Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

flonicamid

EC number: N/A
CAS number: 158062-67-0

CLH-O-0000002561-80-03/F

Adopted

5 June 2013

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CLH-O-0000002561-80-03/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: flonicamid

EC number: N/A

CAS number: 158062-67-0

The proposal was submitted by **France** and received by the RAC on **21/06/2012.**

In this opinion, all classifications are given firstly in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS) and secondly, according to the notation of 67/548/EEC, the Dangerous Substances Directive (DSD).

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **21/06/2012**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **6/08/2012**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Boguslaw Baranski**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation.

The RAC opinion on the proposed harmonised classification and labelling was reached on **5 June 2013** and the comments received are compiled in Annex 2.

The RAC Opinion was adopted by **consensus**.

OPINION OF THE RAC

The RAC adopted the opinion that **flonicamid** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry										
Dossier submitters proposal		flonicamid (ISO); *N*-(cyanomethyl)-4-(trifluoromethyl)pyridine-3-carboxamide		158062-67-0	Acute Tox. 4	H302	GHS07 Wng	H302		
RAC opinion		flonicamid (ISO); *N*-(cyanomethyl)-4-(trifluoromethyl)pyridine-3-carboxamide		158062-67-0	Acute Tox. 4	H302	GHS07 Wng	H302		
Resulting Annex VI entry if agreed by COM		flonicamid (ISO); *N*-(cyanomethyl)-4-(trifluoromethyl)pyridine-3-carboxamide		158062-67-0	Acute Tox. 4	H302	GHS07 Wng	H302		

Classification and labelling in accordance with the criteria of DSD

	Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
Current Annex VI entry								
Dossier submitters proposal		flonicamid (ISO); *N*-(cyanomethyl)-4-(trifluoromethyl)pyridine-3-carboxamide		158062-67-0	Xn; R22	Xn R: 22 S:		
RAC opinion		flonicamid (ISO); *N*-(cyanomethyl)-4-(trifluoromethyl)pyridine-3-carboxamide		158062-67-0	Xn; R22	Xn R: 22 S: (2-)46		
Resulting Annex VI entry if agreed by COM		flonicamid (ISO); *N*-(cyanomethyl)-4-(trifluoromethyl)pyridine-3-carboxamide		158062-67-0	Xn; R22	Xn R: 22 S: (2-)46		

SCIENTIFIC GROUNDS FOR THE OPINION

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The acute toxicity of flonicamid was evaluated for the oral, dermal and inhalation routes of exposure.

In rats, flonicamid exhibits an oral LD_{50} of 884 and 1768 mg/kg in males and females, respectively (Ridder *et al.*, 2001a).

By the dermal route, no deaths occurred in rats in response to a limit dose of 5000 mg/kg by semi-occluded topical application for 24 h (dermal LD_{50} > 5000 mg/kg);

By inhalation, no deaths occurred in young adult rats exposed for 4 hours (via inhalation; nose only) to flonicamid at concentration of 4.9 mg/L (inhalation LC_{50} > 4.9 mg/L).Taking into account that flonicamid exhibited an acute oral toxicity with an LD_{50} between 200 and 2000 mg/kg, the substance meets the criteria for classification according to Regulation (EC) No 1272/2008 (CLP) (oral (mg/kg bw) 300 < ATE ≤ 2000) as Acute Tox. 4 - H302, and Xn; R22 according to Directive 67/548/EEC (Dangerous Substances Directive; DSD).

The dermal LD_{50} lies above the classification cut-off of 2000 mg/kg under both CLP and DSD, and therefore no classification is proposed.

No deaths occurred after inhalation exposure, at a concentration marginally lower than the classification cut-off of 5 mg/L/4h under both CLP and DSD; nevertheless no classification is proposed.

Comments received during public consultation

The Spanish and Danish MSCAs supported the proposed classification of flonicamid as Acute Tox. 4 - H302 (Harmful if swallowed) and Xn; R22 (Harmful if swallowed) according to CLP and DSD, respectively.

RAC Assessment and comparison with the classification criteria

Taking into account the oral LD_{50} values for male and female rats equal to 884 and 1768 mg/kg bw, respectively, the RAC agreed with the opinion of the dossier submitter since these values are within the classification criteria for Acute Tox. 4 – H302 (Harmful if swallowed) according to CLP, and Xn; R22 according to DSD.

The high dermal LD_{50} value of > 5000 mg/kg for rats and the LC_{50} value for inhalation of > 4.9 mg/L in conjunction with no deaths or adverse clinical signs, shows that flonicamid should not be classified for acute toxicity for these exposure routes.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

No specific target organ toxicity has been identified after single exposure and hence, no classification is required under either CLP or DSD.

Comments received during public consultation

No comments were received concerning STOT SE.

Assessment and comparison with the classification criteria

The RAC agreed with the opinion of the dossier submitter that no classification was required.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier submitter's proposal

No specific target organ toxicity has been identified after single exposure and hence, no classification is required under either CLP or DSD.

Comments received during public consultation

No comments were received concerning STOT SE.

Assessment and comparison with the classification criteria

The RAC agreed with the opinion of the dossier submitter that no classification was required.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Results from a test carried out according to OECD TG 404 on six adult male New Zealand White rabbits are available. In that study, 0.5 g of flonicamid technical was applied to the clipped intact thoracic dorsal skin (6 cm^2) under a semi-occlusive dressing for 4h. After removal of the patch, there were no erythema/eschar formation or oedema on either test or vehicle control sites throughout the study. The EU primary irritation indices were 0.0 for both erythema/eschar formation and oedema.

Since the mean scores after 24 to 72 hours for erythema and oedema were below the criteria for classification and labelling, the dossier submitter concluded that flonicamid does not meet the classification criteria for skin irritation under either CLP or DSD.

Comments received during public consultation

No comments were received concerning skin corrosion/irritation

RAC Assessment and comparison with the classification criteria

The RAC agreed with the proposal of the dossier submitter that flonicamid does not have skin corrosive or irritation properties and therefore should not be classified for this hazard class.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

In a study carried out according to OECD TG 405, six male New Zealand White rabbits were each given a single instillation into the lower conjunctival sac of the right eye of 0.1 ml (approx. 70 mg) unformulated flonicamid technical (batch n° 9809; purity 98.7%). The upper and lower eyelids were held together immediately after administration to minimize the loss of test material. The left eye remained untreated to serve as a reference control. The individual mean 24 – 72 h scores did not exceed 0.7 for conjunctival effects and were 0.0 for both corneal and iridial effects in all animals.

Since the mean scores after 24 to 72 hours for corneal opacity, iritis, conjunctival redness and conjunctival oedema were below the criteria for classification and labeling, the dossier submitter concluded that flonicamid should not be classified for eye corrosion/irritation under either CLP or DSD.

Comments received during public consultation

No comments received concerning eye corrosion/irritation.

RAC Assessment and comparison with the classification criteria

The RAC agreed with the proposal of the dossier submitter that flonicamid does not have eye corrosive or irritation properties and therefore should not be classified for this hazard class.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

In a study conducted with technical flonicamid in 40 male Guinea pigs, in line with the guideline US-EPA OPPTS 870.2600, using the maximisation test method of Magnusson & Kligman, a challenge test revealed that 2 out of 20 flonicamid treated animals showed mild erythema at 24 and 48 h after challenge, and 2 out of 20 control animals showed mild erythema at 48 h only. On the other hand, DNCB [ECHA Secretariat: presumably 2,4-Dinitrochlorobenzene used as a positive control] produced mild to intense erythema in 9 out of 10 treated animals at 24 h and in 10 animals at 48 h, compared to a zero incidence of positive reactions in the DNCB control group. The severe response induced by DNCB demonstrates the acceptability/reliability of the study.

