Committee for Risk Assessment

RAC

Annex 2

Response to comments document (RCOM)

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

flonicamid

EC number: -
CAS number: 158062-67-0

CLH-O-0000002561-80-03/A2

Adopted

5 June 2013

ANNEX 2 - COMMENTS AND RESPONSE TO COMMENTS ON CLH PROPOSAL ON FLONICAMID

COMMENTS AND RESPONSE TO COMMENTS ON CLH: PROPOSAL AND JUSTIFICATION

ECHA has compiled the comments received via the internet that refer to several hazard classes and entered them under each of the relevant categories/headings as comprehensively as possible. Please note that some of the comments might occur under several headings, when splitting the information provided is not reasonable.

Substance name: Flonicamid
EC number: -
CAS number: 158062-67-0
Dossier submitter: France

GENERAL COMMENTS

Date	Country	Organisation	Type of Organisation	Comment number
02/07/2012	Hungary		Individual	1
Comment received				
Flonicamid induces 17 beta estradiol decrease. It has strong adverse effects (delay in vaginal opening, reduced ovary and uterus weights, increase gestation duration). Flonicamid should be considered as an endocrine disruptor.				
Dossier Submitter's Response				
Potential hormonal disrupting effects were discussed by an expert group during peer review of the active substance under plant protection product regulation. Indeed, some concern about potential anti-oestrogenic effects of flonicamid was raised in the rat reproduction study (Takahashi, 2002b). At the highest tested dose (1800 ppm), the findings included: delayed vaginal opening in the F1 generation, reduced absolute and relative uterus weight in the F1 generation, reduced absolute ovary weight in the P generation. Equivocal results in some hormone levels were also observed: isolated increased LH at 300 ppm, increased LH and FSH and decreased 17β-estradiol at 1800 ppm. Further investigations detailed in the CLH report enable the expert group to conclude that these findings can be considered not adverse, taking into account the fluctuations of hormone levels in untreated animals at different sampling times and the lack of variations after dietary administration of flonicamid for 28 or 90 days. Nevertheless this point could further be discussed when regulatory criteria identifying an endocrine disruptor substance will be defined.				
RAC's response				
Due to lack of regulatory criteria for endocrine disruptors, the significance of these effects is assessed in the sections Reproductive toxicity in the opinion document.				
Date	**Country**	**Organisation**	**Type of Organisation**	**Comment number**
19/07/2012	Spain		MSCA	2
Comment received				
p 20. Conclusions on classification and labelling The Spanish CA supports the proposed classification of flonicamid as Xn, R22: Harmful if swallowed (limits 200< LD50 ≤ 2000 mg/kg bw) and as Acute Tox 4 (H302: Harmful if swallowed) (limits 300< LD50 ≤ 2000 mg/kg bw) according to DSD and CLP classification criteria, respectively. This classification is based on the LD50 value in male (LD50 = 884 mg/kg bw) and female (LD50 = 1768 mg/kg bw) obtained in the oral toxicity study in rats (Ridder WE, 2001a).				
Dossier Submitter's Response				
Agreed.				
RAC's response				
Agreed.				
Date	**Country**	**Organisation**	**Type of Organisation**	**Comment number**

20/07/2012	Denmark		MSCA	3
Comment received				
Denmark agrees to the proposed classification as Acute Tox 4; H302.				
Dossier Submitter's Response				
Agreed.				
RAC's response				
Agreed.				
	Country	**Organisation**	**Type of Organisation**	**Comment number**
06/08/2012	Germany		MSCA	4
Comment received				
1.3 Proposed harmonised classification and labelling based on Regulation (EC) No 1272/2008 and/ or DSD criteria Concerning labelling based on Directive 67/548/EEC we like to remark the following: - The S-phrase "(S2-)" should be added in case the substance may be available for the consumer/ general public. - Taking into consideration our further comments below concerning carcinogenicity and reproductive toxicity the assignment of "S36/37" can be agreed to. Normally, if any, only "S22" and/or "S24" may be appropriate S-phrases for a "R22"-substance.				
Dossier Submitter's Response				
Indeed, the labelling with S36/37 is not needed for a substance classified with R22; this was a mistake. In accordance to our proposal that flonicamid requires only classification in respect with oral acute toxicity Xn, R22, we consider that the appropriate S-phrases are S2 and S46 for general public, as mentioned in the CLH report (2.4.2.).				
RAC's response				
S-phrases are not decided by RAC.				

