1991) administered doses up to 8000 ppm (approximately 560 mg/kg bw/day) resulting in a 44% decrease in final bodyweight, and a 12% anaemia in males (15% in females). Anaemia is a clear and consistent toxicological effect of isoproturon and is the basis of the STOT-RE proposal in the CLH report. It is reasonable to assume the animals studied by Sarkar et al (1997) were more severely affected than those of Wragg (1991) as a result of the higher dose; consequently, it is unsurprising that function of any organ (including testes) is shown to be compromised by comparison to an untreated control. Indeed, the top dose was of sufficient severity that the top dose might not be repeatable under modern ethical standards.

The CLH report states (p47) that histological changes were also reported at 400 mg/kg bw/day. This contrasts with the text of Sarkar (pp 132-133), which states changes were significant only at the top dose. The testicular lesions are unrepeatable in any of the adequate repeat-dose studies (although only Wragg, 1991, investigated doses this high; at which systemic toxicity was marked).

Sarkar et al (1997) provides no evidence for specific testicular toxicity. It is comprehensively unclear why the CLH report allocates greater weight to this study, than to numerous better-quality repeat dose studies, some of which are GLP- and guideline-compliant.

3. Apparent effects on pregnancy

The pregnancy deficits are secondary to non-specific maternal toxicity, and not a cause for classification. The effects seen (decreased mating index in one study only, decreases in implantation site numbers and in pregnancy rate) are recognised in the Guidance on the Application of CLP Criteria as being influenced by (or indicators of) maternal toxicity. They occurred at dose levels of marked maternal toxicity as evidenced by bodyweight and presumed haemolytic anaemia. These findings were not considered sufficiently specific for classification at the first approval of isoproturon, and since they do not form the basis of the classification proposal in the CLH report, are not addressed in further detail.

References not cited in the CLH report:

Sarkar SN, Chattopadhyay SK, Majudmar AC (1995) Subacute toxicity of urea herbicide, isoproturon, in male rats. Indian Journal of Experimental Biology 33, 851-856. ECHA note - The following attachment was submitted with the comment above: *1206507.uk0-8261 IPU Reprotox comment v2.docx*
Dossier Submitter's Response
No new toxicological data have been submitted by BCS-CGNS Isoproturon Task Force for the evaluation of reproductive toxicity. The comment for CLH Public Consultation submitted by BCS-CGNS Isoproturon Task Force is registered. In the CLH report all repeat-dose studies were considered concerning testicular pathology. However, reproductive toxicity (reduced mating index, pregnancy rate, number of implantations, litter size, and pup weight) was only seen in the two-generation reproduction toxicity studies in rats at dose levels of 400 ppm or above, along with parental toxicity (reduced body weight gain and feed consumption). There was also evidence of histopathological changes in the testes (retarded spermatogenesis) in few F1 animals at 200 ppm and above. In a supplementary published study there was evidence of an affected spermatogenesis in rats possibly based upon impaired androgen biosynthesis at high doses. Reproductive toxicity was observed at clear parental toxicity. However, there is no clear evidence to conclude that the observed reproductive toxicity is solely produced as a non-specific secondary consequence of parental toxicity. Considering the fact that effects are only observed at dose levels also inducing parental toxicity, and the fact that effects are not consistently observed in all generations, we agree that there is only some evidence for effects on fertility and thus with classification in Cat 2. The reproductive toxicity potential was discussed during the pesticide peer review expert meeting. Reliability of the database was considered, too. The European Food Safety Authority concluded that: "the majority of the experts considered that reproductive toxicity seen in rats can be due to reduced male fertility suggesting that classification as 'Reproductive toxicity Cat. 2 (H361f: Suspected of damaging fertility)' would be required for isoproturon, as proposed by the RMS." (Conclusion on the peer review of the pesticide risk assessment of the active substance isoproturon, EFSA Journal 2015;13(8):4206)
RAC's response
Thank you for the comment. Your position and the response from the DS have been noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Spain		MemberState	10
Comment received				
There is evidence that reproduction toxicity seen in rats is due to reduced male fertility. In the two-generation reproduction toxicity studies histopathological changes in the testes revealed retarded spermatogenesis. The results of a supplementary published study confirm an affected spermatogenesis in rats possibly based upon impaired androgen biosynthesis at high doses. Reproductive toxicity was observed at clear parental toxicity. We are in agreement with the dossier submitter that, there is no clear evidence to conclude that the observed reproductive toxicity is solely produced as a non-specific secondary consequence of parental toxicity.				

