CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: FLONICAMID

EC Number: **Not allocated**

CAS Number: 158062-67-0

Index Number: **not allocated**

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1. Substance identity: flonicamid

Substance name:	Flonicamid
EC number:	Not allocated
CAS number:	158062-67-0
Annex VI Index number:	Not harmonised
Degree of purity:	≥ 960 g/kg
Impurities:	Toluene ≤ 3 g/kg Other impurities : See confidential annex

1.2 Harmonised classification and labelling proposal

Table 2. The current Annex VI entry and the proposed harmonised classification: flonicamid

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	-	-
Current proposal for consideration by RAC	Acute tox. 4- H302	Xn R22
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute tox. 4- H302	Xn R22

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Table 3. Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None		None	No classification warranted
2.2.	Flammable gases	None		None	Not adequate
2.3.	Flammable aerosols	None		None	Not adequate
2.4.	Oxidising gases	None		None	Not adequate
2.5.	Gases under pressure	None		None	Not adequate
2.6.	Flammable liquids	None		None	Not adequate
2.7.	Flammable solids	None		None	No classification warranted
2.8.	Self-reactive substances and mixtures	None		None	No data
2.9.	Pyrophoric liquids	None		None	Not adequate
2.10.	Pyrophoric solids	None		None	No data
2.11.	Self-heating substances and mixtures	None		None	Not adequate
2.12.	Substances and mixtures which in contact with water emit flammable gases	None		None	No data
2.13.	Oxidising liquids	None		None	Not adequate
2.14.	Oxidising solids	None		None	No classification warranted
2.15.	Organic peroxides	None		None	Not adequate
2.16.	Substance and mixtures corrosive to metals	None		None	No data
3.1.	Acute toxicity - oral	Acute tox. 4 H302		None	
	Acute toxicity - dermal	None		None	Data conclusive but not sufficient for classification
	Acute toxicity - inhalation	None		None	Data conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	None		None	Data conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	None		None	Data conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	None		None	Not evaluated

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
3.4.	Skin sensitisation	None		None	Data conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	None		None	Data conclusive but not sufficient for classification
3.6.	Carcinogenicity	None		None	Data conclusive but not sufficient for classification
3.7.	Reproductive toxicity	None		None	Data conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	None		None	Data conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	None		None	Data conclusive but not sufficient for classification
3.10.	Aspiration hazard	None		None	Not evaluated
4.1.	Hazardous to the aquatic environment	Not Classified	-	None	Data conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	-	-	None	No data

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: warning
Hazard statements: H302: harmful if swallowed
Precautionary statements: not harmonised
Pictogram: GHS 07

Proposed notes assigned to an entry: none

Table 4. Proposed classification according to DSD

Hazardous property	**Proposed classification**	**Proposed SCLs**	**Current classification [1)]**	**Reason for no classification [2)]**
Explosiveness	None		None	No classification warranted
Oxidising properties	None		None	No classification warranted
Flammability	None		None	No classification warranted
Other physico-chemical properties *[Add rows when relevant]*				
Thermal stability				Using a 99.7 % purity test item, flonicamid is stable at 25°C for 36 months (see Klocek Nye, C., 2002a)
Acute toxicity	Xn R22		None	
Acute toxicity – irreversible damage after single exposure	None		None	Data conclusive but not sufficient for classification
Repeated dose toxicity	None		None	Data conclusive but not sufficient for classification
Irritation / Corrosion	None		None	Data conclusive but not sufficient for classification
Sensitisation	None		None	Data conclusive but not sufficient for classification
Carcinogenicity	None		None	Data conclusive but not sufficient for classification
Mutagenicity – Genetic toxicity	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – fertility	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – development	None		None	Data conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	None		None	Data conclusive but not sufficient for classification
Environment	Not Classified	-	None	Data conclusive but not sufficient for classification

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: Xn
R-phrases:R22
S-phrases: S36/37

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Flonicamid is not listed in the Annex I of the 67/548/EC Directive.

2.2 Short summary of the scientific justification for the CLH proposal

Flonicamid has been shown to be harmful if swallowed in an acute toxicity study

2.3 Current harmonised classification and labelling

No previous harmonised classification in Annex VI of CLP.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

No registration dossier was available for flonicamid on 21 February 2012. It is noted that in the self classification notified by manufacturers and importers, the classification for oral acute toxicity applied is the same between notifiers and with the proposed harmonized classification: for oral acute toxicity, all the notifiers apply a classification in category 4, H302.

2.4.2 Current self-classification and labelling based on DSD criteria

Flonicamid is currently classified with Xn R22 at the national level. Flonicamid is currently labelled at the national level with S2: « keep out of the reach of children » and S46: « if swallowed, seek medical advice immediately and show this container or label ».

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Flonicamid is an active substance in the meaning of Directive 91/414/EEC. In accordance with Article 36(2) of the CLP Regulation, flonicamid shall be subjected to harmonised classification and labelling. Therefore, this proposal considers all physical, human health and environmental hazards.

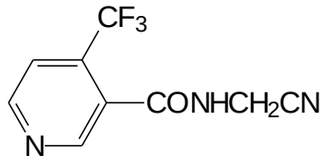
Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5. Substance identity

EC number:	Not allocated
EC name:	-
CAS number (EC inventory):	Not allocated
CAS number:	158062-67-0
CAS name:	*N*-(cyanomethyl)-4-(trifluoromethyl)-3-pyridincarboxamide
IUPAC name:	*N*-(cyanomethyl)-4-(trifluoromethyl)-nicotinamide
CLP Annex VI Index number:	Not harmonised
Molecular formula:	$C_9H_6F_3N_3O$
Molecular weight range:	229.16
Structural formula:	

1.2 Composition of the substance

Table 6. Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
flonicamid		≥ 960 g/kg	
Toluene		≤ 3 g/kg	

Current Annex VI entry:

No harmonised classification

Table 7. Impurities (non-confidential information)

Impurities are confidential. See confidential annex.

Table 8. Additives (non-confidential information)

None

1.2.1 Composition of test material

Above 98.7 % for the physico - chemical properties of flonicamid

1.3 Physico-chemical properties

Table 9. Summary of physico - chemical properties of flonicamid

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance	Solid powder Light beige (TGAI)* Off white (PGAI)* Munsell color system (21.9°C) 10YR9/1	Pelton, J.A., 2000a	observation
Melting/freezing point	157.5°C PAI (99.7%)	Sweetapple, G.G., 1999a	Measured
Boiling point	306-320°C PAI (99.7%)	Tognucci, A. 2002a	Measured
Relative density	1.54 g/mL (at 20°C)	Sweetapple, G.G., 1999a	Expressed as density. Measured
	1.531 g/mL (at 20°C)	Pelton, J.A., 2000a	
Vapour pressure	30°C: 6.48 x 10-6 Pa 40.1°C: 4.40 x 10-5 Pa 50.1°C: 2.31 x 10-4 Pa 2.55 x 10-6 Pa at 25°C 9.43 x 10-7 Pa at 20°C	Schetter, J.E., 1999a	Vapor pressure measured at 30°C, 40.1°C and 50.1°C to extrapolate to 25°C
Surface tension	47.3 mN/m at 25±1°C 47.0 mN/m at 40±1°C Surface active TGAI (98.7%)	de Ryckel, B., 2002a	measured at 90% of water solubility.
Water solubility	5.2 g/L at 20°C PAI (99.7%) (no pH provided. Only stated that study has been performed in an unbuffered water)	O´Donnell, R.T., 1999b	Measured using shake flask method
Partition coefficient n-octanol/water	Log Pow = -0.24 at 20°C	Dudones, L.P., 1999b	calculated value (pH not measured) HPLC method
Flash point	Not required (solid)	-	-
Flammability	Not highly flammable TGAI (98.7%)		Measured
Explosive properties	No explosive properties	Schmiedel, U., 2001a	Expert statement + measured
Self-ignition temperature	No self-ignition	de Ryckel, B., 2002a Schmiedel, U., 2001b	Measured
Oxidising properties	Not oxidizing	Schmiedel, U., 2001b	Expert statement
Granulometry	no data	-	
Stability in organic solvents and identity of relevant degradation products	No data-		
Dissociation constant	pKa=11.60 at 20±1°C	Beckwith, R.C., 1999a	Measured
Viscosity	Not required (solid)	-	-
Henry's law	4.2 x 10^{-8} Pa x m^3/mole	Schetter, J.E.,	Calculated from

<table>
<tr><td>constant</td><td></td><td>1999a</td><td>vapour pressure and solubility in water</td></tr>
<tr><td>Solubility in organic solvent</td><td><table>
<tr><th></th><th>PAI (99.7%) g/L at 20°C</th><th>TGAI (98.7%) g/L at 20°C</th></tr>
<tr><td>Acetone</td><td>163.5</td><td>157.1</td></tr>
<tr><td>Ethyl acetate</td><td>34.2</td><td>34.9</td></tr>
<tr><td>Methanol</td><td>104.3</td><td>89.0</td></tr>
<tr><td>Dichloromethane</td><td>4.5</td><td>4.0</td></tr>
<tr><td>Toluene</td><td>0.55</td><td>0.30</td></tr>
<tr><td>Hexane</td><td>0.0002</td><td>0.0003</td></tr>
<tr><td>n-Octanol</td><td>3.0</td><td>2.6</td></tr>
<tr><td>Acetonitrile</td><td>132.8</td><td>111.4</td></tr>
<tr><td>Isopropyl alcohol</td><td>18.7</td><td>14.7</td></tr>
</table></td><td>O`Donnell, R.T., 1999c Dudones, L.P., 1999a</td><td>Measured</td></tr>
<tr><td>UV/VIS absorption (max.) incl. ε ‡ (state purity, pH)</td><td>ε (L/(cm x mol)) : 3870, 3890, 13200 and 4190 at respectively 265 nm in neutral solution, 266 nm in acidic solution, 204 and 270 nm in basic solution (99.7%)</td><td>Malinski, M.F., 1999a</td><td>Measured</td></tr>
<tr><td>Storage stability at 25°C and 5°C</td><td>Start: 99.7%
after 36 month: 99.8% at 25°C
99.8% at 5°C</td><td>Klocek Nye, C., 2002a</td><td>Measured</td></tr>
</table>

*TGAI : technical grade active ingredient (<98 % purity)
PGAI : pure grade active ingredient (> 98 % purity)

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant in this dossier

2.2 Identified uses

Potatoes, wheat, apples/pears, peaches

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10. Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
EEC A 10 (flammability)	Not highly flammable TGAI		de Ryckel, B., 2002a
UN Recommendations on Transport of Dangerous Goods (Explosive properties)	No explosive properties		Schmiedel, U., 2001a
EEC A 16 (Self-ignition temperature)	No self-ignition		de Ryckel, B., 2002a
Expert statement (Oxidising properties)	Not oxidizing		Schmiedel, U., 2001b

3.1 Explosive properties

Flonicamid is a stable organic substance. None of these components or grouping are associated with explosive hazards. All are stable groupings in high oxidation states. A study (Schmiedel, U., 2001a) has been performed indicating no explosive properties.(see Table 9 on chemical and physical properties).

3.2 Flammability

Flonicamid is an organic compound. De Ryckel, B's study (2002a, has demonstrated that using a 98.7 % test purity item, flonicamid is neither highly flammable nor auto-inflammable As such, flonicamid is not likely to undergo self heating under bulk storage conditions .(see Table 9 on chemical and physical properties).

The determination of flash point is not relevant because the active substance is a solid.

So we can conclude that flonicamid is not highly flammable.

3.3 Oxidising potential

Oxidising compounds are materials that can easily transfer oxygen to other compounds i.e. they contain weakly bound oxygen, for example NO_3 and peroxides. Bound oxygen must also become available through a low energy degradation route with a low energy of activation. The oxygen in flonicamid is bound to stable amide structural groupings with strong oxygen bonds. The decomposition temperature of flonicamid is high (306-320°C) indicating a high energy of activation. Flonicamid is therefore considered stable under the conditions of oxidation.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

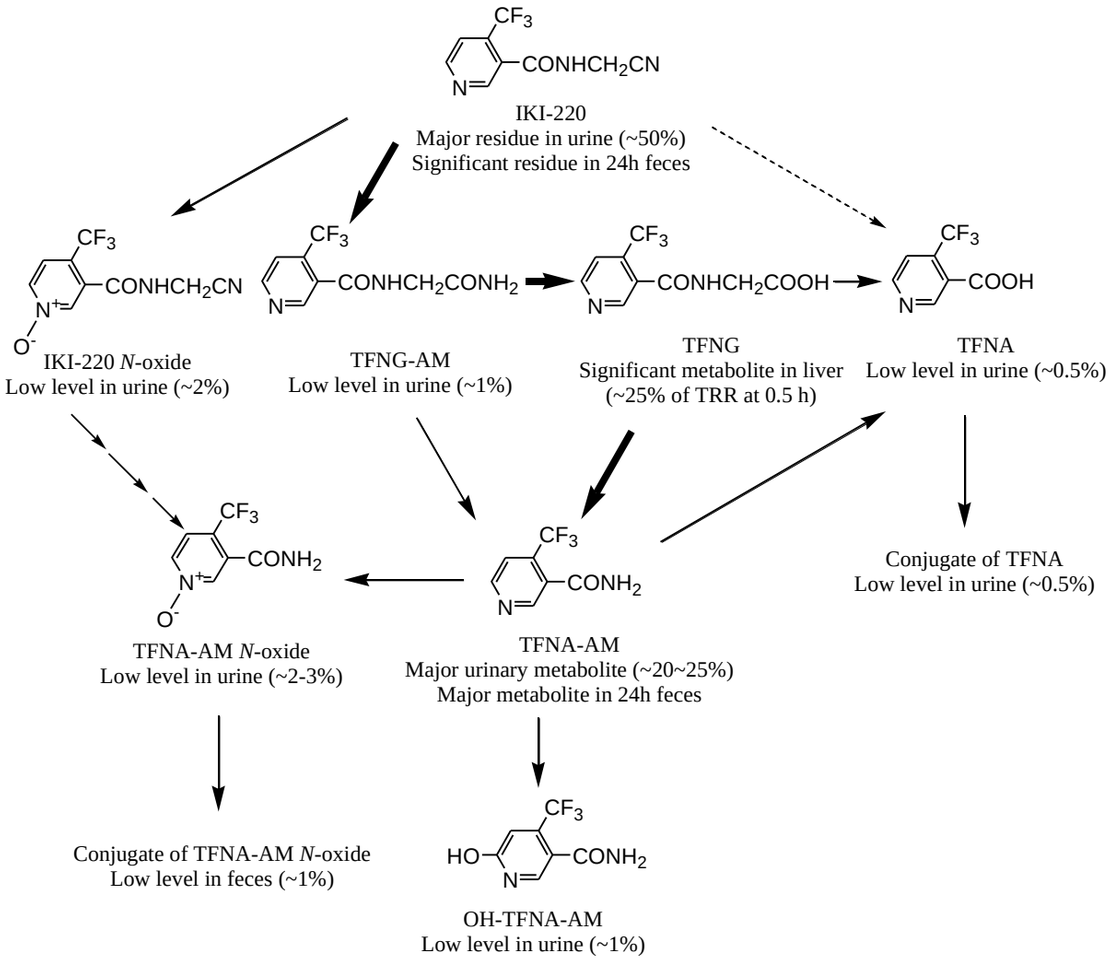
4.1.1 Non-human information

Following pilot excretion and pharmacokinetics experiments after single oral gavage (0.85 – 50 mg/kg bw), the absorption, distribution, metabolism and excretion of Flonicamid (IKI-220) using ^{14}C radiolabel was investigated in Sprague Dawley derived rats which were given single oral gavage (2 or 400 mg/kg bw) and repeated doses of 2 mg/kg bw for 15 consecutive days. Identification and characterisation of the metabolites in the various samples collected in the experiments was done under a separate study.

In the rat, flonicamid is rapidly and almost completely absorbed (> 93%) and is rapidly eliminated from plasma following single oral doses of 2 or 400 mg/kg and multiple oral doses of 2mg/kg for 14 consecutive days. None is detected in expired air. Excretion occurs predominantly in the urine, mostly within 24h and total urinary elimination after 7 days accounts for 72 - 78% of the administered dose for both sexes at both dose levels. Fecal excretion and biliary elimination are minor routes, accounting for less than 7% and ≤ 4.63 % of the administered dose, respectively, both after single oral low or high dose (except that biliary excretion is slightly more prolonged in males at 400 mg/kg).
Elimination follows first order kinetics and the pharmacokinetics of flonicamid is not markedly influenced the dose level; however, at 400 mg/kg, males exhibit a slight prolongation of T_{max} (0.9 h *vs.* 0.5 h) and $T_{1/2}$ of elimination (11.6 h *vs.* 4.5 - 6.8 h) due to an extended plateau in plasma concentration. The AUC values are approximately proportional to dose level in both sexes and the AUC ratios are 263 and 265 in males and females, respectively, for a dose ratio of 200. The maximum plasma concentrations (C_{max}) are comparable in both sexes at 2mg/kg (2.07 / 2.11μg-eq/g), but at 400mg/kg the C_{max} for females (367.6μg-eq/g) is higher than for males (249.6μg-eq/g), probably in relation with the prolonged plateau in plasma concentrations in males at 400 mg/kg. The ratio of C_{max} values shows an approximate proportionality to dose, 120 and 174 in males and females, respectively, but the ratio in males is depressed as a consequence of the prolonged plateau in plasma concentrations at 400 mg/kg.

The tissue distribution of [^{14}C]IKI-220 is not affected by dose level or sex; following a single oral low or high dose and multiple oral low dose (2 mg/kg for 14d) of [^{14}C]IKI-220, radioactivity is rapidly and widely distributed throughout the tissues, in which the levels are broadly in line with blood concentration, but higher peak concentrations occur in liver, kidneys, adrenals and thyroid tissue and ovarian tissue. Radioactivity is rapidly cleared from the tissues and there is no accumulation in any tissue.

The metabolic profile in urine is generally quantitatively and qualitatively similar between the sexes and after single and multiple doses of 2mg/kg, suggesting that no induction of metabolism would occur. In urine, the major residue is IKI-220 (up to 72% AD after 48 h) and a major urinary metabolite is TFNA-AM (up to 27.3% AD) and all other metabolites each occur at ≤ 3.7% AD. In the feces, the predominant residues are IKI-220, TFNA-AM and TFNA (each < 1.2% AD) and TFNA-AM *N*-oxide conjugate also occurs after administration of 400mg/kg (up to 1.0% AD). In the bile, the predominant residue is IKI-220 (≤ 3.3% AD) and in the liver, the metabolic profile is generally quantitatively and qualitatively similar after single and multiple doses of 2mg/kg : the predominant residue is IKI-220 (≤ 2.35% AD) and 2 minor, metabolites occur (TFNG at ≤ 1.10% and TFNA-AM at ≤ 1.19% AD).