CARCINOGENICITY

Date	**Country**	**Organisation**	**Type of Organisation**	**Comment number**
02/07/2012	Hungary		Individual	5
Comment received				
Effect in lung warrants a classification. There is species differences observed. However this cannot be explained (only) by the pourcentage of Clara cells. The mechanistic data showing no difference of Clara cells number between the different strains but differences on mitogenic effect of flonicamid and isoniazid could be explain by strain genetic background sensitivity. Another hypothesis would be that cell division occurs in different cell types including/than Clara cells. Therefore the effects observed cannot only be explained by clara cell numbers. The applicability of the effects observed to human cannot be overrulled and should be taken into account for classification.				
Dossier Submitter's Response				
See response to comment number 7.				
RAC's response				
The argumentation is much broader than relying only on number of Clara cells. There are data indicating that sensitivity to induce proliferation of bronchial epithelial cells in CD-1 mice, a first step of carcinogenicity of non-genotoxic Flonicamid in this strain, is much higher than such a sensitivity in two other tested strains of mice as well as in rats, and this proliferation in CD-1 mice is reversible after the end of 28-day exposure. The first step, i.e. epithelial cell proliferation, was not observed in other mice strains or in rats. Thus carcinogenic effects observed in CD-1 mice are species and strain specific.				
Date	**Country**	**Organisation**	**Type of Organisation**	**Comment**

				number
19/07/2012	Spain		MSCA	6

Comment received

p 101. Summary and discussion of carcinogenicity / Conclusions on classification and labelling. The Spanish CA agrees with the proposal of the dossier submitter that not classification is required on carcinogenicity for flonicamid, under either DSD or CLP Regulation.
The Spanish CA also agrees with the conclusions of the studies that assess the mechanism of lung tumour induction produced by flonicamid. This substance has a cell proliferation effect in bronchiolar epithelium of the lung which is specie-specific, strain-specific (CD-1 mouse). Thus, the sequence of increased proliferation followed by hyperplasia, leading to adenomas and ultimately carcinomas does not seem to be extrapolated to humans.

Dossier Submitter's Response

Agreed.

RAC's response

Agreed.

Date	Country	Organisation	Type of Organisation	Comment number
06/08/2012	Germany		MSCA	7

Comment received

Flonicamid is clearly carcinogenic in CD-1 mice (up to ca. 60% tumour incidence) but not in Wistar rats. Several mechanistic studies are provided to support a non-genotoxic, threshold MOA for tumour development in mice associated with increased cell proliferation of lung Clara cells in response to (presumably) mitogenic stimuli. This MOA is considered not relevant to humans because of the lesser content of Clara cells in human lungs (10-20% vs. ca 80% in mice) and their potentially lower capability to adversely respond to mitogenic signals.
While the proposed MOA seem to be sufficiently supported in mice, the rationale that this MOA won't be operative in humans is rather weak. Clara cells are present in human lungs in the same region where carcinogenicity in mice occurs (although in lesser amount), and there is no clear evidence that they will not be subject to increased cell proliferation (as demonstrated in rats). It should be also noted that the high dose male rats from the carcinogenicity study demonstrated some incidence of lung damage broadly defined as "lung masses" (regarded as histopathologically heterogenic and not treatment-related). While no signs of cytotoxicity are noted, other mechanisms for proliferative cell damage cannot be completely excluded (i.e., effects on apoptosis, oxidative stress etc.). The demonstrated strain-specificity of cell proliferation despite almost equal Clara cell content in the 3 mouse strains might be indicative of other mechanisms involved in development of lung tumours. Here, a more detailed comparison of the background lung tumour incidences between the strains could be helpful. For Crl:CD-1 BR mouse, historical control incidence of adenomas from 18 month studies is reported as 7.53% in males (1.92-12.00) and 6.49 % in females (0-15.38); for carcinomas, the incidence was 5.84% in males (0-21.15) and 4.03% in females (0-9.62). A closer analysis on the reported ranges might reveal potential sensitivities towards tumour development in this specific strain.
Further, the reference to Isoniazid might not be very relevant for the discussion since both chemicals have quite different functional groups (apart from some degree of structural similarity). Additional elaboration is needed before a conclusion can be drawn with regard to the stated analogy to Isoniazid.
Overall, a more formal application of the IPCS framework for MOA analysis and its human relevance (Boobis et al., 2006; 2008) as a part of the presented WOE approach might be helpful.