Based on the data presented, we support to classify isoproturon for reproductive toxicity in category 2 (H361f: Suspected of damaging fertility) according to the CLP criteria.
Dossier Submitter's Response
The comment is registered.
RAC's response
Noted

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Sweden		MemberState	11
Comment received				

The Swedish CA supports classification of Isoproturon (Cas No. 34123-59-6) in Repr. 2; H361f as specified in the proposal.

No detailed data are presented in the CLH-report on adverse effects on sexual function and fertility or on adverse effects on the development of the offspring of Isoproturon. Therefore, we consider that there is not sufficient information to make an independent assessment of reproductive toxicity of Isoproturon in the current CLH-report. A higher level of details of the reproductive toxicity data is available in the DAR, however, also in this report there is a lack of transparency in the presentation of data.

Moreover, we note that reasoning of the comparison with criteria with available data is lacking for both adverse effects on sexual function and fertility and adverse effects on the development of the offspring.

Adverse effects on sexual function and fertility:
Based on available data presented in the DAR, we agree that there is some evidence for adverse effects on sexual function and fertility. Statistical significant findings to support classification for adverse effects on sexual function and fertility from four two-generation reproductive toxicity studies in rat include:
- reduced mating index at 40 mg/kg bw/day in F1 females
- reduced pregnancy rate in F1 females at 40 mg/kg bw/day
- reduced number of implantations in both F0 and F1 at 134-263 mg/kg bw/day
- decreased litter size in both F0 and F1 at 134-263 mg/kg bw/day

In addition, histopathological changes in the testes were observed in a few males (retarded spermatogenesis) starting at 20 mg/kg bw/day and observed at 40 mg/kg in all three studies by Bhide.

The findings of reduced mating index, reduced pregnancy rate and histological changes in testes were observed at 40 mg/kg bw/day where parental toxicity was evident (significantly reduced body weight in F1 males was 23-28% compared to control and in F1 females 17-25% compared to control at 40 mg/kg bw/day). Reduced number of implantations and decreased litter size was reported at 134-263 mg/kg bw/day were severely reduced weights by 20-31% compared to control was reported in F1 females compared to control, but only moderately decreased in F0 females (11% decrease compared to control).

Adverse effects on the development of the offspring:
In general, level of detail reported is insufficient to make an independent assessment of the data and evaluation against classification-criteria, both in the CLH-report and in the

DAR. Actual data for maternal weight and weight gain/loss and other signs of maternal toxicity should be reported and described more clearly than just described as e.g. "decreased" to be considered in the evaluation for developmental toxicity. This is lacking in the current report. This is important for deciding on the relevant classification category.

The SE CA propose that that Repr. 2 for adverse effects on the development of the offspring may be taken into consideration based on the observation of increased resorptions (40-65% increase compared to control) in two out of four PNDT rat studies where the effect was dose dependent in one case at 250 mg/kg bw/day and 500 mg/kg bw/day (but only statistically significant at the highest dose) (Katadre 1991) and not statistically significant at 320 mg/kg bw/day in the Dickhaus & Heisler (1987) study. In addition, resorptions were also reported in one out of two studies in rabbit at 100 mg/kg bw/day (embryonic resorptions, not statistically significant; Fritz et al. 1978).