Considering that no specific effect is reported in humans, and that flonicamid induced a mild erythema in 10% of the Guinea pigs being below the 30 % threshold for a Guinea pig maximisation test, no classification is required for flonicamid under either CLP (including the criteria defined in the 2nd ATP) or DSD.

Comments received during public consultation

No comments were received concerning skin sensitisation.

RAC Assessment and comparison with the classification criteria

The RAC agreed with the proposal of the dossier submitter that flonicamid does not have skin sensitization properties and therefore should not be classified for this hazard class.

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

The repeated dose toxicity has been studied after oral and dermal exposure. No repeated dose toxicity study was performed using the inhalation route of exposure.

Oral route:

The short term effects of flonicamid after oral administration were studied in rats (28- and 90-day studies), in dogs (28/35-day, 90-day, and 1-year studies) and in mice (90-day study). The target organs were the liver (rats, mice), the kidney (rats) and the haematopoietic system (anaemia, mice).

In the rat studies, the adverse effects on the kidneys were considered to be mediated by the male rat specific protein, alpha-2-microglobulin, and were not regarded as relevant to humans. Therefore, the short term NOAEL in rats was 60 mg/kg bw/d from the 90-day study. In the dog studies, the relevant NOAEL was 8 mg/kg bw/d, based on reduced body weight gain, reduced thymus weight in males (90-day study), and mild anaemia (1-year study). In the mouse study, the NOAEL was 15.3 mg/kg bw/d based on hepatocellular hypertrophy and splenic extramedullary haematopoiesis (related to anaemia).

Dermal exposure:

In a 28-day percutaneous study in rats, the NOAEL was higher than 1000 mg/kg bw/d (highest dose tested).

Comparison with criteria

The dossier submitter compared the effects observed in the repeated dose toxicity studies with the CLP criteria for classification as STOT RE. The dossier submitter concluded that no significant toxic effects, of relevance to human health, were observed in a 90-day repeated-dose study conducted in experimental animals within the guidance value ranges of 10-100 mg/kg/d.

Similarly, using the DSD classification criteria the dossier submitter concluded that in the reported studies there was no 'serious damage' (clear functional disturbance or morphological change which has toxicological significance) caused by repeated or prolonged exposure by an appropriate route, in a 90-day repeated-dose study conducted in experimental animals at a dose ≤ 50 mg/kg/d. When interpreting the results of a sub-acute (28-days) toxicity test, this value should be increased approximately three-fold.

Since the findings in the repeated dose studies do not meet the classification criteria according to either CLP or DSD, no classification for specific target organ toxicity - repeated exposure (STOT RE) or for repeated dose toxicity under the DSD is proposed.

Comments received during public consultation

One comment was received from an individual from Hungary noting that the hepatotoxic effects observed with flonicamid occur at doses not warranting classification.
However the structural similarity of flonicamid with ionazid, known to release N-acetyl hydrazine (a human hepatotoxic substance) should lead to an assessment of the metabolism of flonicamid to ensure that it does not release any N-acetyl hydrazine or similar substance.
In the response to the comment concerning release of N-acetyl hydrazine (human hepatotoxic) the dossier submitter noted that flonicamid was not shown to release N-acetyl hydrazine or similar substance in any of the toxicokinetics and metabolism studies performed.

Another comment was submitted on kidney toxicity observed in males. It is claimed to be mediated via an alpha-2-microglobulin mechanism. However, vacuolation of kidneys is observed in females together with impairment of different clinical chemistry 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 an alpha-2-microglobulin mechanism. Therefore a classification as STOT RE might be appropriate based on some findings in males observed at concentrations of 100 ppm (equal to 7,47 mg/kg bw/d) and higher in a 28-day rat study (the type of findings in the core data need to be evaluated in detail for: species specificity, type of lesions and applicability of the alpha-2-microglobulin mode of action (MoA)).
In their response to this comment, the dossier submitter noted that there is strong evidence that kidney nephropathy is in fact mediated by an alpha-2-microglobulin mechanism; and thus this nephropathy is not regarded as relevant for humans. The hyaline droplets deposition, granular casts, tubular basophilia were observed in the male rat only. Immuno-histochemical staining of the kidneys demonstrated that the hyaline droplets and granular casts reacted positively to the alpha-2-microglobulin antibody.
Furthermore, lesion morphology of findings observed in females displayed a clear difference from lesions observed in males; the main kidney effect observed in females being vacuolation of renal tubular cells in the 90-day rat study.

Other animal 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 Maximum Tolerable Dose (MTD) and are thus not considered relevant. In the 90-day mouse study, although some clinical chemistry findings (not statistically significant) were observed, no associated histo-pathological lesions were seen. Therefore, the dossier submitter responded that is the data was not sufficient to classify flonicamid as STOT RE for kidney toxicity.

RAC Assessment and comparison with the classification criteria

The RAC agreed with the conclusion of the dossier submitter that flonicamid does not exert significant specific target organ toxicity after repeated exposure or repeated dose toxicity which meets either the CLP or the DSD classification criteria and therefore it should not be classified for this hazard class.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

The potential of technical flonicamid to induce gene mutation or chromosomal damage was tested in a standard battery of studies including *in vitro* point mutation assays in bacterial and mammalian cells, *in vitro* and *in vivo* clastogenicity studies (incl. a mouse micronucleus test), and *in vivo* unscheduled DNA synthesis (UDS) and comet assays.

- Flonicamid technical does not produce gene mutations in prokaryotic or eukaryotic cells *in vitro*, either in the presence or absence of a mammalian metabolic activation system.
- It is not clastogenic in an *in vitro* cytogenetics assay in Chinese Hamster CHL cells.
- It is not clastogenic in the *in vivo* mouse micronucleus test; although there was no effect on the PCE/NCE ratio in the latter study, there is evidence from the tissue distribution study that the concentration of flonicamid technical in bone marrow is similar to the blood concentration for at least 24 h after administration of a single dose; therefore, the assay is to be considered as a valid assessment of *in vivo* clastogenic activity, in as much as the study was carried out at dose levels approaching the MTD.
- Flonicamid does not produce DNA damage, as assessed by unscheduled DNA synthesis in rat hepatocytes and the electrophoretic migration of DNA derived from mouse colon, liver and lung tissue.

It is concluded by the dossier submitter that flonicamid does not exhibit primary genotoxic properties at the DNA, gene and chromosome levels of genetic organization.

Comparison with criteria

The complete battery of *in vitro* and *in vivo* genetic toxicology studies conducted with flonicamid indicates no genotoxic potential. Flonicamid does not meet the criteria for classification.

Comments received during public consultation

No comments were received concerning germ cell mutagenicity.

RAC Assessment and comparison with the classification criteria

The RAC agreed with the conclusion of the dossier submitter that flonicamid did not induce genotoxic or mutagenic effects which could meet the CLP or DSD classification criteria; and therefore it does not have inherent mutagenic properties and should not be classified for this hazard class.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

According to the dossier submitter, flonicamid does not fulfil the criteria for classification for carcinogenicity, and no classification is required for flonicamid under either CLP or DSD.