References:
Boobis AR, Cohen SM, Dellarco V, McGregor D, Meek ME, Vickers C, Willcocks D, Farland W. IPCS framework for analyzing the relevance of a cancer mode of action for humans. Crit Rev Toxicol. 2006; 36(10):781-92.
Boobis AR, Doe JE, Heinrich-Hirsch B, Meek ME, Munn S, Ruchirawat M, Schlatter J, Seed J, Vickers C. IPCS framework for analyzing the relevance of a noncancer mode of action for humans. Crit Rev Toxicol. 2008; 38(2):87-96.
Charles River Laboratories (1995) Spontaneous Neoplastic Lesions in the CrI:CD-1®BR Mouse (http://www.criver.com/sitecollectiondocuments/rm_rm_r_lesions_crl_cd1_br_mouse.pdf)

Dossier Submitter's Response

The results of investigative studies support the fact that, in the CD-1 mouse, flonicamid induces cell proliferation in the Clara cells of bronchiolar epithelium of the lung by a mitogenic mechanism. Indeed, Clara cells, which account for 80% of the terminal bronchiolar cells, undergo a reversible hypertrophy/hyperplasia and morphological elongation in response to the administration of flonicamid, whereas the area surrounding activated Clara cells shows no evidence of secondary tissue responses such as necrosis or inflammatory changes. The response to flonicamid exhibits both species-specificity and mouse strain-specificity: flonicamid does not elicit a lung epithelial cell proliferation effect in Wistar rats, in which the proportion of Clara cells is only approximately 35% in the lung, and in B6C3F1 and C57/6J mice. The proportion of Clara cells in the terminal bronchiolar region of mouse lung was similar among the 3 mouse strains (namely 80%) and proliferation was only seen in CD-1 mouse, but this correlates with the published spontaneous incidences of bronchiolar-alveolar tumour incidences. Indeed, a total of 59 studies were reported in a Charles River Laboratories document. These 78 to 104-week studies were conducted between 1987 and 2000 in 11 different laboratories, using male and/or female Crl:CD-1 (ICR) mice from different Charles River Laboratories production sites. The incidence of spontaneous bronchio-alveolar adenoma in male and female CD-1 mouse is reported as 2-42 % and 1.67-26.67 % respectively; in the male and female CD-1 mouse the incidence of spontaneous bronchio-alveolar adenocarcinoma is reported as 1.43-26 % and 0.77-18.37 % respectively. The CD-1 mouse has thus a considerably higher spontaneous rate of lung lesions than does the B6C3F1 mouse (2-28% and 0-14% for lung adenoma in males and females respectively, 0-17% and 0-6% for lung carcinoma in males and females respectively (Rao, 1988)), which has a higher incidence than the C57BL mouse (3% for adenomas and carcinomas combined in males and in females (Rao, 1988)).

The aging CD-1 mouse has a high incidence of spontaneous bronchiolar-alveolar tumours and two characteristics of the lung epithelium, proportion of immature Clara cells and their sensitivity to stimulated cell division, appear to account for the sensitivity of mice to the development of tumours of the terminal bronchiolar epithelium. Furthermore, in published literature, analysis of genetic markers linked to the pulmonary adenoma susceptibility 1 (*Pas 1*) locus revealed the presence of the *Pas 1* susceptibility allele in a high percentage of CD-1 mice (95-98%), providing a molecular genetic explanation for the high susceptibility of CD-1 mice to spontaneous and chemically-induced lung tumorigenesis compared to other strains (Manenti *et al.,* 2003).