4.1.2 Human information

No data available

4.1.3 Summary and discussion on toxicokinetics

In rats, flonicamid is rapidly and almost completely absorbed (>80% within 24h) after oral administration and is rapidly eliminated from the plasma. It is widely distributed throughout the tissues with higher peak concentration occuring in liver, kidneys, adrenals and thyroid tissue. It is rapidly cleared from the tissues and there is no accumulation in any tissue nor in the carcass.

The elimination follows first order kinetics. Excretion occurs predominantly via urine mostly within 24h and total urinary elimination after 7 days accounts for 72 - 78% of the administered dose. Fecal elimination amounts to ~5%, almost all of which is eliminated within 48 h. Biliary elimination is a minor route of excretion (~4%). Flonicamid is not excreted in expired air.

The metabolism is not extensive, flonicamid being the major residue in urine (up to 72% of administered dose after 48 h), bile and liver. Among 10 isolated metabolites, the main one is TFNA-AM (~25% in urine).

4.2 Acute toxicity

Table 11. Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral OECD 401	LD_{50} = 884 mg/kg (M) 1768 mg/kg (F)	-	Ridder W. E., Yoshida M. and Watson, M. (2001a)
Percutaneous OECD 402	LD_{50} > 5 000 mg/kg	-	Ridder W. E., Yoshida M. and Watson M. (2000a)
Inhalation OECD 403	4-h LC_{50} > 4.9 mg/L (nose only)	-	Paul G. R., Bowden A.M., Coombs D.W. (2000)

4.2.1 Non-human information

4.2.1.1 *Acute toxicity: oral*

Reference: Ridder W. E., Yoshida M. and Watson, M. (2001a)
GLP: Yes
Guidelines: OECD 401; US-EPA OPPTS 870.1100; JMAFF 59 NouSan n° 4200

Two experiments (range finding study and main study) were conducted on groups of Sprague-Dawley-derived rats [Crl:CD®(SD)BR®(IGS) strain; 9 w old at dosing; bw range = 172-259g] which were given, by gavage single oral doses of 1000, 2000 or 5000 mg/kg bw (range finding study; 2-3 rats /sex/dose level) and 625, 1250, 2500 or 5000 mg/kg bw (main study; 5 rats/sex/dose level) of flonicamid technical in 0.5% aqueous methylcellulose (10 mL/kg bw).

Rats of the range finding study were observed for mortality at 1h and 4h post dosing and thereafter twice daily for 7 days and then killed but not subjected to necropsy.

Rats of the main study were observed twice daily for morbidity/mortality and clinical signs were recorded at 1h, 2.5h and 4h post-dosing and daily thereafter during the 14d observation period. Bw were recorded before dosing, on the day of treatment and weekly thereafter and on the day of death or sacrifice. All rats found dead and all rats surviving at the end of the 14d observation period were necropsied for examination of major organs and tissues.

All deaths occurred between 2.5 and 48 h post-dosing in 0/5, 5/5, 5/5, 5/5 males and in 0/5, 1/5, 4/5 and 5/5 females of the 625, 1250, 2500 and 5000 mg/kg bw groups, respectively.

The main clinical signs recorded in rats that died within 4h post dosing included decreased activity, loss of mobility, laboured breathing, prostration, convulsions, cold to touch and hypersensitivity to noise; subsequently, clinical signs occurred mainly in females at all dose levels and comprised laboured breathing, rales, prostration, cold to touch, tremors, anogenital staining and few or no feces; ataxia was a common finding in females.

Survivors gained weight during the observation period, with the exception of 1 female in each of the 625mg/kg and the 2500mg/kg groups that had weight loss at d14.

Gross lesions at necropsy considered to be treatment-related were confined to dark red-black foci on the serosal surface of the stomach in 1 male at 1250 mg/kg, 2 females at 2500 mg/kg and 2 females at 5000 mg/kg. All other gross findings were non-specific (diffuse dark red color in lungs of 1 female, red discharge and/or staining in the anogenital area in 2 females, dark red black spots in the thymus in 2 females).

The acute oral LD50 values in rats were 884 (males) and 1768 (females) mg/kg bw.

4.2.1.2 *Acute toxicity: inhalation*

Reference: Paul G. R., Bowden A.M., Coombs D.W. (2000)
GLP: Yes
Guidelines: OECD 403; US-EPA OPPTS 870.1300; Japan MAFF, 59 NouSan n°4200

Groups of 5/sex young adult Sprague-Dawley-derived rats (approx. 7-8 w old; bw range: range 173 - 210g) were exposed continuously for 4h by inhalation (nose only) 4h to a test atmosphere containing a particulate aerosol generated from flonicamid technical (batch n°. 9809; purity 98.7%) at nominal concentrations of 0 (air only) and 20.9 mg/L (equivalent to gravimetrically determined mean concentrations of 0 and 4.9mg/L).

The exposure equipment comprised a fast Wright dust feed generator connected, via a glass elutriator used to reduce the amount of non respirable particulate by sedimentation, to a cylindrical exposure chamber with a continuous airflow system. Parameters were adjusted to give a flow rate of 15L/min and a target concentration of 5 mg/L.

Exposure chamber temperature and relative humidity were measured at 30-min intervals and air flow was continuously monitored throughout the exposure period. Oxygen concentration in the chambers was recorded at 30-min. intervals throughout exposure. The nominal exposure concentration was calculated from the total mass of flonicamid dispersed by the dust generator and the total airflow through the exposure system during the 4h exposure. The actual exposure concentration was measured gravimetrically in 6 samples, each of 2 L, withdrawn from the breathing zone at approx. hourly intervals throughout the exposure period with an additional sample taken after approx. 30 min. The particle size distribution of the atmosphere was determined gravimetrically using a 9-stage cascade impactor in 0.5L atmosphere samples withdrawn after 88; 140 and 207 min. exposure.

Clinical signs were recorded several times during exposure, at 1h and 2h post-exposure and at least twice daily thereafter during a 14d observation period. Morbidity/mortality were recorded twice daily throughout the study. Individual bw were recorded prior to exposure and weekly during the observation period. The food consumption was measured at weekly intervals and water consumption was evaluated daily. All animals were subjected to necropsy; the lungs with larynx and trachea, the liver and kidneys were weighed and then discarded.

Aerosol concentrations of flonicamid measured approximately hourly in the breathing zone were 4.17; 4.72; 5.01; 5.24; 4.70 and 5.21mg/L, and provided a time-weighted mean aerosol concentration of 4.9mg/L (the mean chamber concentration was 23.4% of the nominal concentration, reflecting the loss of test material due to impaction and deposition within the exposure system).

The particle size distribution analysis showed that approx. 66% of particles were < 7 µm in aerodynamic diameter and could then be considered to be within the respirable range; the MMAD was 4.8 ± 2.4µm i.e. slightly different than the acceptable range.

No deaths occurred in any groups.

Clinical signs included exaggerated breathing in some treated rats (from 30 min. after the start of exposure and in all treated rats within 3h, persisting until d2 of the observation period) and brown staining around the snout and jaws in all treated rats post-exposure (persisting up to d2 in the females). All other clinical signs (soiling of fur with excreta) occurred in both treated and control rats and were therefore, considered not to be related to treatment.

No treatment-related effects on bw gain, food and water consumption were seen.

There were no treatment related macroscopic findings at necropsy, but 2 treated rats showed congestion of the lungs (minimal in 1male, severe with small dark foci on the lungs in the other male). The weights of the lungs, liver and kidneys were not affected by exposure to flonicamid.

Although the mean measured concentration during the 4h inhalation exposure was marginally lower than the limit concentration of 5 mg/L for classification, no death or adverse clinical signs were seen; it can then be concluded that the 4-h LC_{50} value is greater than 4.9mg/L air by nose-only inhalation of aerosol particles in the respirable range (MMAD ± GSD: 4.8 ± 2.4 µm). Consequently, flonicamid does not deserve classification with respect to inhalation exposure.

4.2.1.3 *Acute toxicity: dermal*

Reference: Ridder W. E., Yoshida M. and Watson M. (2000a)
GLP: Yes
Guidelines: OECD 402; US-EPA OPPTS 870.1200; JMAFF, 59 NouSan, no. 4200

Two experiments (range finding study and main study) were conducted on groups of Sprague-Dawley-derived rats [Crl:CD®(SD)BR®(IGS) strain; 9 w old at dosing; bw = 202 - 299g (range finding study); 212 - 333g (main study)] which were given single dermal application (on clipped unabraded intact dorsal skin; approx. 10% of body surface) of 5000 mg/kg bw [2 rats /sex in the range finding study; 5 rats/ sex in the main study] of flonicamid technical (Batch n° 9809; purity 98.7%) moistened with distilled water under semi-occlusive dressing; after 24h exposure, the skin was gently wiped using distilled water to remove residual material.

Rats from the range-finding study were observed for viability at 1h and 4h post dosing and therefater twice daily for 7 days; bw was recorded 1 day before dosing, the day of dosing and at termination; all rats were sacrificed on d7 and no necropsy was performed.

Rats from the main study were observed twice daily for morbidity/mortality during the 14d observation period; clinical signs were recorded at 1h; 2.5h and 4h post dosing and daily thereafter; bw were recorded shorthy before dosing , on the day of dosing and at weekly intervals thereafter. All rats were sacrificed on d14 and subjected to necropsy.

No deaths occurred during the observation periods, both in the range finding and the main study.

Minor, treatment-related clinical signs were seen both sexes and included coloured material around the nose and eyes (until d3 and d1, respectively), and anogenital staining (on d1 only). No treatment-related clinical signs occurred after d3.

Bw was increased in all rats at end of the 14d observation period (males: 38.6 ± 15.8 g; females: 21.2 ± 10.8g) ,with the exception of one male that showed a weight loss of 3g during w1 post dosing.

There were no gross lesions at necropsy other than one male with localised hair loss.

The dermal LD_{50} value is greater than 5000 mg/kg bw. Flonicamid does not deserve classification with respect to dermal exposure.

4.2.1.4 *Acute toxicity: other routes*

No data

4.2.2 Human information

No data available

4.2.3 Summary and discussion of acute toxicity

The acute toxicity of flonicamid was evaluated following 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; no deaths occur in response to a limit dose of 5000 mg/kg by semi-occluded topical application for 24h (dermal LD_{50} > 5000 mg/kg); the 4-hour nose only LC_{50} is >4.9 mg/L.

4.2.4 Comparison with criteria

Flonicamid exhibited an acute oral toxicity 200 < LD_{50} < 2000 mg/kg, according to Dir 67/548 EC this substance meets the criterion for classification with Xn R22.

Rationale for classification in respect with acute oral toxicity: flonicamid exhibited an LD_{50} of 884 mg/kg bw, therefore this substance meets the CLP criterion for classification "oral (mg/kg bw) 300 < ATE ≤ 2000".

The dermal LD50 lie above the classification cut-off of 2000 mg/kg under both Directive 67/548/EEC and regulation (EC) 1272/2008; therefore no classification is proposed.

The inhalation LC50 lies marginally lower than the classification cut-off of 5 mg/L/4h (4.9 mg/L/4h) under both Directive 67/548/EEC and regulation (EC) 1272/2008; nevertheless no classification is proposed (see 4.2.1.2).

4.2.5 Conclusions on classification and labelling

According to dir. 67/548 EC, flonicamid requires classification in respect with oral acute toxicity with Xn R22.

Flonicamid requires classification in respect with oral acute toxicity with "H302; hazard category 4, harmful if swallowed"

4.3 Specific target organ toxicity – single exposure (STOT SE)

No specific target organ toxicity identified after single exposure.

These data indicate that no classification is required under either Directive 67/548/EEC or the CLP Regulation.

4.4 Irritation

4.4.1 Skin irritation

Table 12. Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
OECD 404	Not irritating	-	Ridder W. E., Yoshida M. and Watson M. (2000b)

4.4.1.1 *Non-human information*

Reference: Ridder W. E., Yoshida M. and Watson M. (2000b)
GLP: Yes
Guidelines: OECD 404; US-EPA OPPTS 870.2500; JMAFF, 59 NouSan, no. 4200

Six adult male New Zealand White rabbits (10 - 12 w old; bw = 2.6 to 2.8 kg at dosing) were applied 0.5 g of flonicamid technical (batch n° 9809; purity 98.7%) wetted with approx. 1 mL distilled water to the clipped intact thoracic dorsal skin (6cm^2) under semi-occlusive dressing for 4h. As a vehicle control, distilled water was applied to a similar area of skin on the opposite side of each rabbit under semi-occlusive dressing. After removal of the patch, each test and control site was wiped with warm water.

Bw were recorded prior to treatment; all rabbits were observed twice daily for mortality/morbidity during the observation period and all were sacrificed at termination; no necropsy was performed.

The application sites were examined and skin reactions were evaluated approx. 1 h; 24h; 48h and 72 h after patch removal. Erythema/eschar formation and oedema at the application sites were recorded and graded according to the Draize scale. Primary irritation indices (PII) were calculated using EU criteria and US-EPA criteria.

All 6 rabbits survived until termination and no abnormal clinical or dermal findings were seen.

There were no erythema/eschar formation and oedema on both test and vehicle control sites throughout the study which was therefore terminated after the 72h examination. The EU primary irritation indices were 0.0 for both erythema/eschar formation and oedema.

Flonicamid was not found irritating in a skin irritation study and does not require classification with respect to skin irritation.

4.4.1.2 *Human information*

No data available

4.4.1.3 *Summary and discussion of skin irritation*

Flonicamid is not a skin irritant

4.4.1.4 *Comparison with criteria*

1) Criteria for classification under Directive 67/548/EEC:

A substance shall be classified irritant for the skin if it causes significant inflammation which persists for at least 24 hours after an exposure period of up to four hours on the rabbit. That is to say that the mean value of the scores for either erythema and eschar formation or oedema formation calculated over all the animals tested is 2 or more or if the mean value of 2 or more calculated for each animal separately has been observed in two or more animals. Inflammation of the skin is also significant if it persists in at least two animals at the end of the observation time.

2) Criteria in the CLP classification:

A substance shall be classified as irritant in category 2 if in at least 2 of 3 tested animals mean value for erythema/eschar or for oedema is between 2.3 and 4.0 from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions. If inflammation persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling, substance shall be also considered as irritant.

3) Comparison with criteria:

Here, mean scores 24 to 72 hours for erythema and oedema were below the criteria for classification and labelling.

4.4.1.5 *Conclusions on classification and labelling*

In this context, flonicamid does not support classification for skin irritation under either directive 67/548/EEC or CLP regulation criteria.

4.4.2 Eye irritation

Table 13. Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD 405	Not irritating	-	Ridder W. E., Yoshida M. and Watson M. (2000c)

4.4.2.1 *Non-human information*

Reference: Ridder W. E., Yoshida M. and Watson M. (2000c)
GLP: Yes
Guidelines: OECD 405; US-EPA OPPTS 870.2400; JMAFF, 59 NouSan, n° 4200

Six male New Zealand White rabbits (10 - 12 w old; bw 2.6 to 2.8 kg at dosing) were each given a single instillation into the lower conjunctival sac of the right eye of 0.1mL (approx. 70mg) 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.

All rabbits were observed twice daily for mortality/morbidity throughout the study period and all were sacrificed without necropsy examination

Both eyes of all rabbits were examined for ocular irritation reactions approx. 1h; 24h; 48h and 72h after instillation and ocular reactions of the cornea, iris and conjunctivae were recorded and graded for severity

according to the Draize scale. Following the 24h; 48h and 72h examinations, the eyes of all rabbits were further examined under UV light after treatment with fluorescein. Individual irritation indices for EU classification were calculated for corneal opacity, iridial damage and conjunctival redness and chemosis by calculating the mean score for each parameter for the 24h; 48h and 72h observations.

All rabbits survived until termination.

No corneal and iridal effects were seen in any animal at any observation.

Conjunctival effects, including redness (grade 2), chemosis (grades 1-2) and discharge (grades 1-3), were observed in all animals at 1h post application. Redness became less severe at 24h post application and completely cleared within 72h post application. Chemosis cleared in most of the animals within 24h post application. Discharge cleared in all the animals within 24h post application. No additional ocular findings or non-ocular effects were observed in any of the animals throughout the study. The individual mean 24 - 72h scores did not exceed 0.7 for conjunctival effects.

Ocular examinations using fluorescein, revealed no corneal epithelial damage in any of the animals at any observation. Therefore, mean scores for corneal and iridial effects in all animals were zero.

Table 14. Eye irritation individual mean scores (24 - 72 h)

Observation	**Mean individual scores (24 - 72 h) in animal:**					
	1	**2**	**3**	**4**	**5**	**6**
Corneal opacity	0.0	0.0	0.0	0.0	0.0	0.0
Corneal area involved	-	-	-	-	-	-
Iris	0.0	0.0	0.0	0.0	0.0	0.0
Conjunctival redness	0.7	0.7	0.3	0.3	0.3	0.0
Conjunctival chemosis	0.0	0.3	0.0	0.0	0.0	0.0

Flonicamid was not found irritating to the rabbit eye and does not deserve classification with respect to eye irritation.

4.4.2.2 *Human information*

No data available

4.4.2.3 *Summary and discussion of eye irritation*

Flonicamid is not an eye irritant.

4.4.2.4 *Comparison with criteria*

1) Criteria for classification under Directive 67/548/EEC:

A substance shall be classified as a substance which could provoke irritating to eyes if it causes, when applied to the eye of the animal, significant ocular lesions which occur within 72 hours after exposure and which persist at least 24 hours.

Ocular lesions are significant if the mean scores of the eye irritation test have any of the following values:

- Cornea opacity equal to or greater than 2 but less than 3,
- iris lesion equal to or greater than 1 but not greater than 1.5,
- redness of the conjunctivae equal to or greater than 2.5,

- oedema of the conjunctivae (chemosis) equal to or greater than 2.

Or if 3 animals are tested, if the lesions on 2 or more animals, are equivalent to any of the above values axcept that for iris lesion the value should be equal to or greater than 1 but less than 2 and for redness of the conjunctivae the value should be equal to or greater than 2.5.