The oral long-term toxicity of flonicamid was investigated via dietary administration in a combined chronic toxicity and carcinogenicity study in Wistar rats, and in oncogenicity studies in the CD-1 mouse.

Rat study

In a combined chronic toxicity and carcinogenicity study (Kuwahara, 2002c, see BD) male and female Wistar rats (5-6 weeks old at start of dosing) were administered 0; 50 (males only); 100 (males only); 200 (males and females); 1000 (males and females) and 5000 ppm (females only) flonicamid technical in the diet for 104 weeks (main group: 52 rats/sex) or for 52 weeks (satellite group no. 1: 14 rats/sex) or for 26 weeks (satellite group no. 2: 10 rats/sex). The doses were calculated to be: 1.84; 3.68; 7.32 and 36.5 mg/kg bw/d in males from the 50; 100; 200 and 1000 ppm groups, respectively and 8.92; 44.1 and 219 mg/kg bw/d in females in the 200; 1000 and 5000 ppm groups, respectively.

Neoplastic findings:
No consistent cause of death was evident in animals that died prior to scheduled sacrifice (decedents). The nature and incidence of all tumor types were similar in all groups of animals scheduled to be killed after 104 weeks, including the decedents. Statistically significant changes in tumor incidences between treated and control groups were confined to reduced incidences of anterior pituitary adenoma in males at 1000 ppm and mammary gland adenoma in females at 5000 ppm. Treatment-related rare tumor types did not occur in either sex, and the multiplicity of tumors and latencies did not indicate a treatment-related effect in either sex at any dose level.

The lung masses observed in 5 out of 52 decedents and terminally killed male rats exposed at the dose of 36.5 mg/kg bw/d (1000 ppm) were described as tumours of various histopathological types and were concluded to be not treatment-related. In one male rat such a lung mass was histopathologically diagnosed as hyperplasia of alveolar epithelial cells, in a second rat as adenoma, in a third one as adenocarcinoma, in fourth rat as malignant methothelioma (mesothelioma) and in a fifth rat as histiocytic sarcoma. Different types of diagnosed tumours indicate that they were not related to the same neoplastic process and were not treatment-related. These lung tumors were not observed in females exposed to a dose of 44.1 mg/kg bw/d (1000ppm) or at the higher dose of 219 mg/kg bw/d (5000 ppm).

The squamous cell carcinomas in the nasal cavity of males rats (3.8% and 11.8% in the control and 1000 ppm group, respectively) and in the nasal cavity of females rats (5.8% in 5000 ppm group) were neither dose-related nor with statistical significance and were observed unilaterally and without pre-neoplastic lesions. These tumours were re-evaluated as occurring in the nasolacrimal duct. Historical control data from the testing facility of The Institute of Environmental Toxicology (IET) included four studies conducted in Jcl:Wistar Rats (205 males and 204 females). In these studies, the incidence of squamous cell tumors of the nasolacrimal duct ranged from 0 to 10% (mean 4.32%) in male rats and from 0 to 4.1% (mean 0.98%) in females. Thus, the frequency of tumors in the nasolacrimal duct in rats exposed to flonicamid were not statistically different from concurrent control animals and very close to historical control incidences in male and female rats.

After scrupulous analysis of all available data in the study (Kuwahara, 2002c, see BD) the dossier submitter concluded that no carcinogenic potential was found after dietary administration of flonicamid for 104 weeks at dose levels up to 36.5 and 219 mg/kg bw/d in male and female rats, respectively.

Mouse studies
Two carcinogenicity mouse studies (Ridder, 2003a; Nagaoka, 2004, see BD) were analysed by the dossier submitter.

First mouse study
In the Ridder study (2003a, see BD) groups of Swiss mice (Crl:CD-1® (ICR) BR VAF/Plus strain, 60 animals/sex, were administered 0, 250, 750 and 2250 ppm of flonicamid technical in the diet for 78 weeks. Satellite control and high dose groups of 10 mice/sex were sacrificed at 26 weeks or 52 weeks. The average test substance intake was 29, 88 and 261 mg/kg bw/d in males and 38, 112 and 334 mg/kg bw/d in females from the 250, 750 and 2250 ppm groups, respectively.

Survival was not affected by treatment at any dose level and no specific cause of premature death was discernible.

Neoplastic findings
Lung: Treatment-related statistically significant increased incidences of primary lung tumors occurred in both sexes at all dose levels.

The incidence of alveolar/bronchiolar adenoma in control CD-1 male mice and those exposed to flonicamid at concentrations of 250, 750 and 2250 ppm were 9/60, 26/60, 24/60 and 32/60

respectively, while in CD-1 female mice frequencies were 9/60, 21/60, 30/60 and 25/60, respectively. Thus, there was a similar, statistically significant increase in frequency of lung adenomas in male and female mice; however, this increase was not dose-dependent.

The incidence of alveolar/bronchiolar carcinoma in control CD-1 male mice and those exposed to flonicamid at concentrations of 250, 750 and 2250 ppm were 2/60, 4/60, 9/60 and 10/60, respectively, while in CD-1 female mice frequencies were 0/60, 3/60, 4/60 and 5/60, respectively. Statistically significant increases were observed in male mice exposed at 750 and 2250 ppm and in female mice exposed at 2250 ppm.

It was noted that there was a considerable increase of frequency of focal alveolar/bronchiolar hyperplasia in all exposed male and female mice, which is important for elucidation of the mode of action (MoA).

The dossier submitter noted in the conclusion that aging CD-1 mice, as used in this study, have a high incidence of spontaneous bronchiolo-alveolar tumours and that 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 this mice strain to the development of tumours of the terminal bronchiolar epithelium.

Other organs and tissues: No treatment-related neoplastic effects occurred in any other tissue at any dose level.

Second mouse study
The additional mouse oncogenicity study (Nagaoka, 2004) was designed to determine a NOAEL for flonicamid when administered to the same strain of mouse at lower dose levels than the previous study, using the same production batch.

Groups of 50/sex Swiss mice (Crj:CD-1® (ICR) strain were administered 0, 10, 25, 80 and 250 ppm of flonicamid technical in the diet for 78 weeks (equivalent to 0, 1.2, 3.1, 9.9 and 30.2 mg/kg bw/d in males and 0, 1.4, 3.6, 11.8 and 36.3 mg/kg bw/d in females).

Neoplastic findings:
Lung: Single or multiple alveolar/bronchiolar adenomas of the lungs were statistically significantly increased only in males at 250 ppm (21/50 vs. 8/50 in control group). Also total number of mice with primary lung tumors was statistically increased in male mice exposed at 250 ppm (27/50 vs. 11/50 in control group).

The incidences of bronchioloalveolar adenocarcinoma in male and female CD -1 mice exposed to flonicamid in the diet for 78 weeks, at doses of 0, 10, 25, 80 and 250 ppm, were not statistically increased in the Nagaoka study (2004), see BD, when comparing with concurrent control groups.

The latency of pulmonary tumor formation in these males was not affected by treatment since the first pulmonary neoplastic changes were seen in decedent mice after 64, 75, 68, 53 and 56 weeks of treatment in the control, 10, 25, 80, and 250 ppm groups, respectively.

The incidences of pulmonary epithelial adenomas and carcinomas in males treated at up to 80 ppm (9.9 mg/kg bw/d), and in females at all dose levels up to 250 ppm (36.3 mg/kg bw/d), were not significantly different from the control incidences.