Human lung is considered to be the most mature with respect to epithelial cell differentiation because the Clara cell content is very low at 22% and 11% in respiratory and terminal bronchioles respectively, compared to 80% in the CD-1 mouse. Moreover, no relationship between the therapeutic uses and the occurrence of human lung tumours was observed in epidemiological studies on isoniazid, a structurally similar compound to flonicamid, which exhibits species-specificity and mouse strain-sensitivity effect on lung cell proliferation (IARC, 1987).

Furthermore, flonicamid is not mutagenic or genotoxic at the DNA, gene and chromosome levels.

It is therefore concluded that the mechanism of lung tumours formation in mice which follows the sequence of increased proliferation, hyperplasia, adenomas and then carcinomas, is not relevant to humans and classification for carcinogenicity is not needed.

References:

Spontaneous neoplastic lesions in the Crl:CD-1 (ICR) mouse in control groups from 18 month to 2 year studies; March, 2005, Charles River Laboratories

Rao, G. N. *et al*., (1988): Mouse strains for chemical carcinogenicity studies: overview of a workshop, Fund. Appl. Toxicol., 10, 385 - 394

Manenti G, Galbiati F, Noci S, Dragani TA, Outbred CD-1 mice carry the susceptibility allele at the pulmonary adenoma susceptibility 1 (*Pas1*) locus. Carcinogenesis. 2003 Jun;24(6):1143-8.

IARC (1987): Monographs on the evaluation of the carcinogenic risk of chemicals to human, Suppl. 7, 227 - 228.

RAC's response

Agreement with and support to the Dossier Submitter response.

MUTAGENICITY: no comments received

TOXICITY TO REPRODUCTION

Date	Country	Organisation	Type of Organisation	Comment number
02/07/2012	Hungary		Individual	8

Comment received

Some viceral abnormalities (especially on the lung) should be discussed regarding the toxic effect of Flonicamid on Lung + the rarity of such findings.

Dossier Submitter's Response

Increased incidence (not statistically significant) of abnormal lung lobation, absent lung and small lung were noted in the rabbits during the developmental study. The incidence of absent and small lung lie within the laboratory and published historical control data, as stated in the amended table provided in comment n°10. Abnormal lung lobation was observed in the middle and high dose groups. However the feature of this malformation is not the same among individuals: fusion of the lobes occurred in the right lung of the 2 middle-dose fetuses and in the left lung in the 2 high-dose fetuses. The background control incidence of abnormal lung lobation has been reported in the literature by Nakatsuka et al. (1997) as combined data (0 to 32.59 %) and as individual incidence at each testing facility (0-1.30 ; 0-23.31 ; 13.27-20.99 ; 0-2.33 ; 0-3.14 ; 0-0.80 ; 0-2.94 ; 0-2.44 ; 0-32.59 ; 0-2.59 ; 0-1.92 ; 0-1.70 %). These data indicate that the incidence of this anomaly in most testing facilities is almost similar to IET laboratory, although the values in 3 facilities are higher. Furthermore the incidence of this anomaly in the 7.5 and 25 mg/kg bw/d groups falls in the range of control data from all facilities except one and is well within the range of 0 to 32.59 %.

Reference:

Nakatsuka et al., Japan pharmaceutical manufacturers association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. Cong Anom, 37:47-138, 1997

RAC's response

The observed malformations were very rare, without dose-response relationship and within historical control data of the same laboratory. Thus they were not considered to be related to flonicamid treatment.

Date	Country	Organisation	Type of Organisation	Comment number
20/07/2012	Denmark		MSCA	9

Comment received

Denmark disagrees with the proposal.
Denmark proposes classification of Flonicamid as Repr.2; H361D, due to increased occurence of visceral malformation in the rabbit at non-maternally toxic level. This classification was also recommended by the expert group in the peer review under the ppp directive (91/414). Further justification for the Danish position can be found in the attached document.