In both cases all scores at each of the reading times (24, 48 and 72 hours) for an effect should be used in calculating the respective mean values.

2) Criteria in the CLP classification :

A substance shall be classified as a substance which could induce reversible eye irritation, classified in Category 2 (irritating to eyes), if when applied to the eye of an animal, a substance produces:

- At least in 2 of 3 tested animals, a positive response of:

Corneal opacity ≥ 1 and/or

Iritis ≥ 1 and/or

Conjunctival redness ≥ 2 and/or

Conjunctival oedema ≥ 2

Calculated as the mean scores following grading at 24, 48, and 72 hours after instillation of the test material, and which fully reverse within an observation period of 21 days.

3) Comparison with criteria:

Here, mean scores 24 to 72 hours for corneal opacity, iritis, conjunctival redness and conjunctival oedema were below the criteria for classification and labeling.

4.4.2.5 *Conclusions on classification and labelling*

In this context, flonicamid does not support classification for eye irritation under either directive 67/548/EEC or CLP regulation criteria.

4.4.3 Respiratory tract irritation

4.4.3.1 *Non-human information*

No data

4.4.3.2 *Human information*

No data available

4.5 Corrosivity

Flonicamid is not a corrosive substance.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 15. Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
US-EPA OPPTS 870.2600	Not sensitizing	-	Ridder W. E. and Watson M. (2000)

4.6.1.1 *Non-human information*

Reference: Ridder W. E. & Watson M., (2000)
GLP: Yes
Guidelines: US-EPA OPPTS 870.2600

A sensitization study was conducted with flonicamid technical (batch n°9809; purity 98.7%) using the Magnusson & Kligman method on groups of 10-20 males guinea pigs (Hartley albino strain, 4-7 w old; mean bw: 512,1g [441 - 620g] at time of intradermal induction).

- Range finding studies: The vehicle and concentrations of flonicamid technical for induction and challenge administration were selected from the results of 4 preliminary range finding experiments using a total of 16 guinea pigs which were given intradermal injection with 0.1 mL of the vehicle alone and 0.1; 1; 5 and 10% flonicamid (w/v) in mineral oil, or topical application of the vehicle and 10; 25; 50 and 75% flonicamid (w/v) in mineral oil. Dermal scores at the induction sites were recorded 24 and 48 h after injection or patch removal.

- Main study: Intradermal and topical induction doses of flonicamid technical or DNCB (batch n° 10040738 ; purity > 98%) were applied to shaved areas of dorsal skin (2 x 4cm) of the male guinea pigs. Control group animals received vehicle only for induction but were challenged in a similar manner to the respective test groups.

Table 16. : Study design

Group no. / description	Number of animals	Concentration applied (%w/v)			
		Induction		Challenge	
		intradermal	topical	left flank	right flank
1 / flonicamid sensitisation (test)	20	10	50	10	0 (vehicle)
2 / flonicamid control	20	0 (vehicle)	0 (vehicle)	10	0 (vehicle)
3 / DNCB sensitisation (test)	10	0.1	0.1	0.08	0 (vehicle)
4 / DNCB control	10	0 (vehicle)	0 (vehicle)	0.08	0 (vehicle)

- On d1, each guinea pig was given a total of 6 intradermal injections i.e. 0.1 mL of FCA (1:1 dilution in distilled water) for 2 dorsal sites in test and control groups; 0.1 mL of flonicamid (10% w/v in mineral oil) or 0.1 mL of mineral oil or 0.1 mL DNCB in mineral oil for 2 other dorsal sites in tests, vehicle control and positive control groups; and 0.1 mL of flonicamid in 1:1 (v:v) FCA:distilled water

or 0.1 mL 50% (w/v) mineral oil in 1:1 FCA:distilled water or 0.1 mL DNCB in 1:1 (v/v) FCA: distilled water for the 2 remaining sites of tests, vehicle control and positive control groups.

- On d8, the test animals were treated topically (0.4 mL of dosing substances applied to a filter paper placed on 2x 4 cm scapular areas) under occlusive dressing for 48h with 50% flonicamid (w/v) in mineral oil or 0.1% DNCB (w/v) in mineral oil or mineral oil only. The test sites were wiped with water to remove traces of test substances after patch removal.
- On d21, both test groups were challenged topically (0.4 mL of dosing substances), under occlusive dressing for 24h, with 10% flonicamid (w/v) in mineral oil or 0.08% DNCB (w/v) in mineral oil (left side) and vehicle alone (right side). On removal of the patches, the challenge sites were wiped with water, dried and scored on a 4-point scale for dermal reactions 24 and 48h after removal of the challenge dressings.
- All animals were observed twice daily for morbidity/mortality during the study period; clinical observations were recorded weekly and individual bw were recorded pre-test and following challenge.

Based on these experiments of the range finding study, the concentrations of flonicamid selected for the main study were 10% (w/v) for intradermal induction and 50% (w/v) for topical induction application and 10% for topical challenge, mineral oil being selected as the vehicle. From a separate experiment using 0.1; 0.15 and 0.20% DNCB (w/v) in mineral oil, the selected concentrations of DNCB were 0.1% for inrtradermal and topical inductions and 0.08% for challenge.

In the main study, all guinea pigs survived and all gained weight throughout the study period.

Following intradermal induction injection, the flonicamid test animals showed localised dermal reactions at all sites ranging from moderate (grade 2) to intense (grade 3) erythema, with oedema, but without necrosis or ulceration. The flonicamid control animals showed moderate to intense erythema at all sites in response to FCA with 2 animals also showing necrosis after 48 h. The mineral oil vehicle produced mild (grade 1) erythema only.

The DNCB positive control groups showed mild to intense erythema with infrequent necrosis, but no ulceration.

Following topical induction application, 8 of the 20 flonicamid test guinea pigs and 2 of the 20 controls guinea pigs exhibited mild erythema but without further dermal reactions. On the other hand, 9/10 DNCB test animals showed intense erythema (score 3) at 24 and 48h after patch removal, with oedema, necrosis and eschar formation in most animals at 24 - 48h.

After challenge, 2/20 flonicamid test animals showed mild erythema at 24 and 48 h after challenge and 2/20 control animals showed mild erythema at 48h only. On the other hand, DNCB produced mild to intense erythema in 9 animals at 24 h and 10 animals at 48h, compared to a zero incidence of positive reactions in the DNCB control group.

Table 17. Incidence of dermal reaction scores following challenge application

Group n° / description	**Dermal score**	**Time (hours)**	**No. of animals responding:**	
			Test site	**Control site**
1 / flonicamid sensitisation (test)	0	24	18	20
		48	18	20
	1	24	2	0
		48	2	0
	2	24	0	0
		48	0	0
	3	24	0	0
		48	0	0
2 / flonicamid control	0	24	20	20

		48	18	20
	1	24	0	0
		48	2	0
	2	24	0	0
		48	0	0
	3	24	0	0
		48	0	0
3 / DNCB sensitisation (test)	0	24	1	10
		48	0	10
	1	24	3	0
		48	3	0
	2	24	4	0
		48	3	0
	3	24	2	0
		48	4	0
4 / DNCB control	0	24	10	10
		48	10	10
	1	24	0	0
		48	0	0
	2	24	0	0
		48	0	0
	3	24	0	0
		48	0	0

The severe response induced by DNCB demonstrates the acceptability of the study. Flonicamid technical induced a mild erythema in 10% of the guinea pigs which is below the 30% threshold; moreover, a similar response was observed in the irritation control group; therefore IKI-220 should be considered as devoided of dermal sensitizing potential in the guinea pig maximization test and no classification is required.

Under the conditions of the M & K test, flonicamid is regarded as non-sensitising and no classification is required.

4.6.1.2 *Human information*

No data available

4.6.1.3 *Summary and discussion of skin sensitisation*

Flonicamid is not sensitizing.

4.6.1.4 *Comparison with criteria*

The CLP criteria for classification of substances for skin sensitization are as follow:
Substances shall be classified as skin sensitisers (Category 1) where data are not sufficient for sub-categorisation in accordance with the following criteria:
(a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or
(b) if there are positive results from an appropriate animal test

Substances classified in sub-category 1a are substances showing a high frequency of occurrence in humans and/or a high potency in animals that can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.

Substances classified in sub-category 1b are substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals that can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.

Considering that no specific effect is reported in human, and that flonicamid induced a mild erythema in 10% of the guinea pigs which is below the 30% threshold for a guinea pig maximisation test, no classification is required for flonicamid under either Directive 67/548/EEC or the CLP Regulation (including the criteria defined in the 2nd ATP).

4.6.1.5 *Conclusions on classification and labelling*

Flonicamid is not a skin sensitizer to guinea-pig in the maximisation test and therefore no classification is warranted under either Directive 67/548/EEC or the CLP Regulation (including the criteria defined in the 2nd ATP).

4.6.2 Respiratory sensitisation

4.6.2.1 *Non-human information*

No data

4.6.2.2 *Human information*

No data available

4.7 Repeated dose toxicity

Table 18. Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
28-d dietary (range finding study) **Wistar Rat** 0, 50, 100, 500, 1000 5000 ppm (10000 ppm F only) Equivalent to 0, 3.6, 7.5, 36.5, 74, 353, 642 mg/kg bw/d Method B7	↓ bw gain / food intake, Liver ; hypertrophy, ↑ weight & functional changes (both sexes); Kidney: ↑ weight, hyaline degeneration in males at ≥7.5mg/kg bw/d	Histopathological examination performed on liver, kidney and spleen only	Kuwahara (2002a)
28-d capsule (range finding study) **Beagle Dog** 0, 2, 10, 20/50mg/kg/d OECD 409	Death (1male at 50 mg/kg on day 2) 50 mg/kg bw/d >> MTD	Duration of treatment was 28 or 35 d; number of animals: 2/sex/group; these deviations did not affect the outcome of the study	Ridder (2001b)
90-d dietary toxicity **Wistar Rat** 0, 50, 200, 1000, 2000ppm (M), 0, 200, 1000, 5000ppm (F) OECD 408	Liver: hypertrophy, ↓ plasma triglycerides, (both sexes), ↑ weight (F) Kidney: males: granular casts / tubular basophilia (≥1000ppm), hyaline droplets deposition (≥200ppm) , NOAEL other effects 60 mg/kg bw/d; females: vacuolation of renal tubular cells (NOAEL 1000 ppm /72.3 mgKg bw/d)		Kuwahara (2002b)
90-d dietary (range-finding study) **CD-1 Mouse** 0, 100, 1000, 7000ppm OECD 408	Liver : hypertrophy (M), splenic extramedullary hematopoiesis at 1000ppm (M+F) Changes in electrolyte homeostasis and anemia (M+F; 7000ppm) NOAEL 15.3 mg/kg bw/d	No ophthalmoscopy performed; only liver, kidneys and spleen were weighed at necropsy; only bone marrow, liver, kidneys, gross lesions and spleen were subjected to histopathological evaluation	Ridder. (2001c)
90-d capsule toxicity **Beagle Dog** 0, 3, 8, 20mg/kg/d and 50mg/kg/d (F) OECD 409	50 mg/kg bw/d > MTD: morbidity / renal tubular vacuolation at 50mg/kg/d (F only) ↓ thymus weight without histological correlate in males at 20mg/kg bw/d considered non-adverse effects NOAEL 8mg/kg bw/d (M & F)		Ridder (2001a)
52-w capsule toxicity **Beagle Dog** 0, 3, 8, 20 mg.kg/d OECD 452	Mild anemia ↓ bw gain NOAEL 8mg/kg bw/d (M & F)		Ridder (2003b)
28-d dermal toxicity **SD Rat** 0, 20, 150, 1000mg/kg/d OECD 410	No effects	Deviation in clinical chemistry analysis: only female rats were fasted from approx. 16-24h prior to blood collection	Ridder (2001)

4.7.1 Non-human information

4.7.1.1 *Repeated dose toxicity: oral*

Reference: Kuwahara M. (2002a)
GLP: Yes
Guidelines: 92/69/EEC (method B.7).
Deviations: histopathological examination was performed on liver, kidney and spleen only

Groups of 6/sex Wistar rats (6-w old at start of dosing; mean bw: 146 g for males and 118 g for females) were administered dietary concentrations of 0; 50 (males only); 100; 500; 1000; 5000 and 10 000 ppm (females only) of flonicamid[1] for 4 weeks. Two satellite groups of 2 male rats (0 or 5000ppm) were used for immunohistochemical analyses.

Test diets were prepared every 4w during the treatment period; samples of each dose level were analysed for homogeneity and test substance concentration; samples from the 50 and 10 000 ppm were analysed for test substance concentration after storage (14 d in ambient air + 35 d in dark and cold container + 3 d at room temperature in dark and finally 15 d in animals room).

All rats from the main and satellite groups were observed at least once daily for clinical signs and a detailed clinical examination was performed at least once a week. Bw were recorded pre-dose, weekly throughout the study and at necropsy. Food consumption was measured for 3 consecutives days/week.

Urine analysis (specific gravity, glucose, ketones, occult blood, pH, protein, urobilinogen) was carried out on all rats at w4. Hematology (Ht, Hb, RBC, MCV, MCH, MCHC, PLT, total leukocytes counts) and blood chemistry analyses (ALP, GOT, GPT, GGT, CPK, creatinin, BUN, total protein, albumin, globulins, A/G ratio, glucose, total cholesterol, triglycerids, total bilirubin, calcium, inorganic phosphorous, sodium, potassium, chloride) were performed on all rats of the main groups after 28 d of treatment.

All main study rats were necropsied after 28 d of treatment. A detailed *post mortem* examination of major organs and tissues was performed, selected organs were weighed and samples of 18 major organs / tissues[2] and gross lesions were preserved. Histopathological examinations were conducted on preserved kidneys, liver and spleen from all rats.

The rats from satellite groups, used for immunohistochemical determinations, were killed and subjected to whole-body perfusion followed by a complete post mortem gross examination. The kidneys were immunostained with antibody against α-2-microglobulin (α2μG) and examined microscopically.

The recovery rate of test substance in the 50 and 10 000 ppm test diets analysed for stability were 90 and 93%, respectively. A good homogeneity was achieved as shown by the coefficient of variation obtained which was within 5.8 % for each dose level. The mean achieved concentrations of test substance in test diets were within 98-103% of the target concentrations (50; 98; 506; 1015; 5148 and 10 327 ppm for the 50; 100; 500; 1000; 5000 and 10 000 ppm nominals levels, respectively).

No deaths occurred in any group and there were no treatment-related clinical signs at any dose level.

Bw loss occurred in females at 10 000 ppm and reduced bw gain occurred in males at 5000 ppm during the first week of treatment. Although subsequent weekly bw gains in both sexes were comparable to the control

[1] batch n° 9809; purity: 98.7%

[2] Brain* (cerebrum, cerebellum, pons and medulla) ; pituitary ; thymus* ; thyroid with parathyroid; adrenals*; spleen*; heart*; kidneys*; urinary bladder; testes*; epididymes; prostate; seminal vesicles; coagulating glands; ovaries*; uterus including cervix; vagina; liver*; gross lesions (* weighed organs).

groups, the group mean bw in the females were significantly lower throughout the study and were significantly lower in the males for the first 2 w of treatment. No significant bw changes were seen in other group.

The **food consumption** was significantly reduced in females at 10 000 ppm in w1; w2 and w4 and also in males at 5000 ppm during w1 only. The food efficiency in 10 000 ppm females was 17% lower than in controls. The test substance intake during the treatment period was 0; 3.613; 7.47; 36.45; 73.8 and 353.4 mg/kg bw/d for the males from the 0; 50; 100; 500; 1000 and 5000 ppm groups, respectively and 0; 8.36; 41.24; 81.9; 372.6 and 642mg/kg bw/d for the females from the 0; 100; 500; 1000; 5000 and 10 000 ppm groups, respectively.

Table 19. Group mean bw, food consumption and test substance intake

Group mean values												
ppm	**0**		**50**	**100**		**500**		**1000**		**5000**		**10000**
Sex	**M**	**F**	**M**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**F**
Bw (g)												
w-0	146	118	146	146	118	146	118	146	118	146	118	118
w-1	200	140	200	197	143	202	138	198	142	188**	140	117**
w-2	250	161	250	246	165	252	157	246	167	236**	163	136**
w-3	290	177	286	282	179	292	174	284	184	374	179	154**
w-4	320	191	310	309	195	323	190	312	198	303	192	169**
Food consumption (g/rat/d)												
w-1	15.9	11.5	15.8	16.3	12.5	16.1	11.8	16.2	11.9	13.6**	9.9	5.3**
w-2	18.6	13.2	18.9	19.0	14.4	19.0	13.5	19.0	14.7	18.0	13.5	9.5**
w-3	20.1	13.6	20.0	20.0	14.7	20.6	14.2	20.1	14.7	19.1	13.1	11.3
w-4	20.6	14.1	20.0	21.0	15.1	21.3	14.6	20.4	15.0	19.5	13.7	11.5*
total	18.8	13.1	18.7	19.1	14.2	19.3	13.5	18.9	14.1	17.6	12.6	9..4
Test substance intake (mg/kg/bw)												
Main group	-	-	3.613	7.47	8.36	36.45	41.24	73.8	81.9	353.4	372.6	642
Satellite	-									357.3		
* p < 0.05; ** p < 0.01												

Urinalysis changes were confined to slightly more acidic urine in males at 5000 ppm (pH range 6.0 - 8.0) than in male controls (pH range 8.0 - 8.5); although treatment effects on the kideny were seen in males at this dose level, the toxicological significance of this acidulated urine is unclear since all other urine parameters were comparable to the control values in both sexes.

There were some statistically significant changes in **hematological parameters** (increased PLT counts in females at 10 000 ppm; reduced RBC and Ht in males at 5000 ppm) which would indicate some doubtful

anemic effect considering the absence of Hb changes. All other hematological parameters in all treated groups were comparable to control values, except a significant increase in the WBC of females at 1000 ppm, which is to be considered as incidental because of the absence of a dose-response effect.

There were also significant changes in **blood chemistry parameters** indicating a treatment related effect on the liver (increased GGT activity and reduced TG concentration in females at 10 000 ppm; increased total cholesterol in females at 10 000 ppm and in both sexes at 5000 ppm). Other significant changes did not exhibit a clear dose response pattern or were seen in only 1 sex and should be considered as not related to treatment (increased globulins and reduced A/G ratio in females at 10 000 ppm; increased total proteins and albumin and reduced GPT in females at 5000 ppm; reduced Ca levels in males at 5000 ppm).