Decreased maternal body weight gains have been reported at the same dose levels as the observed resorptions (74% of that of control body weight gain at 320 mg/kg bw/day in rat; body weight gain is stated to be significantly decreased at 100 mg/kg bw/day in rabbit but no data is presented), but no data on maternal body weights were reported in the DAR. No adverse maternal effects were reported at 250 mg/kg bw/day and lethargy was reported at 500 mg/kg in the rat study (Katadre 1991) at GD 18-20 (no data on incidence was available). But in this study there was no decrease in body weight gain; on the contrary an in increase in body weight gain was observed. In our view, there is not sufficient support to disregard the findings of increased resorptions since the present picture of maternal toxicity is unclear and it is therefore not possible to make a reliable conclusion. However, if lacking data on maternal toxicity could be supplemented and better described the available data may be considered as some evidence for developmental toxicity.

Dossier Submitter's Response

The comment is registered. The proposal of SE CA for Repr. 2 for adverse effects on the development of the offspring based on the observation of increased resorptions is not supported.

Developmental toxicity was assessed in 5 teratogenicity studies in rats and two teratogenicity studies in rabbits. There was no evidence of developmental toxicity in these studies.

In the rat study of Dickhaus and Heisler (1987, TOX9550735) in the high dose group, the number of resorptions was slightly but not statistically significantly increased. There were no effects on number of implantations, litter size or foetal malformations and variants. Foetal weights and placenta weights were decreased statistically significant in the high dose group only. Maternal toxicity was apparent in the high dose group. Feed consumption in the high dose group was significantly decreased in week 2 and increased in week 3. Body weight gain was decreased statistically significant in the high dose group only. The study is considered acceptable.

In the rat study of Katdare (1991, TOX9500350) the number of resorptions was slightly but not statistically significantly increased at 250 mg/kg bw/d and statistically significantly increased at 500 mg/kg bw/d. However, there were no effects on numbers of implantations, litter size, foetal malformations and variants or foetal body weight. Maternal toxicity (clinical signs) was evident at 500 mg/kg bw/d.

In the rabbit study of Fritz, Becker, and Hess (1978, TOX9551915) there was no evidence of embryotoxicity, fetotoxicity or teratogenicity up to the highest dose level tested (100 mg/kg bw/d). Maternal toxicity, as indicated by decreases in body weight gain and feed consumption was evident in the 100 mg/kg bw/d group. There were no effects on pregnancy rates, number of implantations, number of resorptions, litter size, sex ratio or foetal malformations and variants. The effect "resorption" was statistically significantly increased only at 500 mg/kg bw/d. This dose level was associated with marked severe maternal toxicity in other studies with repeated administration. Therefore no classification for developmental toxicity is proposed. The European Food Safety Authority concluded that "In the developmental toxicity studies, there was no evidence of teratogenicity, … ."
RAC's response
Thank you for the comment. Your position and the response from the DS have been noted.

Date	Country	Organisation	Type of Organisation	Comment number
22.01.2016	Finland		MemberState	12
Comment received				
German CA proposes Isoproturon to be classified for Repr. 2f (suspected human reproductive toxicant) primarily due to its effects on spermatogenesis and female mating index and pregnancy rate. We are of the opinion that the data presented in the CLH report and DAR may warrant classification of Isoproturon for fertility effects. However, to justify classification we would prefer more accurate reporting of the data. Moreover, discussion and comparison with CLP criteria is very limited in the CLH report and thus it is not clear why category 2 is preferred over category 1B. Retarded spermatogenesis was observed by histopathological analyses of the testes in three separate two-generation studies at doses ≥ 20 mg/kg. These studies were conducted in the same laboratory but they are reported as independent studies and were assessed to be acceptable or supplementary studies. In two of these studies decreased pregnancy rate (in one study also decreased female mating index) was also reported and the reporter of the studies stated these effects to be associated with decreased male fertility due to testes lesions. No effects on spermatogenesis were reported in a two generation study conducted in a different laboratory (Becker et al. 1989) but this study revealed decreased number of implantations and reduced litter size at higher doses (134-263 mg/kg). Since classification proposal seems to be primarily based on these three two-generation studies from the same laboratory the hazard assessment would benefit of more accurate reporting. Specifically we wonder whether more specific histopathological data is available, i.e. what specific pathological findings justify the statement "retarded spermatogenesis". Moreover, it is not stated either in the CLH report or in DAR whether any other effects on testes (organ weights, histopathology) where observed e.g. in repeated dose or in carcinogenicity studies. Moreover, we note that classification proposal seems to be partly based on scientific article which is not, at least easily, publicly available.				
Dossier Submitter's Response				

Category 2 is proposed because reproductive toxicity was observed in the two-generation toxicity studies at clear parental toxicity.