*ECHA comment: The document: [**Danish EPA, 16/7-2012 Comments from the Danish CA on the CLH Proposal from France on Flonicamid (CAS no 158062-67-0)**][Flonicamid ECHA høring juli 2012 .doc] was submitted as a separate attachment. The attachment document is copied below. Attachment no. 1*

Danish EPA, 16/7-2012

Comments from the Danish CA on the CLH Proposal from France on Flonicamid (CAS no 158062-67-0)

Denmark agrees with the proposed classification for acute oral toxicity as **Xn; R22/Acute tox 4 H302**. (LD_{50} oral: 884 mg/kg M, 1768 mg/kg F).

In addition to the classification proposed by France, Denmark proposes that flonicamid should be classified for developmental toxicity as **Repr cat 3; R63 / Repr 2; H361D.**

Justification for classification for reproductive toxicity:

Based on the data presented in the DAR (ppp) which appear to be identical to the data in the CLH dossier, the expert group on toxicology under ppp directive recommended the classification as Repr 3; R63.

Rabbit developmental study:
In the rabbit, there was higher incidence of total malformations and especially of visceral malformations compared to concurrent control.

Historical incidences from the literature and from the laboratory (over a very long period of time: 10 years!) are provided for individual most of the external, skeletal and visceral malformation types, leading the French CA to the conclusion that the statistically significant increased occurrence of visceral and total malformations should be discarded.

The Danish CA does not agree that the presented historical data should be used to overrule the specific concurrent control data to discard the effects on development seen the study. We still have concern that flonicamid has a toxic effect on development in rabbits at non-maternally toxic doses of 7.5 and 25 mg/kg bw/day.

The NOAEL for development in this rabbit study should in our opinion be set at 2.5 mg/kg bw/day, as it was decided in the peer review under the ppp directive. Thus the NOAEL dev rabbit would be 2.5 mg/kg bw/day and the NOAEL maternal still 7.5 mg/kg bw/day.

The Danish CA proposes that flonicamid is classified as Repr 3; R63 under the DSD respectively Repr 2, H361D under the CLP regulation, based on clear effects on visceral malformations occurring at non-maternally toxic levels in the rabbit (one species is sufficient according to the criteria for classification as reproductive toxicant).

End of attachment no. 1

Dossier Submitter's Response

The experts of the European Food Safety Authority indeed suggest that the proposal Repr Cat. 3 R63 had to be considered by ECHA because of visceral malformations occurring without maternal toxicity. Nevertheless, before the EFSA meeting, the active substance was peer-reviewed at national level and French experts did not consider necessary to classify flonicamid with R63 for the reasons detailed below.

Increased incidence of visceral malformations was observed in the rabbits at the dose levels of 7.5 and 25 mg/kg bw/d, with a statistically significant difference between the control group at 7.5 mg/kg bw/d only. The type of malformations varies widely among foetuses and incidences of each malformation are low, no statistically significant difference being observed between the control and treated groups. Historical control data (HCD) of the laboratory were provided in the CLH report for the period 1992-2001. The company further provided data from the performing testing facility IET covering the period 1992-2011 (see comment n°10 and attached amended table). As the experimental phase of the study ran between July 2001 and December 2001 and is thus largely covered by the HCD and as these HCD come from an important number of studies and foetuses/litters (29 studies, 4647 foetuses and 583 litters), it is considered appropriate to consider these data. Only the incidences of abnormal lung lobation, absent lung, absent kidney and absent ureter lie above the HCD. Nevertheless, the incidences of these findings are within the HCD reported in the literature by Nakatsuka et al. (1997).

As a conclusion, visceral abnormalities observed in the rabbits can be considered as incidental, as they were never dose-related, were not statistically significantly different from controls and fall within the laboratory and/or published historical control data. No classification is thus required for flonicamid.

Reference:
Nakatsuka et al., Japan pharmaceutical manufacturers association (JPMA) survey on background control data of developmental and reproductive toxicity studies in rats, rabbits and mice. Cong Anom,

37:47-138, 1997

RAC's response

Support to the Dossier submitter's response. The observed malformations were very rare, without dose-response relationship and within historical control data of the same laboratory. Thus they were not considered flonicamid treatment related.