Table 20. Selected group mean hematology and clinical chemistry values

Group mean values												
ppm	**0**		**50**	**100**		**500**		**1000**		**5000**		**10000**
Sex	**M**	**F**	**M**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**F**
Hematological parameters												
RBC (10^6/mm^3)	7.09	6.83	7.19	7.02	7.12	7.08	6.90	6.96	6.80	6.72*	6.78	6.66
Ht (%)	40.5	37.4	40.7	40.1	38.9	39.7	38.3	40.0	37.3	38.0**	37.1	36.0
Hb (g/dL)	14.6	13.9	14.7	14.4	14.4	14.4	14.2	14.4	13.9	14.0	14.1	13.6
PLT (10^3/mm^3)	1072	1070	1012	1073	1051	1118	1105	1067	1122	1134	1168	1253**
WBC (10^3/mm^3)	10.2	6.8	10.2	10.5	6.5	11.2	7.6	10.4	8.4*	10.3	8.1	7.6
Clinical chemistry parameters												
GGT (U/L)	1	2	1	1	2	1	2	1	2	1	2	4**
Chol (mg/dL)	54	50	52	52	48	53	45	56	56	65*	61*	86**
TG (mg/dL)	155	99	138	158	97	144	91	138	103	104	62	45*
Alb (g/dL)	2.92	3.01	2.92	2.83	3.17	2.93	2.98	2.89	3.04	2.13	3.21*	2.84
Glob (g/dL)	3.12	2.91	3.11	3.08	2.87	3.12	2.86	3.13	2.95	3.09	2.97	3.17**
A/G ratio	0.94	1.04	0.94	0.92	1.11	0.95	1.05	0.93	1.03	0.92	1.10	0.90**
Ca (mg/dL)	10.6	10.0	10.2*	10.2*	10.1	10.3	10.0	10.3	10.1	10.1**	10.	10.3
* $p < 0.05$; ** $p < 0.01$												

Gross-pathological examination revealed treatment-related liver enlargement in all females at 10 000 ppm and in 2 rats of each sex at 5000 ppm; dark-colored livers were seen in 2 males of the 5000 ppm group. Pale coloured and enlarged kidneys were observed in 5/6 and 3/6 males of the 5 000 ppm group, respectively. Liver and kidney enlargement were also seen in the 2 males of the satellite 5 000 ppm group. There were no other treatment-related gross lesions at any dose level.

There were significant changes in **organ weights** at the 2 highest dose levels; the group mean absolute and relative liver weights were significantly increased in females at 10 000 ppm and in both sexes at 5000 ppm and the absolute and relative kidney weights were significantly increased in males at 5000 ppm. Females at 10 000 ppm showed also significantly increased relative spleen weight (but the absolute weight was not significantly different from the controls and there was no histopathological correlate) and significantly reduced absolute and relative weights of the adrenals and the ovary that could be related to reduced bw gain and terminal bw.

Histopathological examination revealed treatment-related alterations in the liver for both sexes and kidneys for males only: centrilobular hepatocyte hypertrophy occurred in all females at 10 000 ppm (graded as moderate) and in 6/6 males and 4/6 females at 5000 ppm (graded as slight); increased hyaline droplet formation in the proximal tubular cells occurred in all male from groups treated at dose levels of 100 ppm and higher; the severity of this kidney change was dose-related, varying from slight at 100 ppm and 500 ppm to moderate at 1000 ppm and severe at 5000 ppm. Immunohistochemical staining of the kidneys from 2 control males and 2 males treated at 5000 ppm demonstrated that the hyaline droplets deposited in the proximal tubules reacted strongly to α2μG-antibody, but the number of tubular cells reacting positively was higher in the treated males. Granular casts seen in 2/6 males at 1000 ppm and 4/6 males at 5000 ppm also reacted positively to α2μG-antibody.

Table 21. Pathological findings at necropsy

Group mean values												
ppm	**0**		**50**	**100**		**500**		**1000**		**5000**		**10000**
Sex	**M**	**F**	**M**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**F**
Gross lesions												
Liver												
enlargment	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	2/6	2/6	6/6**
Dark color	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	2/6	0/6	0/6
Kidney												
Pale color	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	5/6**	0/6	0/6
Enlargement	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	3/6	0/6	0/6
Pelvic dilatation	1/6	0/6	0/6	1/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6
Selected group mean organ weights												
Liver												
Abs.(g)	9.14	5.10	8.87	9.25	5.28	9.39	5.12	9.18	5.40	10.27*	5.86**	7.25**
Rel. (%)	3.08	2.83	3.06	3.23	2.85	3.14	2.89	3.19	2.89	3.68**	3.28**	4.45**
Kidney												
Abs.(g)	2.05	1.27	1.96	1.99	1.31	2.10	1.33	2.14	1.25	2.30*	1.33	1.21
Rel.(%)	0.69	0.71	0.68	0.70	0.70	0.70	0.75	0.74	0.67	0.82**	0.74	0.74
Spleen												
Abs.(mg)	667	427	604	643	441	676	439	661	435	670	451	455
Rel. (%)	0.23	0.24	0.21	0.23	0.24	0.23	0.25	0.23	0.23	0.24	0.25	0.28**
Histopathological findings												
Liver												
Hypertrophy[a]	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	6/6**	4/6*	6/6**
Kidney												
Hyaline droplets[b]	0/6	0/6	0/6	3/6	0/6	6/6**	0/6	6/6**	0/6	6/6**	0/6	0/6
Granular casts	0/6	0/6	0/6	0/6	0/6	0/6	0/6	2/6	0/6	4/6*	0/6	0/6

* p < 0.05; ** p < 0.01; [a] centrilobular; [b] increased droplet deposition in proximal tubules

The major findings of the 28 d dietary administration of IKI-200 in rats were the treatment related effects on the liver of both sexes and on the kidney of males; the NOEL was 50 ppm in males and 1000 ppm in females, equivalent to dose levels of 3.6 and 81.9mg/kg bw/day in males and females, respectively; for males, the NOEL is based on the occurrence of increased hyaline droplet deposition in the kidneys at dose levels ≥ 100 ppm and reduced bw gain and food consumption, liver functional changes, enlargement and hepatocellular hypertrophy, and granular casts in the kidneys at 5000 ppm; for females, the NOEL is based on the occurrence of liver functional changes, enlargement and hepatocellular hypertrophy at 5000 ppm and additionally, reduced weight gain and food consumption at 10 000 ppm.

Since there is evidence that the increased hyaline droplet formation in the kidneys is mediated by the male rat-specific protein, α2μglobulin, a relevant NOAEL in the male for all other effects could be established as 1000 ppm, equivalent to a dose level of 73.8 mg/kg bw/d.

Reference: Ridder W. E., Yoshida M. and Watson M. (2001b)
GLP: Yes
Guidelines: OECD 409; US-EPA OPPTS 870.3150
Deviations: the duration of treatment was 28 or 35 d and the number of animals used was 2/sex/group; these 2 deviations did not affect the outcome of the study (range-finding study).

Groups of 2/sex beagle dogs (approx. 5 months old; bw range = 4.0-6.0 kg at start of treatment) were administered orally (capsules) 0; 2; 10 and 50 / 20 mg/kg[3] bw IKI-200 technical[4] for at least 35 consecutive days. The capsule were prepared weekly and stored at approx. 4°C.

Dogs were observed twice daily for morbidity/mortality and once daily for clinical signs of toxicity (1h after dosing) and a detailed clinical examination was performed once pre-test and then weekly throughout the observation period. An ophthalmoscopic examination was performed on all animals pre-test and on all survivors towards the end of the dosing period. Bw were recorded twice pre-dose, weekly throughout the study and at necropsy. Food consumption was recorded daily.

Hematology (Hb, Ht RBC, WBC, differential leukocyte count, reticulocytes count, PLT, MCV, MCH, MCHC), blood chemistry (BUN, creatinin, ALT, AST, ALP, total bilirubin, total protein, albumin, glucose, sodium, potassium, calcium, chloride, inorganic phosphorous, CK, total cholesterol, TG, GGT, globulins, A/G ratio) and urinalysis (volume, specific gravity, occult blood, protein, pH, bilirubin, ketones, glucose, nitrites, urobilinogen, color and appearance, microscopic examination of the sediment) were performed on all animals pre-test and towards the end of the treatment period.

All decedent and surviving dogs were subjected to necropsy and detailed *post mortem* examination of major organs and tissues was carried out. Selected organs were weighed from animals killed at termination and samples of major organs and tissues and all gross lesions were preserved[5] from all dogs. All preserved tissues from animals treated at 0 or 20/50 mg/kg bw/d were processed and examined by light microscopy.

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[3] Since 1 male died after 1 dose of 50mg/kg bw/d, the group was taken off dose for 6 d and treatment re-started at 20mg/kg bw/d; consequently, this group received 28 daily doses

[4] batch n° 9809; purity: 98.7%

[5] adrenals*, bone & bone marrow, brain*, ear, esophagus, eyes with optic nerve, duodenum, jejunum, ileum, cecum, colon, heart* with aorta, kidneys*, gallbladder, larynx, liver*, lungs*, lymph nodes (cervical and mesenteric), mammary gland, nose, pancreas, peripheral nerve (sciatic), pharynx, pituitary gland*, testes*, epididymes*, prostate*, ovaries*, uterus*, cervix, vagina, salivary glands (submandibular), skeletal muscles (biceps femoris), skin, spinal cord

One male dog treated at 50 mg/kg bw/d **died** on the day following the first dose (histopathological examination of this dog showed unspecific changes i.e. foci of agonal hemorrhage in the brain and spinal cord, splenic contraction, and congestion of the lungs and gastric mucosa). All other dogs survived the treatment period at dose levels up to 20mg/kg bw/d.

Treatment-related **clinical signs** occurred in both males receiving 1 dose of 50mg/kg bw/d, including vomiting, few or no feces, decreased activity, excessive salivation, and isolated occurrences of laboured breathing, increased heart rate, ataxia, and contracted pupils. Isolated occurrences of vomiting also occurred in some dogs of both sexes at 10 or 20mg/kg bw/d, but were not likely to be related to treatment because vomiting is a common response to oral administration in dogs. No treatment-related clinical signs occurred at 2mg/kg bw/d and there were no treatment-related ophthalmological findings at any dose level.

Bw gain and **food consumption** were unaffected by treatment at all dose levels. There were no treatment-related effects at any dose level on **hematology, clinical chemistry and urinalysis** parameters. All hematology and clinical chemistry values at all dose levels were within normal limits. Although there were occasional outlying urinalysis parameters, there was no pattern suggestive of a treatment-related effect.

There were no treatment-related **gross findings** or organ weight changes at necropsy. With respect to significant changes in **organ weights**, there were lower testis weights at 10mg/kg bw/d, increased pituitary weights of males at 2mg/kg bw/d and lower thymus weights of females at 10mg/kg bw/d. All these changes should be considered as incidental because of the absence of any dose response pattern and histological corroborates. There were no treatment-related **histopathological changes** in animals treated at 20mg/kg bw/d. Spontaneous lesions and incidental findings occurred in both control and treated animals at essentially comparable incidences. All histopathological alterations were of the type commonly seen in beagle dogs (foci of chronic active inflammation in the liver, mineralization within the collecting tubules of the kidney and foci of minimal interstitial inflammation of the lung).

Table 22. Bw and selected absolute and relative[a] organ weights

	Group mean values							
Dose level (mg.kg bw/d)	**0**		**2**		**10**		**50/20**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Body Weight (kg)								
Pre-test	5.18	4.78	5.10	4.23	5.03	4.63	5.60	5.18
w-3	6.48	5.85	6.63	4.95	6.05	4.45	5.70	6.15
w-5								
Organ weights								
Testes								
(g)	4.316		4.593		2.352*		3.300	
(g/kg bw)	0.673		0.685		0.382**		0.569	
Pituitary								
(g)	0.044	0.047	0.054*	0.048	0.043	0.044	0.047	0.046
(g/kg bw)	0.0068	0.0080	0.0080	0.0092	0.0070	0.0078	0.0081	0.0077
Thymus								
(g)	10.227	11.264	9.882	8.822	9.382	6.500*	6.942	11.175
(g/kg bw)	1.559	0.1901	1.475	1.702	1.519	1.158	1.197	1.929
* $p < 0.05$; ** $p < 0.01$; [a] relative to bw								

(cervical, midthoracic & lumbar), spleen*, stomach, tongue, thymus*, thyroid with parathyroid glands*, trachea, urinary bladder, all gross lesions. [* weighed]

No treatment related effects were seen in beagle dogs following 28 d - oral administration of flonicamid, except unspecific signs of toxicity at the highest dose level (50mg/kg bw/d) which clearly exceeded the maximum tolerated dose level. The NOEL in this study is 20mg/kg bw/d, based on the absence of unequivocal treatment-related effects.

Reference: Kuwahara M. (2002b)
GLP: Yes
Guidelines: OECD 408; US-EPA OPPTS 870.3100; JMAFF, 59 NouSan n° 4200 (1985).

Groups of 12/sex Wistar rats (6w old; bw = 123-138 g in males and 100-109 g in females at start of dosing) were given dietary concentrations[6] of 0; 50 (males only); 200; 1000; 2000 (males only) or 5000 ppm of flonicamid[7] for 13 consecutive weeks.

Test diets were prepared every 4 w during the treatment period; the stability of diets containing 50 or 10 000 ppm flonicamid was confirmed in the preliminary 28d study; chemical analysis for homogeneity and concentrations of flonicamid in diets were carried out by HPLC analysis for each dose level on samples taken from the first and last diet batches; the concentrations in randomly selected preprations (1000 ppm diets prepared on all other occasions) were also analysed twice during the study period to ensure that proper storage procedures were achieved.

All rats were observed at least twice daily for mortality/morbidity and once daily for clinical signs and a detailed clinical examination, including scored observations in the home cage and a standard arena, was performed at least once a week; observations on functional performance were performed on all animals during w12 (motor activity which was measured quantitatively for 1h in 6 / 10 minute intervals using an automated activity recording system; grip strength which was measured using a strain gauge and sensorimotor responses i.e. approach response, auditory response, touch response, tail-pinch response and righting reflex were evaluated semi-quantitatively using a scoring system). Ophthalmoscopic examinations were performed on all rats pre-dose and on all controls and high dose rats (0; 2000 or 5000 ppm) in w13.

Bw were recorded pre-dose, weekly throughout the study and at necropsy. Food consumption was measured for 4 consecutives days/week. Hematology (Ht, Hb, RBC, MCV, MCH, MCHC, PLT, total and differential leukocytes counts, reticulocytes count), blood chemistry (ALP, GOT, GPT, GGT, CPK, creatinin, BUN, total protein, albumin, globulins, A/G ratio, glucose, total cholesterol, triglycerids, total bilirubin, calcium, inorganic phosphorous, sodium, potassium, chloride) and urine analyses (specific gravity, glucose, ketones, occult blood, pH, protein, urobilinogen, volume and appearance, sediment) were performed on all animals during w13.

All rats were subjected to necropsy after 13w of treatment. A detailed *post mortem* examination of major organs and tissues was performed, selected organs were weighed and samples of major organs / tissues[8] and gross lesions were preserved. Sections of all tissues from all animals were examined by light microscopy.

[6] The dose levels were selected from the results of a 28-day dietary study in the rat (Kuwahara, M., 2002a, report no. IET 98-0140), in which the NOEL was 50ppm in males and 1000ppm in females.

[7] batch n° 9808 ; purity = 98.7%

[8] Brain* (cerebrum, cerebellum, pons and medulla), spinal cord (cervical, thoracic and lumbar), sciatic nerve, pituitary gland, thymus*, thyroid with parathyroid glands, adrenals*, spleen*, bone with bone marrow, lymph nodes (cervical and mesenteric), heart* with aorta, salivary glands (submaxillar & sublingual), esophagus, stomach, liver*, pancreas, duodenum, jejunum, ileum, cecum, colon, rectum, pharynx, larynx, lungs kidneys*, urinary bladder, testes*, epididymes*, prostate, seminal vesicles, coagulating glands, ovaries*, uterus*, cervix, vagina, eyes, Harderian gland, skeletal muscle, skin, mammary gland, all gross lesions. [* weighed]

Additional sections of kidneys from 2 control females and 2 females treated at 5000 ppm stained with PAS and oil red O were also examined microscopically.

A good homogeneity was achieved as shown by the coefficient of variation obtained which was within 1-2% for each dose level. The mean achieved concentrations of test substance in test diets checked at 1rst and last preparation were within 100-103% of the target concentrations (50; 203; 1027; 2034 and 5047 ppm for the 50; 200; 1000; 2000 and 5000 ppm nominals levels, respectively); proper storage procedures were achieved since samples taken from the 1000 ppm diets at the 2nd and 3rd preparation were 10% of the target concentration.

No **deaths** occurred in any group of either sex and there were only minor **clinical signs** in all groups including controls. Although increased or decreased grooming, reduced micturition and red peri-ocular adhesions occurred in some treated groups at higher incidence than in the controls, the observations are considered not to be treatment-related because they occurred on one occasion only or showed no dose-relationship. There were no treatment-related effects at any dose level on motor activity, fore-limb and hind-limb grip strength and the sensorimotor reflexes examined; although statistically significant lower motor activity occurred in females at 5000 ppm during 2 of the 10-min. test intervals, the overall activity for the 60-min. test period was not significantly different from the control activity; the same was true for the 1000 ppm males which exhibited a significantly decreased motor activity counts at one 10 min. interval and a significantly increased motor activity at another 10 min. interval. The quantitative grip strength and the scores for all sensorimotor reflex tests of both sexes at all dose levels were comparable to and not significantly different from the control values. There were no treatment-related ophthalmological findings in 2000 ppm males and in the 5000 ppm females.