Taking into account both studies in CD-1 mice, the NOAEL for neoplastic lung lesions for male mice was 9.9 mg/kg bw/d (80 ppm) and for female mice 36.3 mg/kg bw/d (250 ppm).

Historical control data:
Historical control incidences of benign tumours in the Crl:CD-1® (ICR) BR strain were reported in the CLH dossier to be approx. 14% and 8% in males and females, respectively, and the incidence of malignant tumours was reported to be approx. 7% and 4% in males and females, respectively.

A reference source for the historical control data was not given and during public consultation questions were raised regarding the origin of these historical control incidence values.

In the response to comments made during the public consultation, the dossier submitter provided further data (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) on incidences of lung adenoma and carcinoma in CD-1 mouse and other mouse strains as follows:

1. A total of 59 studies were reported in a Charles River Laboratories document published in 2005. 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.

2. The incidence of spontaneous bronchioloalveolar adenoma in male and female CD-1 mouse is reported as 2-42 % and 1.67-26.67 % respectively; and the incidence of spontaneous bronchioloalveolar adenocarcinoma in the male and female CD-1 mouse is reported as 1.43-26 % and 0.77-18.37 % respectively.

While comparing the historical incidences of spontaneous bronchioloalveolar adenoma in CD-1 mouse with those observed in flonicamid-exposed mice it is noted that the spontaneous incidences of 42% in male and 26.67% in female CD-1 mice are only slightly lower than the highest incidence of adenomas observed in male CD-1 mice exposed to flonicamid in the diet for 78 weeks at concentrations of 2250 ppm (53,3%) and female CD-1 mice exposed at 250, 750 and 2500 ppm (incidences of 35%, 50% and 41,6%, respectively; Ridder, 2003a, see BD).

In the Nagaoka study (2004), see BD, incidences of bronchioloalveolar adenoma in male CD-1 mice exposed in the diet for 78 weeks to flonicamid at 0, 10, 25, 80 and 250 ppm were 16%, 22%, 24%, 22% and 42%, respectively, and in exposed female CD-1 mice adenoma incidences were 20%, 16%, 22%, 28% and 26%. Thus, they were well within reported incidence of spontaneous bronchioloalveolar adenomas in CD-1 mouse. It is noted that the incidences of spontaneous bronchioloalveolar adenoma in control rats in the Nagoka study were relatively high; 16% in males and 20% in females, around twice as high as in the Ridder study (9% in males and females).

Mechanistic studies on cell proliferation:
As mentioned above, aging CD-1 mice have a high incidence of spontaneous bronchioloalveolar tumours and posess two characteristics of the lung epithelium that affect tumour incidence; the proportion of immature Clara cells and their sensitivity to stimulated cell division.
Therefore, the mechanistic studies were designed to investigate the mechanism of action of flonicamid in inducing lung tumours in CD-1 mice as postulated in the IPCS Framework for Analysing the Relevance of a Cancer Mode of Action for Humans (2006).

The results of these studies (Nomura, 2003a,b,c,d,e, see BD) are summarised below:

1. Flonicamid elicits a dose-related increase in cell proliferation in the epithelial cells of the terminal bronchiolar region of the lung in CD-1 strain male mice in the dietary concentration range of 250 – 2250 ppm, equivalent to a dose range of 40.9 - 339.3 mg/kg bw/d. The threshold for the flonicamid-induced stimulation of lung epithelial cell proliferation would lie in the range 80 – 250 ppm, equivalent to a dose range of 12.3 - 40.9 mg/kg bw/d; the NOEL for cell proliferation was 80 ppm, equivalent to a dose level of 12.3 mg/kg bw/d (Nomura, 2003a, see BD).

Conclusion: These results indicate that the threshold exposure level for induction of epithelial cell proliferation is below or at the same level as the threshold exposure level necessary for induction of lung adenoma. Thus the induction of cell proliferation is an essential step in inducing lung adenoma and adenocarcinoma in the lungs of CD-1 mice but occurs well before the appearance of lung tumours. It was seen already after few days of exposure while tumors in exposed animals occurred after more than 50 weeks (Nagaoka, 2004, see BD).

It should be noted that there was a considerable increase in the frequency of focal alveolar/bronchiolar hyperplasia and of lung tumours in male and female mice exposed at 250 – 2250 ppm (Ridder, 2003a; Nagaoka, 2004), but hyperplasia or increased frequency of lung tumours were not observed in male and female CD-1 mice exposed to flonicamid at concentrations of 10 – 80 ppm (Nagaoka, 2004, see BD) suggesting that threshold for hyperplasia induction is also a threshold for lung tumor induction in CD-1 mice.

2. Flonicamid elicits an increase in cell proliferation in epithelial cells of the terminal bronchiolar region of the lung in female CD-1 strain mice after 3 or 7 days of treatment at an average dose level of 380 mg/kg bw/d, but not in female rats at an average dose level of 398 mg/kg bw/d (Nomura, 2003b, see BD).

Conclusion: Lack of hyperplasia induction by flonicamid in rats, i.e. the species in which this substance is not inducing lung tumors, might indicate that hyperplasia is essential for induction of tumours by flonicamid as observed in CD-1 Mice.

3. Flonicamid elicits an increase in cell proliferation in epithelial cells of the terminal bronchiolar region of the lung in CD-1 mice after 28 days of treatment at an average dose level of 303 mg/kg bw/d, which is readily and fully reversible within 7 days of the cessation of treatment (Nomura, 2003c).

Conclusion: The cell proliferation induced by flonicamid is reversible after ending the exposure indicating that this is not a permanent lesion.

4. Flonicamid at 2250 ppm, equivalent to a dose of 389 mg/kg bw/d, elicits an increase in cell proliferation in the epithelial cells of the terminal bronchiolar region of the lung in CD-1 male mice. Since its metabolites TFNG, TFNA and TFNA-AM did not affect the BrdU labelling index, the cell proliferation effect of flonicamid is considered to be due to the parent molecule rather than one of the metabolites tested.

Conclusion: Flonicamid as a parent compound can induce cell proliferation in the epithelial cells of the terminal bronchiolar region of the lung in CD-1 male mice, but not its major metabolite. This indicates that interspecies difference in metabolites formation is not essential for the carcinogenic MoA of flonicamid.

5. Cell proliferation of the lung terminal bronchiolar epithelial cells (as indicated by BrdU labelling index) was significantly increased after a 3-day dietary administration of 2250 ppm flonicamid technical (equivalent to 299 mg/kg bw/d) or 2250 ppm of isoniazid for three consecutive days in CD-1 mice, and the greatest increase was seen in isoniazid-treated mice. Treatment with flonicamid did not induced cell proliferation of the lung terminal bronchiolar epithelial cells neither in B6C3F1 nor in C57 mice, but isoniazid treatment produced statistically significant increases in the BrdU labelling index in these two strains.

Contrary to the differential response seen among the three strains of mice with respect to cell turnover, the proportion of Clara cells in the terminal bronchiolar region of mouse lung (estimated by CC-10 staining) was similar among the three strains employed, namely 80% of the terminal bronchiolar cells were Clara cells (Nomura, 2003e, see BD).

Conclusion on mechanistic studies: Flonicamid and isoniazid are both inducers of cell proliferation in bronchiolar epithelium, but flonicamid only in CD-1 mice, while isoniazid in all three strains of

mouse. The presence of Clara cells is not a sufficient condition for induction of cell proliferation, because flonicamid was unable to induce cell proliferation in the epithelium of the other two mouse strains than CD-1. Thus the CD-1 mouse strain has other, probably genetically–dependent, features that enable flonicamid to induce cell proliferation in the lung, and which might be linked to its carcinogenic properties in this strain of mouse. Therefore the induction of cell proliferation in mice by flonicamid, in the light of available data, is strain specific, while in the case of isoniazid it is not strain specific.