Date	Country	Organisation	Type of Organisation	Comment number
06/08/2012	Belgium	ISK Biosciences Europe N.V.	Company-Manufacturer	10

Comment received

(1) Page 135: Main developmental study in rabbits: Relevance of visceral malformations:
For the interpretation of the study (Takahashi (2002d)), observations were assessed against historical control data from the period 1992-2001. The experimental phase of the study itself ran between July 2001 and December 2001. It would be appropriate as well to consider historical control data from before and after this period. Therefore we would like to amend table 102 (page 135 of the CLH Report) to include historical control data from the performing testing facility IET covering the period 1992-2011. The amended table 102 is herewith attached. The extended data better cover the experimental phase of the study, the data are more detailed and therefore provide better insight in the occurrences of findings for the testing facility. The extended historical control data demonstrate that the observed types of malformations are not exceeding the incidence in the historical control data reported by the IET testing facility. Moreover the total number of fetuses with malformations doesn't exceed the total number of fetuses with malformations from the historical control data. In addition to the observation that the type of malformations varied widely among fetuses and that no statistically significant difference was observed between the control and treated groups for incidence of each malformation, it strengthens the conclusions that the malformations occurred independently and are incidental.
The background control incidence of abnormal lung lobation which has been reported in the literature by Nakatsuka et al. (1997) demonstrates that it is one of the common anomalies in Japanese Contract Research Organisations.

(2) Page 137, Summary and discussion of reproductive toxicity: teratogenicity in rat or rabbit:
In the summary it needs to be specified that the increased incidence of additional cervical ribs was only observed for Wistar rats but not in the preliminary study with CD rats at a dose level of 500 mg/kg bw/d. As the increase of cervical ribs were within historical control data and was also not observed in the rabbit and it suggests that the findings were incidental.

(3) page 137, Note on the EFSA conclusion:
The incidences observed remained within historical control data. They did not show a dose response. Incidences were not repeated in other strains or within species. Hence there's no clear indication that they are treatment related.

ECHA comment: The attachment document no. 2 Table 102.zip (Table 102 Type and incidence of visceral abnormalities) was submitted separately.

Dossier Submitter's Response

This complementary information was taken into account for response to comments number 9 and 11.

RAC's response

The provided information was incorporated into an extended Table 102 (see attachment).

Date	Country	Organisation	Type of Organisation	Comment number
06/08/2012	Germany		MSCA	11

Comment received

The studies provided indicate that the observed increase in the incidence of cervical ribs in rats and indications of foetotoxicity in rabbits at maternally non-toxic doses are of most relevance for classification. The findings of extra cervical ribs can be considered as minor defects that alone are not sufficient for classification. In addition, they occur at maternally toxic doses, and are not reproducible in another (dose-range finding) study under similar conditions (same dose range, same specie, other strain; non-GLP). Details on this study are limited; therefore it is difficult to conclude on its usability

in a WOE approach.
Visceral abnormalities in rabbits occur at doses without signs of maternal toxicity, however incidences in specific organs of single animals are low (and not statistically significant). Here, a further discussion on severity and significance of these effects with respect to historical controls from the same and other laboratories as well as assessment of the data on a litter base will be helpful.

Reference: Chernoff N, Rogers JM. Supernumerary ribs in developmental toxicity bioassays and in human populations: incidence and biological significance. J Toxicol Environ Health B Crit Rev. 2004; 7(6):437-49.

Dossier Submitter's Response

In rats:
Agreed that findings of extra-cervical ribs are considered as minor defects not sufficient for classification. Flonicamid elicits an increase in the incidence of cervical ribs at a dose level of 500 mg/kg bw/d in Wistar rats. Nevertheless, only 2 fetuses (of the same litter) out of 60 exhibited cervical ribs with distal cartilage, which is not significant compared to control animals. Other cervical ribs were revealed as completely ossified and rudimentary (or small) ribs which were adjacent to the 7^{th} cervical vertebra uni- or bilaterally. Then most of the supernumerary ribs showed no distal cartilage and are transient variations which disappear postnatally and should not be regarded as a relevant effect (Chernoff, 2004). Moreover these effects were observed at a dose level which caused toxicity to the dams (liver hypertrophy, vacuolation of renal tubular cells and increased placental weight). In addition, these effects were not reproducible in a dose-range finding study performed with the same dose ranges in SD rats. Although this last study is non-GLP and is not fully detailed, these results can be considered to support the weight-of-evidence that increased incidence of extra cervical ribs in Wistar rats is not relevant.