Retarded spermatogenesis was observed in the two-generation toxicity studies of Bhide (1990, 1991). These studies were conducted in compliance with OECD guideline 416. The guideline includes to record e.g. sperm parameters as count, motility, or morphology. In the two-generation toxicity studies of Bhide (1990, 1991) it was distinguished between retarded spermatogenesis and abnormalities in the testes. There was evidence of retarded spermatogenesis in testes in few F1 animals at 200 ppm and above.

All relevant reproductive effects were reported in the CLH report or in the DAR. No reproductive toxicity effects were observed in repeated dose or in carcinogenicity studies.

Considering the fact that effects are only observed at dose levels also inducing parental toxicity, and the fact that effects are not consistently observed in all generations, we agree that there is only some evidence for effects on fertility and thus with classification in Cat 2.

RAC's response

Thank you for the comment. Your position and the response from the DS have been noted.

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Germany	Troy Chemical Company B.V.	Industry or Trade Association	13

Comment received

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 F1 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.

Dossier Submitter's Response

There is evidence of impaired fertility from results in appropriate animal studies. Reproductive toxicity (reduced mating index, pregnancy rate, number of implantations, litter size, pup weight) was seen at dose levels of 400 ppm or above in the two-generation reproduction toxicity studies in rats. In addition there was evidence of histopathological changes in testes (retarded spermatogenesis) in few F1 animals at 200 ppm and above.

Reproductive toxicity was observed at clear parental toxicity. However, there is no clear evidence to conclude that the observed reproductive toxicity is solely produced as a non-specific secondary consequence of parental toxicity. Therefore, classification is proposed.

Considering the fact that effects are only observed at dose levels also inducing parental toxicity, and the fact that effects are not consistently observed in all generations, we agree that there is only some evidence for effects on fertility and thus with classification in Cat 2.

The reproductive toxicity potential was discussed during the pesticide peer review expert meeting. Reliability of the database was considered, too. The European Food Safety Authority concluded that: "the majority of the experts considered that reproductive toxicity seen in rats can be due to reduced male fertility suggesting that classification as 'Reproductive toxicity Cat. 2 (H361f: Suspected of damaging fertility)' would be required for isoproturon, as proposed by the RMS." (Conclusion on the peer review of the pesticide risk assessment of the active substance isoproturon, EFSA Journal 2015;13(8):4206)

RAC's response

Thank you for the comment. Your position and the response from the DS have been noted.

Date	Country	Organisation	Type of Organisation	Comment number
20.01.2016	Netherlands	RIVM	National Authority	14

Comment received

• It is difficult to assess the included information on reproductive toxicity based on the proposal, since there are no descriptions of study set up and no information is provided on the severity of the observed effects, variation among animals, and severity of parental toxicity.

Fertility:
• According to the DAR (in which more detailed study descriptions can be found), retarded spermatogenesis and focal hyperplasia in seminal vesicles and prostate was observed in 3 similar 2 generation studies, but only in 1-3 animals per dose group in each study (Bhide 1990, 1991 and 1991), starting at 200 ppm. At 400 ppm a decrease in either mating index or pregnancy rate was also observed in these studies. Parental toxicity, indicated by a severe decrease in body weight (17-28% below controls) was only visible at 400 ppm.
It is noted that all effects are only observed in F1 parental animals and F2 pups.
• Affected sperm was also observed at 800 mg/kg bw/day and changes in testes

histopathology at 400 mg/kg bw/day and above in rats treated 6 days/ week for 10 weeks. However, no effects on testes histopathology were reported in the available repeated dose toxicity tests.
• Also according to the DAR, in the 2 generation study by Becker the number of implantation sites was significantly reduced in F0 and F1. In addition, parental body weight was reduced at the end of the study (both generations, 11-20% below controls).
• Considering the fact that effects are only observed at dose levels also inducing parental toxicity, and the fact that effects are not consistently observed in all generations, we agree that there is only some evidence for effects on fertility and thus with classification in Cat 2.