Table 24. Summary of motor activity measurements at 12w of treatment

Group (ppm)	Sex	Mean beam count for 12 animals/group at:						
		1-10min	11-20min	21-30min	31-40min	41-50min	51-60min	1-60min
0	Male	2583 ±170	1385±518	370±471	100±224	47±77	163±198	4646±1298
50		2423±186	1279±495	151±145	67±66	36±28	173±192	4128±595
200		2551±177	1586±584	551±486	168±322	144±234	307±483	5307±1663
1000		2402±138*	1382±442	472±366	328±478*	222±34	177±141	4984±1244
2000		2509±176	1279±337	356±285	209±258	149±196	123±262	4625±702
0	Female	2376±181	1855±226	1324±410	800±452	477±501	337±366	7168±997
200		2321±262	1713±317	1057±589	459±444	194±363	435±359	6179±1382
1000		2315±207	1551±317	693±539*	267±303*	155±361	279±341	5260±1201**
5000		2425±215	1503±360*	798±520*	592±546	372±468	400±344	6090±1669
*p < 0.05; ** p < 0.01								

Bw gain of all treated rats was comparable to that of controls for both sexes. The **mean food consumption** was significantly reduced in the 2000 ppm males during w-10 and w-11 and in the 5000 ppm females at w1, w4, w8, w10, w11 and w13, the overall mean food consumption in these groups throughout the treatment period was 6% and 11.0% lower than that of the controls, respectively. In the 1000 ppm females, the food consumption was only reduced at w13. The **average test substance intake** was 3.079; 12.11; 60.0 and 119.4 mg/kg bw/d in males of the 50; 200; 1000 and 2000 ppm groups, respectively and 14.52; 72.3 and 340.1mg/kg bw/d in females of the 200; 1000 and 5000 ppm groups, respectively.

No significant changes were seen in **urinalysis** parameters in any group of either sex. The onlysignificant changes in **hematological parameters** were seen in the 5000 ppm females, which exhibited reduced Ht (3.4%) and increased (2.4%) MCHC and were therefore not considered of toxicological significance. Statistically significant changes **in clinical chemistry** parameters included reduced triglyceride

concentrations and decreased GPT activities in the high dose males (2000 ppm) and females (5000 ppm) and decreased CPK was in males at 2000 ppm.

Table 25. Selected group mean clinical chemistry findings

Group mean plasma values									
Ppm	**0**		**50**	**200**		**1000**		**2000**	**5000**
Sex	**M**	**F**	**M**	**M**	**F**	**M**	**F**	**M**	**F**
Trig (mg/dL)	175±33	88±17	179±42	161±25	97±25	149±22	79±15	140±24*	52±7*
GPT (U/L)	39±4	32±4	46±30	41±3	31±3	36±5	31±4	34±4*	28±4*
CPK (U/L)	134±30	105±16	127±14	125±22	104±18	138±24	99±17	111±15*	99±19
* $p < 0.05$; ** $p < 0.01$									

The only significant finding at **gross pathological examination** was the increased incidence (11/12) of pale kidneys in the 2000 ppm males. Statistically significant increases in both absolute and relative **organs weights** were seen for the liver of the high dose females (5000 ppm) and the kidney of the 1000 and 2000 ppm males and of the 5000 ppm females.

Histopathological examination showed slight to moderate centrilobular hepatocellular hypertrophy in all high dose males (2000 ppm) males and females (5000 ppm) and slight to moderate tubular basophilic change, granular casts in dilated tubules and hyaline droplet deposition in proximal tubular cells in the kidneys of 1000 and 2000 ppm males. Hyaline droplet deposition also occurred in males at 200 ppm but was not accompanied by the degenerative kidney changes seen at higher dose levels. The changes at 1000 and 2000 ppm are suggestive of α2μglobulin-mediated nephropathy. These kidney alterations were not seen in females at any dose level but all 5000 ppm females exhibited showed cytoplasmic vacuolation of the proximal tubular cells. The vacuoles stained negative for glycogen and fat.

Table 26. Selected group mean organ weights and incidence of histopathological changes

Group mean values									
Ppm	**0**		**50**	**200**		**1000**		**2000**	**5000**
Sex	**M**	**F**	**M**	**M**	**F**	**M**	**F**	**M**	**F**
Organ weights									
Liver									
Abs. (g)	11.28 ±0.58	5.80 ±0.58	10.73 ±1.58	11.56 ±0.67	5.87 ±0.47	11.36 ±0.77	5.63 ±0.38	10.21 ±0.85	6.53 ±0.56**
Rel (%)	2.69 ±0.11	2.39 ±0.13	2.69 ±0.24	2.77 ±0.14	2.38 ±0.10	2.72 ±0.09	2.42 ±0.08	2.80 ±0.11	2.84 ±0.15**
Kidney									
Abs. (mg)	2435 ±150	1501 ±131	2647 ±265	2554 ±173	1538 ±127	2678 ±171*	1447 ±88	2793 ±202**	1613 ±97*
Rel (%)	0.58 ±0.03	0.62 ±0.03	0.62 ±0.04	0.61 ±0.04	0.62 ±0.04	0.64 ±0.05**	0.62 ±0.04	0.70 ±0.02**	0.70 ±0.05**
Histopathological changes									
Liver									
Centrilobularhypertrophy	0	0	0	0	0	0	0	12**	12**
Kidneys									
Tubular basophilia	0	0	0	0	0	11**	0	12**	0

Granular casts[c]	0	0		0	0	5*	0	12**	0
Hyaline droplets[a]	0	0	0	8**	0	12**	0	12**	0
Cytoplasmic vacuolation[b]	0	0	0	0	0	0	0	0	12**
* $p < 0.05$; ** $p < 0.01$; [a] deposition in proximal tubular cells; [b] in proximal tubular cells; [c] in dilated tubules									

The male showed the occurrence of increased hyaline droplet deposition in the kidneys at dose levels ≥ 200ppm, granular casts and tubular basophilia in the kidneys at dose levels of ≥ 1000 ppm, and reduced food consumption, reduced plasma triglyceride concentration and hepatocellular hypertrophy at 2000 ppm. In this respect the NOAEL for males would be 50 ppm i.e. 3.1 mg/kg bw. However, since there is evidence that the increased hyaline droplet formation and associated degenerative changes in the kidneys are mediated by the male rat-specific protein, α2μglobulin, a relevant NOAEL in the male for all other effects could be established as 1000 ppm, equivalent to a dose level of 60.0mg/kg bw/d.

The NOAEL in females was 1000 ppm, equivalent to a dose level of 72.3mg/kg bw/d (occurrence of reduced food consumption, reduced plasma triglyceride concentration, liver enlargement, hepatocellular hypertrophy and cytoplasmic vacuolation of renal tubular cells at 5000 ppm).

Reference: Ridder W. E., Yoshida M. and Watson M. (2001c)
GLP: Yes
Guidelines: OECD408; US EPA OPPTS 870.31
Deviations: Since the study was a dose range-finding study for a further carcinogenicity study, no ophthalmoscopy was performed, only liver, kidneys and spleen were weighed at necropsy and only bone marrow, liver, kidneys, gross lesions and spleen were subjected to histopathological evaluation.

Groups of 10/sex Swiss-derived mice (Crl:CD-1®(ICR) BR strain; 7w old & bw range = 20 – 36 g at start of dosing) were administered dietary concentrations of 0; 100; 1000 and 7000 ppm of IKI-200 technical[9] for 13 consecutive w.

Test diets were prepared weekly and stored in a refrigerator; chemical analysis by HPLC for homogeneity was carried out pre-test on the lowest (100 ppm) and highest (7000 ppm) concentrations test batches; stability at room temperature and at approx. 5°C was checked on the same tests batches as for homogeneity and also on samples placed in animal room or in a refrigerator for 4; 4 and 14 days; achieved concentrations were determined on duplicate samples collected from each test diet used during w1; w4; w8 and w13.

All mice were observed twice daily for morbidity/mortality and once daily for clinical signs of toxicity, and a detailed clinical examination was performed weekly throughout the observation period. Bw were recorded pre-dose, weekly throughout the study and at necropsy. Food consumption was recorded weekly. Hematology (Hb, Ht RBC, WBC, differential leucocytes count, PLT, MCV, MCH, MCHC), blood chemistry (ALT, AST, albumin, total protein, globulins, A/G ratio, creatinin, BUN, total bilirubin, glucose, total cholesterol, sodium, potassium, calcium, chloride, inorganic phosphorous) were performed on all mice at termination.

All mice were necropsied after 13w (except 1 high dose female which escaped and was then sacrificed at w2) and a detailed *post mortem* examination of major organs and tissues was performed; liver / gall bladder,

[9] batch n°. 9809; purity = 98.7%

kidneys and spleen were weighed and samples of major organs / tissues[10] and gross lesions were preserved. Sections of bone marrow, liver, spleen and gross lesions from all animals, and kidneys from all animals treated at 0 or 7000 ppm were examined by light microscopy.

A good homogeneity was achieved as shown by the coefficient of variation from 3 analyses of the 100 and 7000 ppm tests batches (0.88-1.17%). Diets from the lowest and highest concentrations (100 or 7000 ppm) of flonicamid technical were found to be stable at room temperature and at +5°C for at least 14 d, at which time 92.9 - 98.7% nominal concentrations remained, respectively.

No **deaths** occurred at any dose level during the study, except 1 female that escaped and was killed in w2. There were no treatment-related **clinical findings** at any dose level, although 1 to 3 mice in all male treated and control groups showed masses in the lower abdominal region which were confirmed at necropsy as preputial gland enlargement.

There was a treatment-related decrease in **bw** gain in both sexes treated at 7000 ppm, with some males showing weight loss during the first 4 w of treatment; bw gain was unaffected by treatment at up to 1000 ppm.

Table 27. Group mean bw and cumulative bw gain at selected intervals

Group ppm	0		100		1000		7000	
Sex	M	F	M	F	M	F	M	F
Mean bw (g)								
w1	29.6±2.0	23.4±0.8	30.4±3.3	23.2±1.5	30.1±1.9	23.3±1.7	30.3±2.1	24.0±1.2
w13	38.3±2.8	31.2±2.9	38.4±3.5	30.4±4.0	39.1±1.9	31.2±3.1	35.9±5.4	29.1±1.6
Mean bw gain[a]								
w1	1.3±0.8	1.0±0.8	1.4±1.0	0.3±2.0	1.6±1.4	0.9±0.9	-0.8±2.4	0.0±1.6
w5	5.0±0.8	4.3±1.3	4.5±1.2	3.8±2.7	5.2±2.8	4.0±0.9	0.8±5.4*	1.8±2.4*
w7	6.3±0.7	5.3±1.3	5.8±1.5	5.3±3.2	6.6±2.9	5.6±0.7	2.9±5.1	3.1±1.7
w13	8.7±1.7	7.8±2.4	8.0±1.6	7.2±4.0	9.0±2.8	7.9±1.8	5.6±6.2	5.2±2.5
* $p < 0.05$; ** $p < 0.01$; [a]bw compared to bw at w -1								

Food consumption at 7000 ppm was reduced in both sexes throughout the treatment period, but the differences from the controls were statistically significant only in females during w 3 - 7. The overall mean food consumption in males and females at 7000 ppm was 5.1 and 6.7% lower than the controls, respectively. The **mean daily intakes** of flonicamid were 0; 15.25 ± 1.63; 153.9 ± 18.3 and 1069 ± 106mg/kg bw/d in males and 0, 20.1 ± 1.62; 191.5 ± 20.5 and 1248 ± 135 mg/kg bw/d in females of the 0; 100; 1000 and 7000 ppm groups, respectively.

The **hematological investigation**s showed a treatment-related anemia in both sexes at 7000 ppm (significant reduction of RBC, Hb, Ht together with significant increase of MCV, MCH and reticulocyte counts. Platelet counts were also slightly, but significantly in males at 7000 ppm. No changes in hematological parameters were seen in either sex at lower dose levels (Table 27).

Significant changes in **clinical chemistry parameters** also occurred only at the highest dose level: increased creatinine and total bilirubin concentrations in both sexes (statistical significance only for males); increased

[10] adrenals, bone (femur), bone marrow (sternum), brain, esophagus, eyes, duodenum, jejunum, ileum, cecum, colon, rectum, heart thoracic with aorta, kidneys*, liver with gallbladder*, lungs, lymph nodes (cervical and mesenteric), pancreas, pituitary gland, testes, epididymes, prostate, seminal vesicles, ovaries, uterus, cervix, vagina, salivary glands (mandibular), skeletal muscles with sciatic nerve, skin, spinal cord (cervical, midthoracic & lumbar), spleen*, stomach, thymus, thyroid with parathyroid glands, trachea, urinary bladder, all gross lesions. [* weighed]

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).

Table 28. Selected group mean hematological and clinical chemistry values after 13 w

Parameter	Males				Females			
	0	**100**	**1000**	**7000**	**0**	**100**	**1000**	**7000**
MetHb (%Hb)	1.51 ±0.91	2.22 ±0.76	1.40 ±0.86	2.38 ±0.82	2.02 ±0.60	1.95 ±0.40	2.49 ±1.91	2.27 0.52±
RBC (10^6/mm^3)	8.92 ±0.72	8.73 ±0.67	9.16 ±0.81	6.86 ±0.66**	8.76 ±1.06	9.01 ±0.63	8.80 ±0.63	7.14 ±0.49**
Hb (g/dL)	14.43 ±1.00	14.52 ±1.05	15.03 ±0.84	12.87 ±1.27**	14.86 ±1.58	15.33 ±0.66	15.04 ±0.80	13.43 ±0.56**
Hematocrit (%)	42.91 ±3.72	42.41 ±2.81	43.92 ±3.76	37.8 ±2.54**	43.0 ±5.16	44.48 ±2.77	43.50 ±2.67	39.1 ±1.59*
MCV (μm^3)	48.10 ±2.10	48.64 ±1.27	47.95 ±1.00	55.37 ±3.03**	49.10 ±1013	49.36 ±1.09	49.97 ±0.83	54.93 ±2.67**
MCH (pg)	16.20 ±0.70	16.64 ±0.45	16.44 ±0.74	18.79 ±0.93**	17.00 ±0.65	17.04 ±0.76	17.10 ±0.89	18.87 ±1.00**
MCHC (%)	33.68 ±0.75	34.23 ±0.78	34.30 ±1.25	33.97 ±1.50	34.63 ±1.18	34.50 ±1.28	34.62 ±1.38	34.36 ±0.56
Platelets (10^3/mm^3)	1163 ±399	1090 ±168	1176 ±140	1013 ±122*	952 ±348	1036 ±61	1090 ±166	1030 ±152
Reticulocytes (%)	1.51± 0.65	2.06 ±0.69	2.40 ±0.73	7.71 ±9.87**	1.98 ±0.81	2.70 ±1.22	2.58 ±1.17	7.26 ±5.86**
Creatinine (mg/dL)	0.460 ±0.097	0.456 ±0.073	0.480 ±0.063	0.560 ±0.052*	0.478 ±0.083	0.470 ±0.067	0.460 ±0.052	0.533 ±0.071
T. bilirubin (mg/dL)	0.300 ±0.047	0.322 ±0.044	0.290 ±0.074	0.450 ±0.085**	0.320 ±0.079	0.290 ±0.057	0.310 ±0.099	0.411 ±0.162
Glucose (mg/dL)	138.8 ±23.2	141.0 ±13.4	145.1 ±23.0	197.6 ±61.2	147.8 ±23.2	147.3 ±14.7	155.0 ±18.6	183.6 ±26.0**
Sodium (mEq/L)	166.6 ±3.4	166.0 ±4.4	167.1 ±3.3	176.0 ±3.5**	169.9 ±4.0	167.0 ±5.2	172.1 ±8.7	175.3 ±4.9
Potassium (mEq/L)	6.020 ±0.516	6.456 ±0.754	6.170 ±0.793	5.070 ±0.698*	5.689 ±1.111	5.660 ±0.729	5.430 ±0.767	4.90 ±0.711
Chloride (mEq/L)	110.4 ±1.3	110.8 ±2.1	111.6 ±1.6	115.0 ±3.4**	111.8 ±4.2	112.6 ±3.6	113.5 ±2.5	116.1 ±3.0
* $p < 0.05$; ** $p < 0.01$								

There were no treatment-related **gross lesions** at necropsy in any treated group; the most common finding in males were the incidences of enlarged preputial glands in male groups, noted clinically as masses in the lower abdominal region (1; 2; 0 and 2, in order of ascending dose level) and the most common finding in females were the incidences of ovarian cysts and enlargment of the uterus and horns without dose response pattern.

The absolute and relative **liver and spleen weights** of both sexes at 7000 ppm were significantly increased (increased spleen weights considered to be associated with the observed anemic changes).

Treatment-related **histopathological changes** occurred in the liver, spleen and bone marrow of both sexes at 7000 ppm, in the liver and spleen of the 1000 ppm males treated and in the spleen of the 1000 ppm females. Hepatocellular hypertrophy occurred in all animals at 7000 ppm (graded as minimal to slight and correlated with the increased liver weight at this dose level) and in 3 males at 1000 ppm (graded minimal). Hypocellularity (graded as minimal to slight) and increased pigment deposition occurred in the bone marrow of a majority of rats of both sexes at 7000 ppm only. Increased incidences of minimal to moderately severe

extramedullary hematopoiesis occurred in both sexes treated at 1000 or 7000 ppm (changes consistent with treatment related anemia and hemosiderin deposition). No treatment-related histopathological changes were seen the kidneys at 7000 ppm so that there were no histopathological correlates for the observed changes in electrolyte homeostasis and plasma creatinine concentrations at 7000 ppm.

Table 29. Selected group mean absolute & relative organ weights and histopathological alterations after 13w

Organ / finding	**Incidence of treatment-related histopathological alterations**							
	Males treated at (ppm):				**Females treated at (ppm):**			
	0	**100**	**1000**	**7000**	**0**	**100**	**1000**	**7000**
Organs weights								
Liver								
Abs. weight[a] (g)	2.021	2.018	2.172	2.449**	1.640	1.501	1.670	1.831
Rel. weight[a] (%)	5.298	5.257	5.561	6.843**	5.317	4.953	5.408	6.410**
Spleen								
Abs. weight (g)	0.112	0.087	0.109	0.164*	0.110	0.108	0.114	0.178**
Rel. weight (%)	0.293	0.227	0.280	0.466**	0.356	0.356	0.370	0.621**
Kidneys								
Abs. Weight (g)	0.590	0.632	0.66	0.607	0.452	0.401	0.404	0.392
Rel. weight (%)	1.547	1.643	1.667	1.715	1.384	1.346	1.321	1.377
Bone marrow:								
- no. examined	10	10	10	10	10	10	10	10
- hypocellularity	0	0	0	8	0	0	0	6
- increased pigment	0	0	0	7	0	0	0	5
Liver:								
- no. examined	10	10	10	10	10	10	10	10
- hypertrophy	0	0	3	10	0	0	0	10
Spleen:								
- no. examined	10	10	10	10	10	10	10	10
- inc. hematopoiesis	2	2	5	10	3	4	7	10
- increased pigment	0	0	0	10	0	0	0	10
* $p < 0.05$; ** $p < 0.01$; [a] including gall bladder								

In mice given 0; 100; 1000 or 7000 ppm in diet for 13w, the NOEL was 100 ppm for both sexes equivalent to dose levels of 15.3 and 20.1mg/kg bw/d in males and females, respectively, based on the occurrence of hepatocellular hypertrophy in males and increased splenic extramedullary hematopoiesis in both sexes at 1000 ppm, and additionally changes in electrolyte homeostasis and anemia with histopathological sequelae in both sexes and hepatocellular hypertrophy in females at 7000 ppm.