Analysis of the genetic susceptibility of CD-1 mouse:
High susceptibility of the CD-1 mouse may be, at least partially, explained with the help of data presented by Manenti *et al.* (2003).

Analysis of literature data on spontaneous lung tumors in CD-1 mice not treated with chemicals revealed a mean tumor incidence of 21.8%, with a range of 8.8 to 61.1%. Male mice showed a significantly higher mean lung tumor incidence compared to females (males 25.2% with a range of 8.8% - 61.1% vs. females 18.2% with a range of 9.2% to 26.6%). Lung tumors that developed in CD-1 mice showed histological characteristics of lung adenomas or carcinomas typical of mouse lung tumors.

In the 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 mouse strains (Manenti *et al.*, 2003)

CD-1 mice are particularly susceptible to chemically induced lung tumorigenesis, as certain chemicals produce carcinogenic effects in lung of CD-1 mice but not in other rodent species. For example, vinyl chloride inhalation induces lung tumors in CD-1 and A/J mice, but not in B6C3F1 mice, rats, hamsters, or in humans.

There is some evidence suggesting that genetic markers located in the human *Pas1* homologous region may affect risk and prognosis of lung adenocarcinoma in humans. However, there is not sufficient data for establishing any role in tumor formation of various gene alleles in the *Pas1* locus in humans (Manenti *et al.*, 2003).

Comparison of carcinogenicity between flonicamid and isoniazid
Another argument supporting that the mechanism of induction of tumours in lungs of CD-1 mice might not be relevant for humans can be found from the comparison of carcinogenicity between flonicamid and isoniazid; although these structurally related compounds do not have common functional groups and should therefore not be grouped for read-across.
The comparison is made because both chemicals induce proliferation of respiratory epithelium in mice; flonicamid only in CD-1 mice, while isoniazid in three strains of mice (CD-1, B6C3F1, and C57).
Isoniazid, despite being recognized as an inducer of lung tumours in mice, does not increase the frequency of cancer in hamster or in humans; and therefore IARC has not classified isoniazid as a suspected human carcinogen, but instead classifies it to group 3 of 'not classifiable regarding its carcinogenicity to humans' (IARC Monographs, 1987, see BD). Thus at least for isoniazid a key step of lung tumour induction in mice (increased cell proliferation of respiratory epithelium) is not induced humans.

Both isoniazid and flonicamid are proven lung tumour inducers in mice only. They both stimulate intensive proliferation of the lung terminal bronchiolar epithelial cells in mice after oral administration, and most probably they share a MoA through a mitogenic mechanism for initiation lung adenoma and adenocarcinoma in CD-1 mice.

Flonicamid and isoniazid are not mutagenic. Assuming that both chemicals share the same mechanisms of lung tumour induction in mice, and taking into account that this mechanism is not

effective in inducing lung tumours in humans treated with isoniazid, it is highly probable that it will also be ineffective in humans in case of flonicamid. Particularly since it has been demonstrated that flonicamid does not induce cancer in the lungs or any other organs in rats.

Comparison with criteria
According to the dossier submitter, flonicamid has no carcinogenic potential in rats. The mechanism of lung tumour formation in CD-1 mice is not relevant for humans.

The CLP criteria for classification as a Category 2 carcinogen (Category 3 according to DSD) are as follows:
"*The placing of a substance in Category 2 id done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.*"

The dossier submitter concluded that, on the basis of an extensive assessment of historical control data, mechanistic information and comparison with isoniazid, flonicamid does not have intrinsic properties to induce cancer in rats, and the effects observed in mice have been demonstrated to be species- and strain-specific and not relevant to humans. Therefore the existing experimental evidence does not fulfil the criteria for Carc. 2 - H351 under CLP or Carc. Cat. 3; R40 under DSD.

Comments received during public consultation

The following comments which were received are considered as important:

The Spanish MSCA agreed with the proposal of the dossier submitter that no classification is required for carcinogenicity for flonicamid, under either CLP or DSD.
The Spanish CA also agreed with the conclusion 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 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 relevant to humans

According to the German MSCA, 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.

The German MSCA also commented that while the proposed MoA seems 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). They further commented that it should be 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 were 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 three mouse strains might be indicative of other mechanisms involved in development of lung tumours. The German MSCA considered that 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). 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.

One individual from Hungary stated that mechanistic data showing no difference of Clara cells number between the different strains, but differences on mitogenic effect of flonicamid and isoniazid could be explained by strain genetic background sensitivity. Another hypothesis would be that cell division occurs in different cell types 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 overruled and should be taken into account for classification.

RAC Assessment and comparison with the classification criteria

The RAC agreed with the dossier submitter's analysis and interpretation of the carcinogenicity studies conducted with flonicamid. The RAC also concurs that the results of the MoA studies which strongly support the argument that flonicamid induces cell proliferation in alveolar/bronchiolar epithelium of CD-1 mice, leading to adenomas and carcinoma formation, by a mitogenic mechanism. This mechanism, in which proliferation of epithelial cells in the lung is a key event and a necessary step in carcinogenesis (based on the mechanistic studies reviewed above), is a species and strain specific mechanism. This mechanism does not operate in rats and two other strains of mice. In addition, cell proliferation is rapidly and fully reversible on cessation of treatment, indicating that there is no potential for cumulative effects after e.g. intermittent exposure.

In an overall assessment, RAC is of the opinion that the existing data do not warrant classification of flonicamid for carcinogencity due to the following reasons:

1. Flonicamid is not genotoxic or mutagenic.
2. There is no carcinogenicity of flonicamid in rats.
3. The increase in lung adenomas (benign tumors) in mice (above historical control values) is preceded by a bronchiolar/alveolar epithelial cell proliferation in the CD1 mouse strain with a high spontaneous frequency of lung tumors.
4. The incidences of lung carcinomas (malignant tumors) in flonicamid treated CD-1 mice were within the historical control data.
5. Flonicamid does not exert cytotoxic effects leading to prolonged inflammation; therefore it is postulated that, at doses above a threshold of 80 ppm in the diet, in CD-1 mice flonicamid increases the frequency of mitotic division leading to epithelial cell proliferation, as was seen in the Ridder (2003a) and Nagaoka (2004) studies.
6. The mechanistic studies demonstrated that cell proliferation in CD-1 mice is seen already after a few days of exposure to flonicamid at the same doses that are carcinogenic after long-term exposure (250 ppm and above) and it is not seen at lower doses (80 ppm and below) known to not increase a frequency of lung tumors in mice.
7. The proliferation of bronchiolar/alveolar epithelial cells is reversible after cessation of exposure.
8. Flonicamid does not induce cell proliferation in the alveolar/bronchiolar epithelium of two other strains of mice known to have a much lower incidence of spontaneous lung tumours than CD-1 mice (Nomura, 2003e, see BD) indicating that a mitogenic mechanism of lung tumorigenesis in CD-1 mice is not operating in B6C3F1 and C57BL mice.
9. The mitogenic mechanism of lung tumorigenesis is also not operating in rats, since it has been shown that flonicamid does not induce proliferation of cells in the alveolar/bronchiolar epithelium in female rats at doses that are carcinogenic in CD-1 mice (Nomura, 2003b).
10. The incidence of lung carcinoma in humans is 2-3 orders of magnitude lower than in CD-1 mice which seem to indicate that humans are less sensitive to factors inducing lung tumorigenesis.
11. Since alveolar/bronchiolar epithelium proliferation under influence of flonicamid is not induced in two other mouse strains and in rats it is concluded that this proliferation is strain/species specific, and is unlikely to occur in humans.