Development
• In 1 teratogenicity study in rats, a dose-related increase in resorptions was observed (significant at the highest dose). Skeletal effects (increased number of unossified calcanei and unossified phalangeal nuclei of the fore-limb) were also observed in 1 teratogenicity study in rabbits (top dose only). No effects (except for fetal body weight) were observed in the studies with rabbits.
• A decreased fetal body weight gain was noted in several studies. In the 2nd generation of the 2 generation studies by Bhide body weight gain was decreased from pnd 0-21, however, it cannot be excluded that this is caused by a direct effect of isoproturon intake via the diet instead of a developmental effect. The increased post-partum loss of entire litters in 4 out of 24 dams at 2000 ppm could also be a developmental effect. Is this effect significant and outside historical control incidence? Nevertheless, in the 2 generation study by Becker, fetal bw was also reduced at pnd 1 and 4. In addition, fetal body weight was reduced in 1 of the 4 teratogenicity studies in rats and both teratogenicity studies in rabbits.
• In conclusion, embryo/foetotoxicity (reduced weight) has been reported, although at doses that also induced maternal toxicity: reduced body weight, up to 28% below controls. Effects on fetal weight can very well be the result of reduced maternal weight. Other effects (increased resorptions and skeletal effects were only observed in 1 study (and are therefore inconsistent results) and also at maternally toxic doses. We therefore agree with the conclusion that there is not enough evidence for developmental effects and thus with no classification.

Dossier Submitter's Response
The comment is registered.
RAC's response
Noted,

OTHER HAZARDS AND ENDPOINTS – Specific Target Organ Toxicity Repeated Exposure

Date	Country	Organisation	Type of Organisation	Comment number
25.01.2016	Spain		MemberState	15
Comment received				
In subchronic oral studies haemolytic anaemia was observed at or above dietary concentrations of approximately 800 ppm (62 mg/kg /day) in rats (Wragg et al 1991), 500 ppm (38 mg/kg bw/d) in dogs (Scholz & Brunk 1973, Bhide 1990) and 150 mg/kg bw/d in monkeys (Bhide 1984). The severity of the anaemia increased dose-dependently and was associated with Heinz bodies, methemoglobinaemia, hyperplastic bone marrow, extramedullary hematopoiesis and increased hemosiderin in liver, kidneys and bone marrow, indicating toxic haemolytic anaemia.				

Haematotoxicity (decrease in Hb by 20 % along with haemosiderin deposition in reticular cells and Kupffer cells of the liver) was seen below the equivalent guidance value for oral 90-day studies (≤ 100 mg/kg bw/d). Therefore, it is considered to sufficiently fulfil criteria for severity to warrant classification proposed for STOT-RE 2 (oral) ("H373: May cause damage to organs (blood) through prolonged or repeated oral exposure) according to CLP criteria.
Dossier Submitter's Response
The comment is registered.
RAC's response
Noted,

OTHER HAZARDS AND ENDPOINTS – Hazardous to the Aquatic Environment

Date	Country	Organisation	Type of Organisation	Comment number
22.01.2016	Finland		MemberState	16
Comment received				
We support the proposed classification for environmental hazards Aquatic Acute 1 – with M-factor of 10 and Aquatic Chronic 1 – with M-factor of 10 for Isoproturon (ISO); 3-(4-isopropylphenyl)-1,1-dimethylurea.				
Dossier Submitter's Response				
Thank you for your comment and agreement with environmental classification and labelling.				
RAC's response				
Noted.				