Reference: Ridder W. E. and Watson M. (2001a)
GLP: Yes
Guidelines: OECD 409; US-EPA OPPTS 870.3150

Groups of 4/sex beagle dogs (approx. 6 months old; bw range: 4.7 - 7.6 kg at start of dosing) were administered orally (capsules) 0; 3; 8 and 20 mg/kg bw/d[11] of flonicamid technical[12] for at least 90 consecutive days; in addition, a group of 4 females was given 50 mg/kg bw/d of the test substance for 90 days.

[11] The dose levels were selected from the results of the 28-day study on dogs (Ridder (2001a), report n°. 010871-1) in which a dose level of 50mg/kg bw/day clearly exceeded the MTD and the NOEL was 20 mg/kg bw/d.

[12] batch n° 9809; purity : 98.7%

Dogs were observed twice daily for morbidity/mortality and once daily for clinical signs of toxicity (1h after dosing), and a detailed clinical examination was performed once pre-test and then weekly throughout the observation period. An ophthalmoscopic examination was performed on all animals pre-test and on all survivors at end of the dosing period. Bw were recorded twice pre-dose, weekly throughout the study and at necropsy. Food consumption was recorded daily during pre-test and test period. Hematology (Hb, Ht RBC, WBC, differential leukocyte count, reticulocytes count, PLT, MCV, MCH, MCHC), blood chemistry (BUN, creatinin, ALT, AST, ALP, total bilirubin, total protein, albumin, glucose, sodium, potassium, calcium, chloride, inorganic phosphorous, CK, total cholesterol, TG, GGT, globulins, A/G ratio) and urinalysis (volume, specific gravity, occult blood, protein, pH, bilirubin, ketones, glucose, nitrites, urobilinogen, color and appearance, microscopic examination of the sediment) were performed on all animals pre-test, at w6 and and prior to termination.

All decedent and surviving dogs were subjected to necropsy and detailed *post mortem* examination of major organs and tissues was carried out. Selected organs were weighed and samples of major organs and tissues and all gross lesions were preserved[13] from all dogs. All preserved tissues from animals treated at 0; 20 or 50mg/kg bw/d were processed and examined by light microscopy.

One female treated at 50mg/kg bw/d was killed on d21 following severe anorexia, vomiting, ataxia, decreased activity, diarrhoea and bw loss. All other animals **survived** for the duration of the study, but a further female treated at 50mg/kg bw/d, that also showed vomiting, anorexia, bw loss and isolated occurrences of ataxia and tremors, was taken off dose for an unspecified period and made a full recovery from weight loss and anorexia.

Treatment-related **clinical signs** at lower dose levels were confined to episodes of vomiting in dogs given 20mg/kg bw/d. One male and one female at 20mg/kg bw/d also displayed ataxia on 2 and 1 occasions, respectively. There were no treatment-related ophthalmological findings at any dose level

Females at the 50mg/kg bw/d exhibited significant **bw** loss for the first 5 - 10 w of dosing, and bw gain occurred thereafter when supplemental food was provided; however, the overall bw gain was reduced by 66.8% compared with that of controls. Transient and less marked bw loss occurred in both sexes at 20mg/kg bw/d (cumulative bw changes were significantly different from controls in males for w1- w3 and in females for w5; overall bw gains were 27.7 and 51.9% lower than that of control males and females, respectively). Only a minimal and transient bw loss occurred in both sexes at 8mg/kg bw/d during w11. The bw gains of both sexes at 3mg/kg bw/d were not significantly different from the controls throughout the treatment period.

The **food consumption** was significantly reduced in females at 50 mg/kg bw/d during w3-w9 and at 20mg/kg bw/d during w5 and w7; the food consumption of males at 20mg/kg bw/d and of both sexes at lower dose levels was not significantly different from that of controls.

Table 30. Group mean cumulative bw gain and food consumption at selected intervals

Group	**0 mg/kg /d**		**3 mg/kg /d**		**8 mg/kg /d**		**20 mg/kg /d**		**50 mg/kg /d**
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**F**

[13] adrenals*, bone & bone marrow, brain*(including cerebrum, cerebellum,and medulla/pons), ear, esophagus, eyes, duodenum, jejunum, ileum, cecum, colon, heart* with aorta, kidneys*, gallbladder, larynx, liver*, lungs*, lymph nodes (cervical and mesenteric), mammary gland, nose, pancreas, peripheral nerve (sciatic), pharynx, pituitary gland*, testes*, epididymes*, prostate*, ovaries*, uterus*, cervix, vagina, salivary glands (submandibular), skeletal muscles, skin, spinal cord (cervical, midthoracic & lumbar), spleen*, stomach, tongue, thymus*, thyroid with parathyroid glands*, trachea, urinary bladder, all gross lesions. [* weighed]

mean bw gain (kg) after									
w-1	0.23	0.01	0.11	0.06	-0.02	-0.16	-0.25**	-0.18	-0.33*
w-2	0.45	0.28	0.43	0.10	0.10	0.04	-0.08*	-0.08	-0.36*
w-4	1.00	0.64	1.05	0.51	0.64	0.20	0.50	-0.04	-0.45**
w-8	1.83	1.03	1.93	0.93	1.36	0.61	1.09	0.18	-0.12*
w-13	2.71	1.85	2.98	1.70	2.2	1.48	1.96	0.89	0.80
Mean food consumption[a] (g/day) during:									
w1	209.6	197.2	226.1	196.5	208.0	212.0	199.7	215.2	210.4
w3	247.5	188.7	229.2	188.9	183.4	174.2	156.9	169.0	151.4
w5	276.5	217.4	258.6	200.5	228.8	201.4	208.0	178.5	149.3*
w7	265.2	242.4	276.1	229.4	250.9	226.9	239.2	199.1*	154.8**
w9	286.2	257.3	264.6	220.4	252.6	215.6	247.4	187.9**	148.3**
w11	321.9	275.4	293.3	227.5	256.3	248.9	252.0	217.5	172.4**
w13	273.8	289.1	297.5	247.5	247.4	240.8	262.7	207.1	244.0
* $p < 0.05$; ** $p < 0.01$; [a] mean of means									

Treatment-related **hematological** changes were only seen in the 50 mg/kg bw females which exhibited significantly reduced RBC and significantly increased reticulocyte counts in w7, but not at termination; all other hematological parameters at all dose levels and at both sampling intervals were not significantly different from control values, except for monocyte counts which were significantly reduced only in males at 3 and 20 mg/kg bw/d group during w7.

Significant changes in **clinical chemistry** parameters were only seen in the 20 mg/kg bw/d males in which the total protein concentration was significantly elevated at w7 (7.7% over the control value) but not at termination; such a minor and transient change without histopathological correlate should be considered as non adverse.

There were no treatment-related effects on **urinalysis** parameters at any dose level at either sampling interval although interpretation of the data was hampered by water contamination of some urine samples.

There were no treatment related gross lesions at **necropsy**, except the brown spot in the pyloric region of the stomach a single female at 50mg/kg bw/d suggesting focal hemorrhage associated with test substance.

Significant changes in **organ weights** occurred in high dose males (20 mg/kg bw/d) in which absolute and relative weights of the thymus (63.1 - 67.2% lower) were reduced; this finding may be related to treatment, but it should not be considered of toxicological significance, because there was no histopathological correlate and it was not seen in high dose females nor in males at lower dose levels; relative lung weights were increased in high dose females. Other significant organ weight changes were also seen in males of the 8mg/kg bw/d group which exhibited increased absolute and relative prostate weights and in males of the 3mg/kg bw/d group which exhibited increased absolute weights of the adrenal glands, epididymides and prostate gland; however, all these changes should not be considered as treatment related because they occurred in low and mid dose groups, but not in high dose males (20mg/kg bw/d).

Histological examination showed various changes that may have been related to treatment in the 50 mg/kg bw/d females (mild oedema of the pancreas, mild thymic involution and mild vacuolation of the tubules of the kidney cortex in the female prematurely killed at d21; renal tubular vacuolation in another female and showed hemorrhage at the ileo-colic junction in a third one). No treatment-related findings were observed at 20 mg/kg bw/d in either sex (specifically, there were no alterations in the thymus gland of male animals at 20mg/kg bw/d to account for the marked decrease in thymus weight). A neoplasm seen in the kidney (well circumscribed tubular cell adenoma) of 1 female from the 3 mg/kg bw/d is to considered as incidental.

Table 31. Selected organ weights and of histopathological alterations

Group	**0 mg/kg /d**		**3 mg/kg /d**		**8 mg/kg /d**		**20 mg/kg /d**		**50 mg/kg /d**
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**F**

Organ weights									
Adrenals									
Absolute. (g)	0.796	1.075	1.301*	1.106	1.032	1.018	1.191	1.085	1.183
Relative (%)	0.091	0.148	0.137	0.147	0.122	0.144	0.152*	0.168	0.179
Thymus									
Absolute. (g)	11.206	7.572	8.032	5.827	11.686	7.527	3.673**	7.258	7.202
Relative (%)	1.278	0.971	0.867	0.791	1.333	1.064	0.472**	1.125	1.093
Prostate									
Absolute. (g)	1.907		3.387*		3.947*		2.082		
Relative (%)	0.219		0.362		0.452*		0.266		
Lung									
Absolute. (g)	71.33	59.49	80.42	62.66	66.61	60.50	63.48	55.01	62.87
Relative (%)	8.129	7.441	8.471	8.510	7.812	8.588	8.222	8.530	9.480*
histopathological alterations (Incidence / no. of animals examined)									
Thymic involution	0 / 4	0/4	-	-	-	-	0/4	0/4	1[a] / 4
Ileo-colic hemorrhage	0/4	0/4	-	-	-	-	0/4	0/4	1/4
Renal tubular vacuolation	0/4	0/4	-	-	-	-	0/4	0/4	1[a] + 1 / 4
* $p < 0.05$; ** $p < 0.01$; [a] animal killed prematurely on day 21									

The dose of 50mg/kg bw/d to females clearly exceeded the MTD. The NOEL is this study was 8mg/kg bw/day in both sexes, based on the occurrence of reduced bw gain and food consumption in both sexes and reduced thymus weight in males at 20mg/kg bw/d.

Reference: Ridder W. E. and Watson M. (2003b)
GLP: Yes
Guidelines: OECD 452; US-EPA OPPTS 870.3150

Groups of 6/sex beagle dogs (6 months old at start of dosing; bw at start = 4.5 – 7.5 kg) were administered orally, by capsules, 0; 3; 8 and 20 mg/kg bw/d of flonicamid[14] technical for at least 52w. Capsules were prepared weekly and stored under refrigerated conditions.

Dose levels were selected from the results of the 90 days study (Ridder, 2001a) in which the dose level of 50mg/kg bw/d to females clearly exceeded the maximum tolerated dose level; the NOEL in this study was 8mg/kg bw/d for both sexes.

All dogs were examined twice daily for morbidity/mortality; clinical signs were checked once daily at 1h after dosing and a detailed clinical examination was performed pre-test and weekly throughout the treatment period. An ophthalmoscopic examination was performed on all dogs pre-test and in w 52. Bw were recorded twice pre-dose, weekly throughout the study and at necropsy. Food consumption was recorded daily.

Hematology (Ht, Hb, RBC, total and differential leukocyte counts, Ptl, MCV, MCH, MCHC, reticulocyte count), blood chemistry (BUN, creatinin, ALP, ALT, AST, total bilirubin, total protein, albumin, globulins, A/G ratio, glucose, sodium, potassium, chloride, calcium, inorganic phosphorous, CPK, total cholesterol, GGT) and urinalysis (volume, specific gravity, occult blood, protein, pH, bilirubin, ketones, glucose, nitrite, urobilinogen, color and appearance, microscopic examination of the sediment) were performed on all animals pre-test, at 3-month intervals throughout the treatment period and prior to termination.

All surviving animals were subjected to necropsy and detailed *post mortem* examination of major organs[15] and tissues. Selected organs were weighed and samples of major organs and tissues and all gross lesions

[14] batch n° 9809 ; purity = 98.7%

were preserved from all animals. A female decedent was subjected to necropsy within 2 h of death and a full tissue list was examined microscopically. Preserved tissues from the animals treated at 0 or 20mg/kg bw/day, and gross lesions from all animals, were examined by light microscopy.

Mortality and clinical examinations: One female treated at 3 mg/kg bw/day died during the first week of treatment; this death was not considered as related to treatment because necropsy showed findings suggestive of severe pneumonia. All other animals survived the scheduled treatment period. Treatment-related clinical signs were confined to vomiting in several dogs at the 8 and 20 mg/kg bw/d dose levels, generally during the first w of dosing only. One animal of each sex at 20 mg/kg bw/day exhibited occasional decreased activity and an isolated occurrence of ataxia, but these findings were likely related to general debility following episodes of vomiting. There were no abnormal ophthalmological findings at any dose level after 52 w.

Bw and food consumption: Bw gain was significantly reduced in females at 20mg/kg bw/d in w2, w3 and w4, although the group mean bw were not significantly different from control values throughout the treatment period; nevertheless, the overall weight gain decrement was 30.4% at termination and should therefore be considered as related to treatment. Overall bw gain was also reduced in females at 8mg/kg bw/d, but differences in weekly bw and bw gains were not statistically significant. There were no treatment-related effects on the bw gain of females at 3 mg/kg bw/d, or in males at any dose level. There were no treatment-related effects on the food consumption of either sex at any dose level.

Table 32. Summary of group mean bw and bw gain (kg)

	Males				**Females**			
(mg/kg/day)	**0**	**3**	**8**	**20**	**0**	**3**	**8**	**20**
Bw (kg)								
Pretest	6.48 ± 0.68	6.48 ± 0.81	6.11 ± 0.37	6.00 ± 0.47	5.35 ± 0.53	5.09 ± 0.54	5.22 ± 0.22	5.52 ± 0.47
w1	6.46 ± 0.63	6.41 ± 0.68	6.07 ± 0.29	5.92 ± 0.47	5.37 ± 0.55	5.03 ± 0.49	5.11 ± 0.21	5.44 ± 0.52
w4	7.08 ± 0.71	7.28 ± 0.62	6.82 ± 0.46	6.63 ± 0.56	6.13 ± 0.61	5.82 ± 0.58	5.68 ± 0.35	5.88 ± 0.51
w20	9.95 ± 1.15	10.13 ±0.85	9.66 ± 0.50	9.92 ± 0.38	8.53 ± 0.92	8.22 ± 0.89	8.04 ± 0.76	7.80 ± 0.91
w30	10.91 ±1.36	11.03 ±1.01	10.80 ±0.76	10.90 ±0.54	9.64 ± 1.22	9.57 ± 1.52	8.97 ± 0.71	8.70 ± 1.16
w40	11.01 ±1.36	11.11 ±1.19	10.98 ±0.87	11.03 ±0.77	9.78 ± 1.43	9.89 ± 2.00	8.83 ± 0.84	8.57 ± 0.97
w52	11.16 ±1.48	11.13 ±1.18	11.24 ±0.76	11.48 ±0.86	10.25 ±1.79	10.57 ±2.18	9.13 ± 1.02	8.93 ± 1.06
Bw gain (kg)								
w1	-0.02± 0.10	-0.07 ±0.16	-0.04 ± 0.20	-0.08 ± 0.14	0.02 ± 0.07	-0.07 ±0.14	-0.11 ± 0.20	-0.08 ± 0.10
w4	0.61 ± 0.23	0.80 ± 0.24	0.71 ± 0.25	0.63 ± 0.37	0.78 ± 0.14	0.68 ± 0.20	0.46 ± 0.39	0.37 ±0.24*
w20	3.48 ± 0.68	3.65 ± 0.58	3.55 ± 0.61	3.92 ± 0.56	3.18 ± 0.78	3.08 ± 0.43	2.83 ± 0.75	2.28 ± 0.65

[15] adrenals*, bone & bone marrow, brain*(including cerebrum, cerebellum,and medulla/pons), ear, esophagus, eyes, duodenum, jejunum, ileum, cecum, colon, heart* with aorta, kidneys*, gallbladder, larynx, liver*, lungs, lymph nodes (cervical, retropharyngeal and mesenteric), mammary gland, nose, nasopharyngeal tissue, pancreas, peripheral nerve (sciatic), pharynx, pituitary gland, testes*, epididymes*, prostate*, ovaries*, uterus*, cervix, vagina, salivary glands (submandibular), skeletal muscles, skin, spinal cord (cervical, midthoracic & lumbar), spleen*, stomach, tongue, thymus*, thyroid with parathyroid glands*, trachea, urinary bladder, all gross lesions. [* weighed]

w30	4.43 ± 0.96	4.56 ± 0.45	4.69 ± 0.92	4.90 ± 0.84	4.29 ± 1.02	4.43 ± 1.10	3.75 ± 0.63	3.18 ± 0.51
w40	4.53 ± 0.99	4.63 ± 0.59	4.88 ± 1.01	5.03 ± 1.03	4.43 ± 1.27	4.75 ± 1.63	3.61 ± 0.75	3.05 ± 0.77
w52	4.68 ± 1.18	4.66 ± 0.70	5.13 ± 0.96	5.48 ± 1.19	4.90 ± 1.62	5.43 ± 1.90	3.92 ± 0.91	3.41 ± 0.80

Laboratory investigations:

Hematological profile: There were no treatment-related effects on the hematological profile after 3 or 6 months of treatment, but after 9 and 12 months there was a suggestion of a mild anemia in both sexes at the highest dose level; males at 20mg/kg bw/d exhibited significantly increased MCV and MCH at the 9 and 12 months time points, although individual values were within the historical control range ; females at 20 mg/kg bw/d also showed reduced RBC, Hb and Hct values after 9 and 12 months of treatment; in addition, reticulocytes were increased in both sexes at 20mg/kg bw/d from 6 months, with statistical significance at 12 months treatment. The female group at 8 mg/kg bw/d also showed statistically significant reduction of RBC, Hb and Ht value after 9 months, but these changes were not seen after 12 months and were not associated with increased reticulocyte counts; furthermore, RBC, Hb and Hct values in females at 8mg/kg bw/d were significantly lower than the controls prior to the start of treatment. Therefore, they were considered to be unrelated to treatment with flonicamid technical. There were no other treatment-related effects on the hematological profile at any dose level.