Taking into account the weight of evidence analysis of the available data above, it is concluded by the RAC that the low increase in frequency of benign lung tumors in highly susceptible mice with a clear threshold for lung benign tumor induction through a mechanism which is not relevant for other strains of mice and for rats, does not constitute even a limited evidence of carcinogenicity. Therefore RAC supports the proposal of the dossier submitter not to classify flonicamid for carcinogenicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

According to the dossier submitter, flonicamid was not found to be toxic for fertility or sexual function nor for development in animal experiments. Thus it does not fulfil the criteria for classification for reproductive toxicity, and no classification is required for flonicamid under either CLP or DSD.

Preliminary and 2-generation dietary reproductive toxicity studies have been performed in rats, and developmental toxicity studies (with associated range-finding studies) have been performed in rats and rabbits by gavage administration. Additional observations on serum gonadotropin and sex hormone levels and estrogen receptor binding affinity were performed on F1 progeny of the 2-generation study to investigate the etiology of the observed effects.

Rat:

In the rat 2-generation reproductive toxicity study, flonicamid in the diet at concentrations of 0, 50, 300 and 1800 ppm (corresponding to a mean daily intake of 3.07-3.39, 18.3-20.7 and 109.1-124.8 mg/kg bw/d, respectively for parental and F1 males, and 4.67-4.95, 28.2-30.5 and 163.6–176.8 mg/kg bw/d, respectively, for parental and F1 females) did not affect fertility or fecundity in either sex. However, effects suggestive of interference with the normal sexual maturation of female progeny were seen at 164 mg/kg bw/d (reduced uterus weights in the F1 & F2 generation and slightly delayed vaginal opening in F1 progeny) but all other aspects of F1 development and, specifically the reproductive capacity, were unaffected by treatment with flonicamid.

Reduced ovary weights were also apparent in P generation rats at the end of lactation, but the relevance of this finding is questionable since ovary weights are not affected in subsequent generations, or in nulliparous females treated for 13 weeks at up to 5000 ppm from 6 weeks of age. Sperm analysis did not reveal any treatment effect on sperm count and morphology.

Investigation of serum gonadotropin and sex hormone concentrations in male F1 progeny revealed no effects on LH, FSH and testosterone concentrations at any dose level up to 125 mg/kg bw/d. However, in females, increased serum LH and slightly reduced serum 17β-estradiol concentrations occurred at dose levels of ≥ 30.5 and 177 mg/kg bw/d, respectively. 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, these variations were considered not to be adverse. Serum progesterone concentration was unaffected by flonicamid. Further investigation of estrogen receptor binding affinity revealed that flonicamid has a very low affinity for both α- and β-receptors.

The only other effects of flonicamid administration identified in the 2-generation study were related to the kidney. Morphologically distinct histopathological alterations occurred in the kidneys of males and females in both the P and F1 generation. These renal lesions were already seen in previous short-term toxicity studies. In males, the kidney changes were considered to be mediated by the male rat-specific protein, alpha-2-microglobulin; and in females the kidney changes consisted of vacuolation of proximal tubular cells. F1 males and females were not more susceptible than the P generation to these renal lesions.

Flonicamid is not teratogenic in either the rat or the rabbit. However, in rats flonicamid elicited a marked increase in the incidence of cervical ribs at a dose level of 500 mg/kg bw/d. At the same dose overt maternal effects were seen, notably liver hypertrophy, vacuolation of renal tubular cells

and increased placental weight. The increased incidence of this skeletal variant is considered to be irrelevant for humans. In rats the maternal NOAEL was 100 mg/kg bw/d, based on effects observed in the kidneys and liver. The developmental NOAEL was also 100 mg/kg bw/d, related to an increased incidence of skeletal variations, namely extra cervical ribs. In rabbits, the maternal NOAEL was 7.5 mg/kg bw/d, based on reduced body weight gain and the developmental NOAEL was 25 mg/kg bw/d. In rabbits, a number of external, skeletal and visceral anomalies were observed; however, they were not dose-related, and they fall within the historical control data and can thus be considered as incidental.

The conclusion of the EFSA peer review of flonicamid arrived at a different opinion. The significance of the occurrence of cervical ribs in rats in the light of the structure (length of the rib) was considered as an adverse effect, even though occurring in the presence of slight maternal toxicity. In addition it was concluded that there were some indications of foetotoxicity in rabbits at a dose level without maternal toxicity (foetuses with one or more visceral malformations). According to the findings of foetotoxicity observed in both species, classification with Repr. Cat. 3; R63 (Possible risk of harm to the unborn child) according to DSD, was proposed.

Comparison with criteria
According to the dossier submitter, flonicamid was not found to be toxic to fertility or sexual function nor for development in animal experiments. Thus it does not fulfil the criteria for classification for reproductive toxicity, and no classification is required for flonicamid under either CLP or DSD.

Comments received during public consultation

The Danish MSCA proposed classification of flonicamid as Repr. 2 - H361d (Repr. Cat. 3; R63), due to increased occurrence of visceral malformation in the rabbit study (Takahashi, 2002d, see BD), at a maternally non-toxic level. Their justification for classification for reproductive toxicity was that based on the data presented in the DAR (Plant Protection Product), which appear to be identical to the data in the CLH dossier, the expert group on toxicology under the PPP Directive recommended classification as Repr. Cat. 3; R63. In the rabbit developmental study, there was a higher incidence of total malformations and especially of visceral malformations compared to concurrent controls. The Danish MSCA did not agree with the dossier submitter that the reported historical control data should be used to overrule the specific concurrent control data and thereby to discard the effects on development seen in the study. They still had concern that flonicamid has a toxic effect on development in rabbits at non-maternally toxic doses of 7.5 and 25 mg/kg bw/d. The NOAEL for development in this rabbit study should in their opinion be set at 2.5 mg/kg bw/d, as it was decided in the peer review under the PPP directive. Thus the NOAEL for developmental toxicity in the rabbit study (Takahashi, 2002d, see BD), would be 2.5 mg/kg bw/d and the NOAEL for maternal toxicity still 7.5 mg/kg bw/d.

They proposed that flonicamid be classified as Repr. 2, H361d under the CLP and Repr. Cat. 3; R63 under the DSD 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).

ISK Biosciences Europe N.V. (company, manufacturer) commented that for the interpretation of the rabbit study (Takahashi, 2002d, see BD), observations were assessed against historical control data from the period 1992-2001. The experimental phase of the study itself ran between July and December 2001. It would be appropriate to consider historical control data from before and after this period as well. They therefore suggested amending Table 102 in the CLH report to include historical control data from the performing testing facility IET covering the period 1992-2011. (ECHA Secretariat note: An extended version of Table 102 was submitted as an attachment to this comment during PC.) According to them the extended data better cover the experimental phase of the study and are more detailed and therefore provide better insight into the occurrences of findings for the testing facility. According to ISK, the extended historical control data demonstrate that the observed types of malformations did not exceed the incidence in the historical control values (HVC) reported by the IET testing facility (HCV IET). Moreover the total number of fetuses

with malformations did not 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.
ISK reported that the background control incidence of abnormal lung lobation which has been reported in the literature by Nakatsuka *et al.* (1997; survey of Japanese Pharmaceutical Association literature (JPMA)) demonstrates that it is one of the common anomalies in animals used by Japanese Contract Research Organisations.