In rabbits:
See response to comment number 9.

Reference:
Chernoff N, Rogers JM. Supernumerary ribs in developmental toxicity bioassays and in human populations: incidence and biological significance. J Toxicol Environ Health B Crit Rev. 2004; 7(6):437-49.

RAC's response

Agree with comments on minor significance of extra-cervical ribs. The comparison of frequency of visceral malformations in exposed animals with those of the historical control of the same laboratory or with the historical control of other laboratories in the same country did not demonstrated the increased frequency.

OTHER HAZARDS AND ENDPOINTS

Specific target organ toxicity – repeated exposure

Date	Country	Organisation	Type of Organisation	Comment number
02/07/2012	Hungary		Individual	12

Comment received

The hepatotoxic effects observed with Flonicamid occur at doses not warranted classification. However the structure similarity of Flonicamid with Ionazid known to release N-acetyl hydrazine (human hepatotoxic) should lead to double checked the metabolism of Flornicamid to ensure that it does not release any release N-acetyl hydrazine or similar substance.

Kidney toxicity observed in males is claimed to be mediated via alpha2-microglobulin mechanism. However, vacuolation of kidneys is observed in females together with impairement of different clin chem parameters such as increased creatinine and total bilirubin concentrations in both sexes (statistical significance only for males); increased mean glucose concentrations in both sexes (statistical significance only for females); elevated sodium and chloride and reduced potassium concentrations in both sexes (statistical significance only in males). These findings in both sexes (and different species) seem to indicate that nephrotoxicity observed in males is not mediated only by alpha2-microglobulin-mechanisms. Therefore a classification as STOT-RE might be appropriate based on some findings in males are observed in doses of 100 ppm [7,47 mg/kg bw/d] and higher in a 28d

rat study. (the type of findings have to be evaluated in detail from the core data: species specificity, type of lesions and applicability of the by α2-microglobulin-MoA).
Dossier Submitter's Response
Liver effects: Flonicamid was not shown to release N-acetyl hydrazine or similar substance in any of the toxicokinetics studies performed with flonicamid. **Kidney effects:** It is not considered adequate to classify flonicamid as STOT-RE for kidney toxicity. Indeed, there is stronger evidence that kidney nephropathy is mediated by the male rat-specific protein, alpha-2-microglobulin, and is thus not regarded as relevant for humans: hyaline droplets deposition, granular casts, tubular basophilia were observed in the male rat only. Immunohistochemical staining of the kidneys demonstrated that the hyaline droplets and granular casts reacted positively to alpha-2-microglobulin antibody. Moreover, lesion morphology of findings observed in females displayed a clear difference from lesions observed in males: the main kidney effect observed in females is vacuolation of renal tubular cells in the 90-d rat study and in the carcinogenicity rat study at the highest dose level. Other species did not show evidence of nephrotoxicity. Indeed, renal tubular vacuolation observed in female dogs in the 90-day study appeared at a dose level exceeding the MTD and are thus not considered relevant. In the 90-d mouse study, although some clinical chemistry findings (not statistically significant) were observed, no histopathological lesions were associated.
RAC's response
Agree with the response of the Dossier submitter.

REFERENCES: none

ATTACHMENTS RECEIVED: 2

Flonicamid ECHA høring juli 2012.doc. Submitted by Denmark/MSCA. Comment is copied in the table.
Table 102.zip (Table 102: Type and incidence of visceral abnormalities). Submitted by Belgium/ ISK Biosciences Europe N.V./Company-Manufacturer. Comment is not copied in the table.