Clinical chemistry and urinalysis parameters: There were no consistent treatment-related effects at any dose level or sampling interval on the plasma clinical chemistry and urinalysis profiles.

Necropsy, organ weights and histopathological examinations: There were no treatment-related gross necropsy findings and organ weight changes at any dose level. There were no histopathological alterations in any of the tissues and organs examined in animals treated at 20mg/kg bw/d. Specifically, there were no treatment-related histopathological alterations in the tissues of the hematopoietic system. All histopathological alterations detected occurred at comparable incidences in the treated and control groups and were considered to be incidental to treatment.

Table 34. Selected group mean hematological parameters

Parameter (units)	Study	**Group mean values**							
	month	**Males**				**Females**			
Doses (mg/kg bw/d)		**0**	**3**	**8**	**20**	**0**	**3**	**8**	**20**
RBC (10^6/mm^3)	Pretest	5.73	5.66	5.67	5.07**	5.75	5.82	5.17*	5.47
	3	5.85	6.09	5.95	5.55	6.17	6.09	5.51	5.59
	6	6.33	6.31	6.04	6.24	6.36	6.45	5.71	5.84
	9	6.92	7.23	6.50	6.37	7.04	7.00	5.89**	6.01**
	12	6.68	6.62	6.39	6.57	6.80	6.61	6.49	5.90
Hb (g/dL)	P	13.9	13.8	13.9	12.8*	14.1	14.3	12.8*	13.8
	3	13.7	14.6	14.1	13.4	14.6	15.0	13.7	13.9
	6	15.0	15.7	14.9	15.6	15.7	16.2	14.7	15.2
	9	16.6	16.9	16.2	16.5	17.5	18.1	15.8*	15.6*
	12	15.6	15.8	15.4	16.4	16.4	16.2	16.4	15.2
Hct (%)	P	40.1	39.9	39.8	36.8*	40.4	41.4	37.1*	39.2
	3	40.7	43.3	42.2	39.6	43.2	44.2	40.1	40.7
	6	44.4	45.8	43.6	45.8	45.9	47.7	42.8	43.8
	9	48.8	52.5	47.2	47.7	51.3	52.5	45.1*	45.6*
	12	45.6	46.4	44.7	47.4	47.4	47.1	46.6	43.1
MCV (fL)	P	69.9	70.6	70.1	72.5	70.4	71.3	71.8	71.7
	3	69.6	71.3	71.0	71.4	70.2	72.6	73.0	72.9
	6	70.2	72.6	72.2	73.4	72.3	74.0	75.1	75.2

	9	70.6	72.6	72.6	74.9**	73.0	75.0	76.5*	76.0
	12	68.2	70.1	69.9	72.1**	69.9	71.4	71.9	73.1
MCH (pg)	P	24.4	24.5	24.5	25.2	24.5	24.6	24.7	25.3
	3	23.5	24.0	23.8	24.2	23.7	24.7	24.8	25.0
	6	23.7	24.9	24.7	25.0*	24.7	25.1	25.8	26.0
	9	24.1	23.6	25.0	25.9*	24.9	25.9	26.7**	26.0
	12	23.4	23.9	24.1	24.9**	24.2	24.7	25.3	25.7*
Retics (%)	3	0.6	0.5	0.4	0.8	0.1	0.1	0.3	0.4
	6	0.6	0.8	0.4	1.1	0.2	0.2	0.3	0.6
	9	0.6	0.5	0.5	1.1	0.4	0.4	0.5	1.1
	12	0.5	0.6	0.8	1.9**	0.3	0.3	0.4	1.0*
* p < 0.05; ** p < 0.01									

No specific target organs were identified. The NOAEL was 8 mg/kg bw/d in both sexes, based on the occurrence of hematological changes suggestive of mild anemia in both sexes, and reduced bw gain in females, at 20 mg/kg bw/day.

4.7.1.2 *Repeated dose toxicity: inhalation*

No data available

4.7.1.3 *Repeated dose toxicity: dermal*

Reference: Ridder W. E. (2001)
GLP: Yes
Guidelines: OECD 410; US-EPA OPPTS 870.3200, JMAFF 59 NouSan n°4200
Deviations: deviation occurred for clinical chemistry analysis, when only female rats were fasted from approx. 16-24h prior to blood collection

Groups of 10/sex Sprague-Dawley-derived rats (Crl:CD®(SD)(IGS)BR strain; 8-9 w old at dosing; bw = 183 - 361g) were treated daily by semi-occluded topical application 6h/d, 7d/w for 28 consecutive days of 0, 20, 150 and 1000 mg/kg bw/d of flonicamid[16] technical to a clipped area of intact dorsal skin (approx. 10% of the body surface at least for controls and high dose rats); test material was suspended in distilled water (treatment volume not specified) and uniformly spread on the skin area; after 6h exposure, the skin was gently wiped with paper towels moistened with warm water.

All rats were observed at least twice daily for mortality/morbidity and detailed clinical open-field examination, including dermal observations was performed weekly; ophthalmoscopic examinations were performed on all animals pre-dose and at the end of the treatment period.

Bw were recorded pre-dose, and at d7; d14; d21 and d28 of study. Food consumption was measured weekly. Hematology (Hb, Ht, RBC, total and differential leukocytes counts, PLT, MCV, MCH, MCHC) and blood chemistry (BUN, creatinin, ALP, ALT, AST, total bilirubin, total protein, albumin, globulins, A/G ratio, glucose, sodium, potassium, chloride, calcium, inorganic phosphorous, CPK, total cholesterol, GGT) were performed on all rats at termination.

All animals were subjected to necropsy and detailed *post mortem* examination of major organs and tissues was performed. Selected organs were weighed and samples of all major organs / tissues and all gross lesions

[16] batch n° 9809; purity = 98.7%

were preserved[17]. All tissues from the animals treated at 0 or 1000 mg/kg bw/day were examined by light microscopy. Gross lesions and treated skin samples were examined from all animals.

There were no **deaths**, nor treatment-related **clinical signs**, nor dermal reactions at the application sites (all dose levels scored zero for erythema), nor ophthalmological findings in either sex at any dose level. There were no treatment-related effects in either sex at any dose level on **bw** gain and **food consumption**, although the female from 150mg/kg bw/d group exhibited significantly higher food consumption than the controls during w3 and w4.

There were no significant changes in the **hematological** parameters in either sex at any dose level, except in the 150mg/kg bw/d group in which males exhibited higher MCHC values and females showed lower Ht values; such changes are to be considered incidental in the absence of a dose-response pattern.

There were no treatment-related effects on the **clinical chemistry** parameters: some significant changes were seen among group but only in one sex and/or without dose-dependency (lower plasma AST activity in 20 and 1000 mg/kg bw/d males, but higher AST activity in 20 mg/kg bw/d females; higher plasma calcium ion concentration in 20 mg/kg bw/d males; and higher CK in 20 mg/kg bw/d females).

There were no treatment-related **gross lesions** at necropsy and no effects on organ weights in either sex at any dose level.

There were no **histopathological changes** attributable to the dermal application of flonicamid technical at dose levels up to 1000 mg/kg bw/d.

Table 35. hematological and clinico chemical parameters at selected intervals

Group	**0 mg/kg /d**		**20 mg/kg /d**		**150 mg/kg /d**		**1000 mg/kg /d**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
Hematological parameters								
RBC (10^6/mm^3)	6.8±0.3	7.2±0.2	6.7±0.5	7.1±0.4	6.8±0.3	6.7±0.6	6.7±0.3	6.8±0.3
Ht (%)	38.1±1.4	42.2±1.3	38.1±2.8	41.9±1.7	37.7±1.9	39.7±2.4*	38.3±2.1	40.2±0.8
MCH(pg)	21.7±0.8	22.3±0.6	22.1±0.8	22.4±0.9	22.1±0.9	22.9±1.4	22.3±0.5	23.0±1.0
MCHC (g/dL)	38.8±0.8	38.2±0.4	39.1±0.6	37.9±0.8	39.6±0.4*	38.6±0.8	39.1±0.6	38.7±0.8
Clinical chemistry parameters								
AST (U/L)	153.2 ±41.2	114.5 ±23.5	11.6 ±26.5*	142.1 ±32.5*	112.8 ±28.9	119.2 ±19.3	119.3 ±17.7*	114.9 ±18.4
Ca (mg/dL)	9.7±0.2	9.3±0.2	10.1±0.3*	9.4±0.2	9.7±0.3	9.4±0.4	10.0±0.4	9.3±0.3

[17] normal and treated skin, adrenals*, brain* (cerebrum, cerebellum, medulla/pons), bone (femur), bone marrow (sternum), esophagus, eyes, duodenum, jejunum, ileum, cecum, colon, rectum, heart* with thoracic aorta, kidneys*, liver*, lungs, lymph nodes (cervical and mesenteric), mammary gland, nose, pancreas, pituitary gland, testes*, epididymes*, prostate*, ovaries*, uterus*, cervix, vagina, salivary glands (both mandibular), skeletal muscles with peripheral nerve (sciatic), spinal cord (cervical, midthoracic & lumbar), spleen*, stomach, thymus*, thyroid with parathyroid glands*, trachea, urinary bladder, all gross lesions. [* weighed]

CK (U/L)	924.7 ±399	437.7 ±171	547.8 ±356.1	877.1 ±398.8*	670.6 ±447.8	511.2 ±224	653.6 ±300.1	448.1 ±144.6
* $p < 0.05$; ** $p < 0.01$								

The NOEL was in excess of 1000 mg/kg bw/day, based on the absence of treatment-related effects at the highest dose level.

4.7.1.4 *Repeated dose toxicity: other routes*

No data available.

4.7.1.5 *Human information*

No data available.

4.7.1.6 *Other relevant information*

No data available.

4.7.1.7 *Summary and discussion of repeated dose toxicity*

Oral route:

The short term effects of flonicamid after oral administration were studied in rats (28 and 90 days), in dogs (28/35 and 90 days, 1 year) and in mice (90 days). The target organs were the liver (rats, mice), the kidney (rats) and the haematopoietic system (anaemia in mice).
In the rat studies, the adverse effects on the kidneys were considered as mediated by the male rat specific protein, α-2 μ-globulin, and were not regarded as relevant to humans. Therefore, the short term NOAEL in rats was 60 mg/kg bw/day from the 90-day study. In the dog studies, the relevant NOAEL was 8 mg/kg bw/day, based on reduced body weight gain, reduced thymus weight in males (90-d), and mild anaemia (1-y). In the mouse study, the NOAEL was 15.3 mg/kg bw/day based on hepatocellular hypertrophy and splenic extramedullary haematopoiesis (related to anaemia).

Inhalation exposure:

No data.

Dermal exposure:

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

4.7.1.8 *Summary and discussion of repeated dose toxicity findings relevant for classification according to DSD*

The findings in the repeated dose studies are not deemed relevant for classification; no classification for repeated dose toxicity proposed according to DSD.

4.7.1.9 *Comparison with criteria of repeated dose toxicity findings relevant for classification according to DSD*

Rationale for classification as R48/22 (Danger of serious damage to health by prolonged exposure):

The 67/548/EEC criteria for classification as R48/22 are as follow:
Substances are classified as R48/22 when significant serious damage (clear functional disturbance or morphological change which has toxicological significance), is likely to be 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.

The findings in the repeated dose studies are not deemed relevant for classification; no classification for repeated dose toxicity proposed according to DSD.

4.7.1.10 *Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification according to DSD*

No classification is considered necessary for repeated exposure.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The same repeated dose toxicity findings are considered relevant for classification as STOT RE. See 4.7.1.7.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Rationale for classification as STOT-RE:

The CLP criteria for classification as STOT-RE are as follow:
“Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which 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.

The findings in the repeated dose studies are not deemed relevant for classification; no classification for repeated dose toxicity proposed according to CLP Regulation.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

No classification is considered necessary for repeated exposure.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 36. Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Point mutation assay *S. typhimurium (TA98; TA100; TA1535 & TA 1537) & E. coli WP2 uvra*	negative	-	Matsumoto (2002a)

OECD 471			
***In vitro* cytogenetics test** Chinese hamster CHL cells 6, 24 and 48-h exposures OECD 473	negative	-	Matsumoto (2002b)
Mouse lymphoma assay L5178Y $TK^{+/-}$ cells OECD 476	negative	-	Matsumoto (2002c)
Micronucleus test Mouse 24-hour sacrifice OECD 474	negative	-	Matsumoto (2002d)
***In vivo* UDS assay** Rat hepatocytes; 2 and 14-h expression times OECD 486	negative	-	Mehmood (2003)
Comet assay Mouse colon, liver, lung 3 and 24-h expression times	negative	No applicable EU guideline ; study performed according to the guideline established at the International Workshop on Genotoxicity Test Procedures, Washington, D.C., March 25 - 26, 1999	Sasaki (2002)

4.9.1 Non-human information

4.9.1.1 *In vitro data*

Reference: Matsumoto K. (2002a)
GLP: Yes
Guidelines: OECD 471; US-EPA OPPTS 870.5100; JMAFF, 12 NouSan no. 8147

flonicamid technical[18] dissolved in DMSO was tested in a plate incorporation assay including a pre-incubation step, using 5 bacterial tester strains: TA98; TA100; TA1535 and TA1537 of of *S. typhimurium* and WP2 *uvrA* of *E. coli* with and without metabolic activation[19].

Doses were selected from a preliminary toxicity test performed on the 5 tester strains using 7 doses (0; 1.2; 4.9; 19.5; 78.1; 313; 1250 and 5000 µg/plate) of test material with and without metabolic activation (single plate was used for each dose level and duplicate plates were used for solvent control and positive controls). The doses of flonicamid used on all 5 tester strain were 0; 61.7; 185; 556; 1667 and 5000 µg/plate and 0; 313; 625; 1250; 2500 and 5000 µg/plate in the 1st experiment and the the 2nd experiment, respectively; both experiments were conducted with and without metabolic activation using triplicate plates. Solvent and positive controls were also prepared in triplicate.

[18] Batch n° 9809; purity = 98.7%

[19] S9 mix prepared from 9000 supernatant of liver homogenate of SD rats treated with phenobarbital (30 mg/kg bw on d-1 and 60 mg/kg bw on d2, d3 and d4 and a single dose of 80 mg/kg bw of 5,6-benzoflavone on d3.

The components of the preincubation assay (0.1mL tester bacterial suspension, 0.1mL test substance solution and 0.5mL S9 mix or buffer) were mixed and incubated for 20 min. at 37°C. After incubation, 2mL molten amino acid-supplemented molten soft agar was added and all plates were then incubated for 48 h at 37°C. After incubation, the plates were enumerated by automatic colony counting. Cytotoxicity was determined by microscopic examination of the background lawn and signs of precipitation were checked visually. No statistical analysis of the results was performed since the test concentrations were clearly negative and the positive controls were clearly positive.

Table 37. Positive control substances and dose levels

Strain	**Without metabolic activation (µg/plate):**		**With metabolic activation (µg/plate):**	
TA 98	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide* (AF-2)	0.1	2-aminoanthracene* (2AA)	0.5
TA 100	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide* (AF-2)	0.01	2-aminoanthracene*	1.0
TA 1535	Sodium azide** (SA)	0.5	2-aminoanthracene*	2.0
TA 1537	9-aminoacridine hydrochloride* (9-AA)	80	2-aminoanthracene*	2.0
WP2 uvrA	2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide* (AF-2)	0.01	2-aminoanthracene*	10
* in DMSO; ** in sterile pure water				

In the **preliminary dose range-finding test**, precipitation of the test substance and cytotoxicity did not occur in any strain at any dose level with or without metabolic activation; therefore, 5000 µg/plate was selected as the highest dose level for both main assays. In the absence of metabolic activation, the mean number of revertant colonies of TA1535 at 4.9µg/plate was twice the solvent control value, but this excess was considered non specific because no dose-response relationship was not evident.

In both **main experiments**, no precipitation of the test substance and no cytotoxicity occurred in any strain at any dose level with or without metabolic activation. None of the 5 strains showed a doubling or more, relative to the solvent controls, of the number of revertant colonies/plate with or without metabolic activation in either assay. The positive controls, AF-2, SA, 9-AA and 2-AA, all produced marked (3-fold or higher) increases in the mutant colony counts over those of the concurrent solvent controls, demonstrating the sensitivity of the tester strains and the activity of the S9 liver fraction. The validity of the study is further confirmed by the fact that all cultures of the tester strains, the test solutions and S9 mix were free of contamination by other bacteria and the numbers of revertant colonies in the solvent controls for all strains were within the laboratory historical control ranges.

Table 38. Number of revertant colonies/plate - dose range-finding test and main tests

Concentration	Number of revertant colonies/plate									
dose range-finding test										
(µg/plate)	TA 100		TA 1535		WP2 uvrA		TA 98		TA 1537	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9

0 (solvent)	105	104	5	8	16	21	16	18	7	13
1.2	108	115	7	5	18	19	10	26	5	14
4.9	90	96	10	8	23	17	20	25	3	12
19.5	101	93	5	9	17	29	16	20	4	10
78.1	115	113	6	10	17	20	21	30	4	10
313	109	91	6	11	25	19	14	29	4	8
1250	106	81	8	6	19	28	19	25	7	8
5000	124	113	5	9	15	14	17	15	6	11
Positive control	535	555	470	120	207	141	508	165	466	65
Experiment 1 (preincubation)										
0 (solvent)	117	126	6	7	18	27	14	24	6	12
61.7	94	126	7	10	15	21	17	27	6	13
185	102	119	11	7	20	26	18	27	5	13
556	117	103	8	8	20	20	14	20	7	13
1667	116	119	9	7	22	24	16	22	8	12
5000	122	113	5	6	15	22	15	21	7	13
Experiment 2 (preincubation)										
0 (solvent)	103	111	6	7	18	20	15	24	6	11
61.7	104	104	8	6	20	20	15	22	4	12
185	134	121	5	6	22	22	13	22	10	13
556	112	109	9	8	23	17	15	25	4	9
1667	117	102	8	8	21	18	18	20	3	12
5000	121	116	6	8	17	15	21	22	7	7
Positive control	467	778	612	132	221	216	574	188	664	83

Flonicamid technical does not induce gene mutations in the strains of *S. typhimurium* and *E. coli* used in the study at concentrations up to 5000 µg/plate.