ISK further stated that in the 'Summary and discussion of reproductive toxicity: teratogenicity in rat or rabbit' there is a need to specify that the increased incidence of additional cervical ribs was only observed in 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 was within historical controls and was also not observed in the rabbit suggests that the findings were incidental.

Regarding the EFSA conclusion, they stated that the incidences observed in rats remained within the historical control data, and did not show a dose-response. Incidences were not repeated in other strains or within species. Hence there was no clear indication that they were treatment-related.

The German MSCA commented that the provided studies 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 species, other strain; non-GLP). Details on this study are limited; it is therefore difficult to conclude on its usability in a weight of evidence 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). They considered that a further discussion on the 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 would be helpful.

RAC Assessment and comparison with the classification criteria

The RAC has considered the additional data provided by the dossier submitter in the response to comments submitted during public consultation.

Incidence of extra-cervical ribs in rats (Hojo, 2002b; reassessed in Hojo, 2006, see BD)

The findings of extra-cervical ribs are considered as minor defects which are 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 (from 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) which were adjacent to the 7th cervical vertebra uni- or bilaterally. The majority of the supernumerary ribs showed no distal cartilage and they are transient variations which disappear post-natally and should hence not be regarded as a relevant effect (Chernoff and Rogers, 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, it is described in detail and these results can be considered to support the weight of evidence that increased incidence of extra cervical ribs in Wistar rats is not relevant for classification.

Visceral malformations in rabbits (Takahashi, 2002d, see BD)

In the analysis of data on developmental toxicity of flonicamid, the following observations were taken into account by the RAC:

1. All observed visceral malformations in rabbit fetuses of dams exposed to flonicamid occur spontaneously with varying frequency in the Japanese testing facility where the study has been performed.
2. The number and frequency of visceral malformations in fetuses was low, not dose-related and was within the historical control values (HCV) in the same laboratory (HCV IET) as well as historical control values reported in the survey of JPMA literature (HCV JPMA; Nakatsuka *et al.* 1997, see BD).

Detailed data (submitted as an attachment -extended Table 102 - during PC) are provided below:

- Abnormal lung lobation: 2 fetuses (1.28%) at a dose of 7.5 mg/kg bw/d, 2 fetuses (1.18%) at a dose of 25 mg/kg bw/d; HCV IET = 0–0.69%, HCV JPMA = 0–32.59%;
- Absent lungs: 1 fetus (0.59%) at dose 25 mg/kg bw/d; HCV IET = 0–0.55%, HCV JPMA = 0–3.1%;
- Small lungs: 1 fetus (0.64%) at dose 7.5 mg/kg bw/d; HCV IET = 0–0.67%, HCV JPMA = 0–1.81%;
- Various other visceral malformations such as membranous ventricular septum defect, interrupted aortic arch, narrowed pulmonary trunk, retroesophageal subclavian artery, absent kidney, small bladder, absent ureter occurred in 1 fetus either in the group of 7.5 mg/kg bw/d or in the group of 25 mg/kg bw/d; HCV IET = 0–1.32%, HCV JPMA = 0–5.0%;
- Undescended testis was found in 1 control fetus (0.57%), 1 fetus at 7.5 mg/kg bw/d (0.64%), 2 fetuses at 2.5 mg/kg bw/d (1.19%) and in no fetuses at 25 mg/kg bw/d. HCV IET = 0–1.28%, HCV JPMA = 0–4.4%.

A Cochran Armitage trend test was performed for incidences of fetuses having visceral malformations, abnormal lung lobation, absent kidney and absent ureter, respectively, and no significant trend was detected in any of the parameters analysed. Moreover, the type of malformations varied widely among fetuses and though exceeding the incidence in the historical control values reported at the IET testing facility, no statistically significant difference was observed between the control and treated groups when the incidence of each malformation, as low as 0/173 – 2/156, was analyzed.

Absent kidney and ureter was found in 2 fetuses that had multiple malformations at the middle and high dose; the accompanying malformations are totally different in these 2 fetuses, suggesting that the malformation syndromes occurred independently and are incidental. Though the incidence of absent kidney exceeds the background control incidence at IET testing facility, it is slightly under the upper limit of the range (0 – 0.69) reported by Nakatsuka *et al*. (1997), see BD.

Comparison with the criteria
In the 2-generation reproductive toxicity study, flonicamid did not affect the estrus cycle, mating behavior, fertility or fecundity of the parental and F1 generation, or viability and physical development of the progeny. The analysis of the developmental studies indicates that flonicamid is not foetotoxic and it does not have intrinsic properties to induce visceral malformations in rabbits or in rats. The observed malformations in rabbits were spontaneous developmental anomalies not related to exposure to flonicamid, the frequency of which did not significantly increase with dose, even though the dose of 25 mg/kg bw/d induced maternal toxicity.

The results obtained in the studies analysed did not meet the CLP criteria for classification as Repr. 2 - Suspected human reproductive toxicant, which are as follows:
"*Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with*

other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."

Taking into account the weight of evidence analysis of available data above, it is concluded by RAC that the results obtained in the analysed studies do not meet the DSD criteria for classification as Repr. Cat. 3; R62 or R63. Therefore RAC supports the proposal of the dossier submitter not to classify flonicamid for reproductive toxicity.

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal The dossier submitter did not propose classification for environmental hazards.

The dossier summarises some information on degradation, bioaccumulation and both acute and chronic toxicity of flonicamid.

Degradation
Degradation of flonicamid was studied in one hydrolysis study (OECD TG 111), in one photolysis study in water (SETAC TG and GLP) and one in soil (SETAC TG and GLP). In addition, a degradation study in two water/sediment systems was also reported. No screening studies were available on ready degradability.

The reported hydrolysis study of flonicamid (Walsh and Murray, 2000) was performed according to OECD 111 guideline and was considered as a GLP study by the dossier submitter. Hydrolysis of pyridyl ^{14}C-flonicamid (> 97.7 % purity) was studied at 1 mg/L in sterile buffers at various pHs (4, 5, 7 and 9) and temperatures (25 and 50°C) up to 120 d and also at 40°C (pH 9) for up to 59 d. Flonicamid was stable at pH 4 and 5. It was also stable at pH 7 at 25°C but showed slow hydrolysis at 50°C (DT_{50} 578 d). At pH 9, flonicamid was hydrolysed at all temperatures and DT_{50} was 204 d at 25°C, 17.1 d at 40°C and 9.0 d at 50°C. The dossier submitter did not provide any overall conclusion on the hydrolytic degradation of flonicamid and its relevance for classification in the CLH report.

Photolysis in water was studied by using pyridyl ^{14}C-flonicamid at 1.1 mg/L in sterile buffer at pH 7, with 2 replicates (Walsh, 2002b). At pH 7, flonicamid was stable in the dark (> 97.3 %) and limited degradation was observed in the light (flonicamid was 93.6 %). In the light, the DT_{50} for flonicamid was calculated to be 267 d at 23°C. At pH 7 and at 23°C.Flonicamid is stable in the dark and slowly degraded in the light (DT_{50} 267 d continuous artificial light).

The soil study on photolysis of flonicamid showed that flonicamid degraded slowly in the dark (DT_{50} = 53.3 d) and turned into TFNG-AM (13.8 % after 15 d). In the light, degradation was faster (DT_{50} = 22.4 d continuous artificial light or about 45 d for 12 h photoperiod) and TFNG-AM was the main degradation product (max. 29.5 % after 15 d). The dossier submitter concluded that photodegradation on soil is expected to be less significant than aerobic degradation.

The reported simulation studies in water-sediment systems (two systems studied at 20ºC in the laboratory) demonstrated that flonicamid has moderate persistence in water and in the total system, with DT_{50} of 30-37 and 36-44 d respectively. The terminal metabolite, CO_2, was the most significant degradation product accounting for 16-59% of the applied radioactivity (AR) at 136-145 d. However, the dossier submitter did provide systematic assessment of the results and their relevance for final conclusion on degradation.

The dossier submitter concluded that flonicamid is considered not readily biodegradable but did not specify a rationale for this conclusion.

Bioaccumulation
The dossier submitter's conclusion on bioaccumulation potential of flonicamid was that no concern over any potential for bioaccumulation could be concluded due to its log Kow value of -0.24 (pH not measured at 20°C).

Acute toxicity
Two short term toxicity studies in fish (OECD TG 203), one in invertebrates (OECD TG 202) and two in algae (OECD TG 201 from 1984 and OECD TG 221) were reported.
No mortality or other signs of toxicity were observed at the test concentration (100 mg/L, mean measured concentration 98% of the nominal) during the 96-hour acute toxicity studies in rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*). Similarly, no dead or immobile individuals were observed in the 48-hour *Daphnia magna* study at the applied test concentration (nominal 100 mg/L, measured concentration at the start and end of the study 98% and 99%, respectively). In the algal test (*Pseudokirchneriella subcapitata*) the E_rC_{50} value was defined to be above the highest test concentration (100 mg/L, measured concentration varied from 94% to 97% of the nominal). In the 7-day test on *Lemna gibba* no effect on the mortality or growth were observed (mean measured test concentration at the highest test concentration 119 mg/L). All other acute studies were indicated to be reliable by the dossier submitter except the study in *L. macrochirus* (no indication of its reliability). In addition, a sediment study on non-biting midge (*Chironomus riparius*) was reported but no symptoms of toxicity were recorded in the larvae, pupae or emerged midges during the study.

The dossier submitter's final conclusion not to propose Aquatic Acute classification for flonicamid was based on the observation that all EC_{50} values were above 100 mg/L.

Chronic toxicity
One chronic toxicity study in fish (OECD TG 210, GLP) and two in aquatic invertebrates (OECD TG 211, GLP) were available. In addition, NOEC values from the same studies that were used in assessing acute toxicity were reported for the algae, plant and non-biting midge.

The chronic toxicity study in fathead minnow (*Pimephales promelas*) embryos showed statistically significant reduction in total length at the highest test concentration, i.e. at 20 mg/L (other test concentrations were 1.2; 2.6; 4.9 and 9.5 mg/L, mean measured concentrations). The test concentrations of flonicamid employed did not have any significant effect on hatching success or mortality. Therefore, the NOEC (33 d) value for fish was determined to be 10 mg/L.

In the first chronic toxicity study on water flea (*Daphnia magna*) five test concentrations were used (3.1; 6.3; 12.5; 25; 50; 100 mg/L, mean measured concentration were from 93% to 101% of the nominal values). Significantly higher mortality of adults was observed at the two highest flonicamid concentrations: 60% at 25 mg/L and 100% at 100 mg/L. Significantly different body length to controls was observed at 12.5 mg/L flonicamid concentration. When compared to the control, statistically significant difference in reproduction rate was observed at the test concentrations of 6.3 to 50 mg/L. Therefore, the NOEC (21 d) value in this study was determined to be 3.1 mg/L.

The second chronic water flea study was performed at six nominal concentrations (1.6; 3.1; 6.3; 12.5; 25 and 50 mg/L, only 6.3 and 12.5 mg/L were measured to be between 97 and 105% of the nominal values). By the test end, 30% mortality was observed at 50 mg/L flonicamid concentration whereas no mortality was observed in other treatments or control. For the mean reproduction rate a clear exposure concentration related response effect was observed from 12.5 mg/L to 50 mg/L. However, the mean reproduction rate of the two lowest flonicamid concentrations was not in line with this observation, since no statistically significant difference to controls was observed at 6.3 mg/L (98.7% of the control rate) and 63.8% (not statistically significant) reproduction rate at 3.1 mg/L. According to the dossier submitter this was considered to be a chance finding and is not a result of flonicamid toxicity. Therefore, the dossier submitter concluded that the NOEC (21 d) in this study was 6.3 mg/L.

The defined NOEC (72 h) value for the algal study (*P. subcapitata*) was 46 mg/L. For *L. gibba* no effects were observed at the used test concentrations and the NOEC value was the highest test concentrations (119 mg/L). The reported chronic study in the non-biting midge resulted in a NOEC (27 d) of 25 mg/L.

The dossier submitter's conclusion not to propose Aquatic Chronic classification was based on the fact that the lowest valid chronic NOEC (*D. magna* 3.1 mg/L) did not meet the criteria for classification.

Comments received during public consultation

No comments were submitted regarding the proposed environmental classification of flonicamid.

RAC Assessment and comparison with the classification criteria

Degradation

No screening studies on the ready biodegradation of flonicamid were reported. However, taking into consideration the results of studies on hydrolysis and water/sediment systems summarised in the CLH report, RAC agreed that flonicamid should be considered to be not rapidly degradable.

Bioaccumulation

Since the log K_{ow} value of flonicamid is equal to -0.24, it is below the criterion value of 4 according to CLP criteria, and well below the criterion value of 3 according to DSD and no potential for bioaccumulation would thus be expected for flonicamid.

Aquatic acute toxicity: All the aquatic toxicity studies of flonicamid were performed according to GLP and according to EPA or OECD guidelines. In toxicity studies for aquatic organisms all LC_{50} and EC_{50} values were above 100 mg/L. Flonicamid should therefore not be classified for aquatic acute toxicity according to CLP or DSD.

Aquatic chronic toxicity: There are adequate chronic toxicity data (NOEC) available for all three trophic levels (fish, invertebrates and algae). The lowest reported NOEC values for flonicamid for different trophic levels were 10.0 mg/L for fish, 3.1 mg/L for invertebrates and 46.0 mg/L for algae.

Since flonicamid is a non-rapidly degradable substance and the reported NOECs for all aquatic organisms (fish, invertebrates, algae) are higher than 1 mg/L (the criterion in CLP), the substance does not fulfil the criteria for classification as aquatic chronic toxicity according to CLP.

Classification according to the criteria of DSD: a substance can be classified for chronic toxicity to the environment, if it has an acute aquatic toxicity of <100 mg/L and is not rapidly degradable, or has a log Kow of ≥ 3. Assignment into the specific chronic aquatic toxicity category depends on the lowest acute aquatic toxicity value. The acute aquatic toxicity obtained during studies for aquatic organisms are all above 100 mg/L. Taking into account all the above information, flonicamid does not fulfil the criteria for classification with long-term aquatic hazard.

Therefore RAC supports the proposal of the dossier submitter not to classify flonicamid for environmental toxicity

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ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. It is based on the CLH report prepared by the dossier submitter; the evaluation performed by the RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and the RAC (excl. confidential information).