Reference: Matsumoto K. (2002b)
GLP: Yes
Guidelines: OECD 473; US-EPA OPPTS 870.5375; JMAFF, 12 NouSan no. 8147 (2000).

Flonicamid[20] technical was tested in an *in vitro* chromosome aberration test in Chinese hamster CHL cells (average generation time ~15 h, modal chromosome n=25) with and without metabolic activation (S9

[20] batch n° 9809 ; purity = 98.7%

fraction prepared from the livers of male SD strain rats previously treated for 4 days with phenobarbital and a single dose of 5,6-benzoflavone). Since test substance was hardly soluble in water, DMSO (> 99%) was used as solvent.

Cytotoxicity was determined in a preliminary growth inhibition test in which cell cultures were exposed to 0 (DMSO 1%); 8.95; 17.9; 35.8; 71.6; 143; 246; 573; 1145 and 2290 µg/mL (highest achievable concentration in DMSO) of test substance; duplicate cultures were used for each concentration of test substance and solvent control. A short term assay was performed with and without metabolic activation using a 6h exposure period and a harvest time of 24h; an additional continuous treatment assay was performed without metabolic activation using a 24h or 48h exposure period. The staining (Giemsa) intensities of the slide preparations were measured optically and the relative cell growth of each culture was evaluated by comparison with the staining densities of concurrent solvent controls.

Two independent *in vitro* assays were performed on similar, duplicate CHL cell cultures (seeded at 1 x 10^5 cells/5mL) at concentrations based on the results of the preliminary study (0; 573; 1145 and 2290 µg/mL; 2290mg/L ≡ 10mM). Short term experiments were carried out with and without metabolic activation using a 6 h exposure period and cells were harvested at 24h; continuous experiments were performed without metabolic activation using a 24h or a 48h exposure period. Additional duplicate cultures at each concentration were prepared for an evaluation of cytotoxicity based on relative cell growth. In the first assays, concurrent positive control substances in DMSO were Mitomycin C (0.1µg/mL) without activation and benzo(a)pyrene (40µg/mL) with activation; in the second assays positive control was Mitomycin C used at final concentrations of 0.1 and 0.05µg/mL for 24h and 48 h continuous exposure, respectively.

Table 39. study protocol

Assay	**Exposure (h)**	**Harvest time (h)**	**+ / - S9**	**Exposure concentrations (µg/mL)**	**Positive controls**
1st	6	24	-	0 (solvent), 573, 1145, 2290	MMC: 0.1 µg/mL
	6	24	+	0 (solvent), 573, 1145, 2290	BaP: 40 µg/mL
2nd	24	24	-	0 (solvent), 573, 1145, 2290	MMC: 0.1 µg/mL
	48	48	-	0 (solvent), 573, 1145, 2290	MMC: 0.05 µg/mL
MMC: Mitomycin C ; BaP: benzo (a) pyrene					

Whenever possible, 100 well spread metaphase figures/culture from two cultures/group were scored blind for specific and non-specific structural aberrations. Diploid metaphases with a typical karyotype were analysed. The chromosome number of polyploid cells (≥ 37 chromosomes) was recorded but not used in the assessment of clastogenicity. Structural aberrations and the numbers of polyploid metaphases were analysed using the chi^2-test.

Preliminary growth inhibition test: flonicamid technical did not inhibit the cell growth up to 50% of the solvent controls at any concentration for the short term and continuous assays. Precipitation of the test substance did not occur in any culture and thus the highest concentration for both the short-term and continuous exposures used in the main assays was 2290µg/mL (10mM).

Main cytogenetic assays:

- Short term assays: in cultures exposed to flonicamid technical for 6 h, no cytotoxicity or test substance precipitation occurred at any dose level. The frequencies of metaphases with structural chromosome

aberrations excluding gaps were in the range 0 - 1.0% at all concentrations both with and without metabolic activation. There were no statistically significant increases in the incidences of aberrations in the test groups when compared with the solvent control group and no statistically significant increases in the frequencies of polyploid metaphases (range 0 - 1.0%) at any exposure concentration.

- Continuous exposure assays: although the relative cell growth of cultures exposed for 24 and 48h were reduced, the reductions amounted to only 24 and 27%, respectively, at the highest dose level used. Continuous exposure for 24 or 48 h did not induce any statistically significant increases in the incidences of chromosome aberrations excluding gaps (range 0 - 1.0%) or polyploid metaphases (range 0 - 0.5%) at any dose level when compared with the solvent control group.

- The frequencies of chromosome aberrations in the solvent control groups were within the historical laboratory control data, and the positive control groups produced statistically significant ($p < 0.001$) aberration incidences of 34.0 - 41.5%. The assays are then considered as valid.

Table 40. Cell growth – (preliminary growth inhibition test and main assays)

Concentration (µg/mL)	**Preliminary assays: relative cell growth (%) for exposures of:**			
	6 hours		**24 hours**	**48 hours**
	- S9	+ S9	- S9	- S9
0 (solvent)	100	100	100	100
8.95	100	102	100	100
17.9	88	105	100	101
35.8	98	107	98	100
71.6	100	112	103	97
143	97	98	103	98
286	102	100	102	94
573	107	103	100	89
1145	105	107	92	83
2290	72	106	74	72
	Main assays: mean relative cell growth (%)			
	6 hours	**24 hours**	**48 hours**	
	- S9	+ S9	- S9	- S9
0 (solvent)	100	100	100	100
573	102	101	91	91
1145	103	102	85	83
2290	104	102	76	73
Positive control	109	88	105	102

Table 41. Numbers of cells with aberrations

time (h)	+ / - S9	Dose (µg/mL)	No. cells scored	No. with Poly ploidy	Number of cells with structural chromosome aberrations								
					Gaps	Chromatid-type		Chromosome-type		Fragment	Other[a]	Totals	
					(g)	ctb	cte	cab	cse	(frg)		+g	-g
Assay 1													
6	-	0	200	1	0	0	0	0	0	0	0	0	0
		573	200	0	0	0	0	0	0	0	0	0	0
		1145	200	0	2	0	0	0	0	0	0	2	0
		2290	200	1	1	0	0	0	0	0	0	1	0
		PC	200	0	7	25	54	1	1	0	0	74	70*
6	+	0 (solvent)	200	0	2	0	0	0	0	0	0	2	0
		573	200	2	2	2	2	0	0	0	0	3	2
		1145	200	1	0	0	0	0	0	0	0	0	0
		2290	200	0	3	0	1	0	0	0	0	4	1
		PC	200	1	4	11	62	2	1	0	0	70	68*
Assay 2													
24	-	0 (solvent)	200	1	1	0	0	0	0	0	0	1	0
		573	200	0	1	0	0	0	0	0	0	1	0
		1145	200	1	0	0	2	0	0	0	0	2	2
		2290	200	0	2	1	0	1	0	0	0	4	2
		PC	200	0	11	22	73	6	0	0	0	89	83*
48	-	0 (solvent)	200	0	0	0	1	1	0	0	0	1	1
		573	200	0	1	1	1	0	0	0	0	3	2
		1145	200	0	1	1	0	0	1	0	0	2	2
		2290	200	1	0	1	1	0	0	0	0	2	2
		PC	200	0	4	23	67	5	0	3	1	80	80*

G: gap; ctb: chromatid breaks; cte: chromatid exchange; cab: chromosom braek; cse: chromosome exchange;
[a] includes multiple aberrations; PC positive control; * $p < 0.001$; +g including gaps; -g excluding gaps

Flonicamid technical is not clastogenic to Chinese hamster CHL cells *in vitro* at concentrations up to 2290µg/mL (≡ 10mM) with and without metabolic activation.

Reference: Matsumoto K. (2002c)
GLP: Yes
Guidelines: OECD 476; US-EPA OPPTS 870.5300

IKI-200[21] technical in DMSO was tested for its potential to induce mutations at TK locus in mouse lymphoma L5178Y $TK^{+/-}$ cells (doubling time = approx. 11 h).

A preliminary cytotoxicity test was carried out with flonicamid technical at 0 (solvent: DMSO 1% in culture medium), 8.95; 17.9; 35.8; 71.6; 143; 286; 573; 1145 and 2290 µg/mL (highest achievable concentration in DMSO) with and without metabolic activation (S9 fraction prepared from the livers of male SD strain rats previously treated for 4 days with phenobarbital and a single dose of 5,6-benzoflavone); a single cell culture was exposed for 3h for each concentration of test substance and for solvent control. After incubation, the number of micro-wells containing viable colonies was counted with the unaided eye using background illumination.

The definitive study was carried out in 2 independent assays with the micro-well method, using duplicate cultures for all test and control treatments, both with and without metabolic activation: 5 concentrations separated by a factor of 3 [0 (solvent ; DMSO 1%); 28.3; 84.8; 254; 763 and 2290µg/mL] were used in the first assay and 5 concentrations separated by a factor of 2 [0 (solvent); 143; 286; 573; 1145 and 2290µg/mL] were used in the second assay. In both assays Cyclophosphamide (3µg/mL in physiological saline) and methyl-methane-sulfonate (10µg/mL in physiological saline) were used as positive control substances in the presence and the absence of metabolic activation, respectively. Approx. 10mL aliquots of cell suspension (1 x 10^6cells/mL) were incubated at 37°C for 3h of treatment with test substance or vehicle or positive controls. A sample of each adjusted culture (2 x 10^5cells/mL) was dispensed in duplicate to microtiter plates and incubated for 12 days, to assess cell survival. The remaining adjusted cultures were incubated at 37°C for a further 2 days expression time. After expression, a sample of each culture was incubated for 11 days for assessment of cell viability. The remainder of the cultures was diluted to 1 x 10^4cells/mL and 3µg/mL of trifluorothymidine (TFT) was added and each TFT-treated culture was dispensed in triplicate to microtiter plates and incubated for 12 days for determination of the mutant frequency. The numbers of positive wells containing viable colonies were counted for all survival and viability plates. Colonies on the mutant frequency plates were classified as large (> ¼ well diameter) or small (< ¼ well diameter). The numbers of wells containing only large, only small and both large and small colonies were counted. Plating efficiency, relative survival, relative total growth and mutant frequency were calculated. Mutant frequencies of test cultures were statistically analysed.

Preliminary cytotoxicity test: The relative survival of treated cultures was within the ranges 77 – 158% and 84 - 147% without and with metabolic activation, respectively, indicating a low level of cytotoxicity at concentrations up to 10mM flonicamid technical. No precipitation of test substance was observed at any dose level.

Main assays: Minimal or no cytotoxicity was observed in the main assays at concentrations up to and including 2290µg/mL. The relative survival and relative total growth at 2290µg/mL were in the ranges 89 - 102% for both main assays with and without metabolic activation. Neither of the indices of cytotoxicity were significantly different from the solvent control group values. In the first assay, the mutant frequencies of the test cultures were in the range 119.0 - 148.4 x 10^{-6} and 40.0 - 85.5 x 10^{-6}, without and with metabolic activation, respectively, compared to solvent control values of 146.4 x 10^{-6} and 60.3 x 10^{-6}, respectively; in the second assay, the mutant frequencies of the test cultures were in the range 71.6 - 101.2 x 10^{-6} and 85.3 - 119.4 x 10^{-6}, without and with metabolic activation, respectively, compared to solvent control values of 90.8 x 10^{-6} and 115.2 x 10^{-6}, respectively. Consequently, there were no statistically significant differences in mutant frequencies between test and solvent control cultures both assay. The proportions of small colonies in all test cultures, in both assays with and without metabolic activation, were comparable to, and not

[21] batch n° 9809 ; purity : 98.7%

significantly different from, the solvent control cultures. The positive control substances, MMS and CP, produced marked increases in the mutant frequencies in both main assays. All other criteria were achieved for a valid assay (absolute plating efficiencies of the solvent controls between 0.6 and 1.4 on the day of treatment and the mutant frequencies of the positive controls are statistically significantly higher than the solvent controls).

Table 43. Plating efficiency, relative cell survival, growth and mutant frequencies - main assays

Concentration (µg/mL)	Metabolic activation	Plating efficiency		Relative survival (day 0)	Relative total growth	Mutant frequency x10^{-6}
		Day 0	Day 2			
Assay 1						
0 (solvent)	- S9	0.82	0.94	100	100	146.4
28.3		0.78	0.90	95	96	142.9
84.8		1.08	0.90	133	119	148.4
254		0.78	0.85	95	97	127.8
763		0.80	0.90	97	98	125.1
2290		0.73	1.13	89	97	119.0
PC		0.78	0.89	95	84	849.6
0 (solvent)	+ S9	0.73	0.89	100	100	60.3
28.3		0.99	0.88	134	97	40.0
84.8		0.75	0.93	102	106	62.1
254		0.82	0.86	112	94	62.1
763		0.79	0.83	108	86	85.5
2290		0.74	0.88	101	87	69.4
PC		0.14	0.26	20	7	1110.1
Assay 2						
0 (solvent)	- S9	0.91	0.88	100	100	90.8
143		0.94	0.84	103	91	78.5
286		0.83	0.99	91	113	71.6
573		0.97	1.02	107	136	76.8
1145		0.91	1.23	99	134	75.9
2290		0.82	1.19	90	117	101.2
PC		0.91	0.95	100	114	587.3
0 (solvent)	+ S9	1.04	0.74	100	100	115.2
143		1.03	0.90	99	123	85.3

286	1.14	0.80	109	138	107.0
573	1.08	0.82	103	132	100.2
1145	1.03	0.77	99	130	119.4
2290	1.06	0.81	102	146	115.7
PC	0.51	0.45	49	42	1590.9

All mutant frequencies of positive control groups statistically significant, but significance levels not reported.

Flonicamid technical does not produce gene mutation at the TK locus in L5178Y mouse lymphoma cells.

4.9.1.2 *In vivo data*

Reference: Matsumoto K. (2002d)
GLP: Yes
Guidelines: OECD 474; US-EPA OPPTS 870.5395; JMAFF 12 NouSan, n° 8147

The *in vivo* micronucleus assay was conducted on groups of 10 (5/sex) ICR mice (Crj:CD-1 strain; 7 w old at treatment; mean bw = 36.2 g for males and 28.2 g for females). The dose levels for the main study were selected from a range-finding toxicity study in which groups of 6 (3/sex) mice (mean bw = 32.0 g for males and 25.5 g for females) were given by gavage 2 doses of 250; 500; 1000 and 2000 mg/kg of flonicamid[22] technical suspended in 0.5% aqueous carboxymethylcellulose at 24h interval; mice were observed for clinical signs during 24 h after the 2nd administration.

The definitive study was carried out on 4 groups of 10 (5/sex) ICR mice which were treated twice (24-h interval) by gavage, with 10mL/kg flonicamid technical suspended in 0.5% aqueous carboxymethylcellulose at dose levels of 0 (vehicle); 250; 500 and 1000mg/kg bw/d (males) and 0; 125; 250 and 500mg/kg bw/d (females). A positive control group of 5mice /sex was given a single administration by gavage of 10mg/kg bw of mitomycin C in physiological saline. All test, vehicle control and positive control animals were killed 24 h after the last dose and 2 bone marrow smears per animal were prepared from the femurs of each mouse. For each mouse, 2000 polychromatic erythrocytes (PCE) were scored for the presence of micronucleated cells. In addition the number of polychromatic erythrocytes (PCE) per 1000 erythrocytes was also determined. The numbers of micronucleated PCEs in the test and positive control groups were analysed statistically.

Results

- **Dose range finding experiment**: There were 4 deaths (1 male and 3 females) at 2000 mg/kg bw/d, and 2 and 1 females died at 1000 and 500 mg/kg bw/d, respectively. Clinical signs in decedents included decreased spontaneous activity, piloerection, tremors, and stupor. The maximum tolerated dose levels in males and females were considered to be 1000 and 500 mg/kg bw , respectively, and were selected as the dose level for the main study.

- **Micronucleus assay**: No deaths nor clinical signs occurred during the test period in both sexes at any dose level. All animals were killed according to schedule. There were no statistically significant increases in the incidences of micronucleated polychromatic erythrocytes in any of the flonicamid technical treated groups of either sex. In contrast, the incidences in both males and females treated with the positive control mitomycin C were significantly higher than the solvent control values. The mean PCE/NCE ratios were unaffected by treatment at all dose levels of flonicamid technical and none was

[22] batch n° 9809 ; purity : 98.7%

significantly different from the control values. In the positive control group, the PCE/NCE ratios were slightly lower than the vehicle control values, but not significantly different. The incidences of micronucleated polychromatic erythrocytes in the vehicle and positive control groups fulfilled the criteria for a valid assay (the mean frequency of micronucleated PCEs for vehicle and positive control groups are less than 0.3% and ≥ 2.0%, respectively).

Table 44. Group mean PCE/NCE ratios and incidences and ranges for micronucleated PCEs

Sex	Test substance	Dose level (mg/kg/d)	N° of mice	Mean MNPCE (% PCE)		Mean PCE (% PCE+NCE)	
				Mean	Range	Mean	Range
Male	Vehicle	0	5	0.19 ± 0.14	0.00 - 0.35	56.3 ± 5.2	49.1 - 61.4
	flonicamid	250	5	0.19 ± 0.07	0.10 - 0.25	53.8 ± 7.0	44.9 - 61.0
	flonicamid	500	5	0.11 ± 0.08	0.05 - 0.25	57.1 ± 7.6	49.2 - 67.1
	flonicamid	1000	5	0.17 ± 0.06	0.10 - 0.25	56.3 ± 3.4	52.6 - 60.9
	MMC	10	5	5.49 ± 2.34***	2.00 - 7.80	45.0 ± 8.6	33.0 - 57.0
Female	Vehicle	0	5	0.09 ± 0.04	0.05 - 0.15	58.2 ± 7.9	45.1 - 66.0
	flonicamid	125	5	0.12 ± 0.12	0.00 - 0.25	59.0 ± 8.2	46.3 - 66.2
	flonicamid	250	5	0.13 ± 0.12	0.00 - 0.30	60.1 ± 6.3	51.3 - 68.1
	flonicamid	500	5	0.16 ± 0.13	0.05 - 0.30	62.6 ± 4.3	56.2 - 67.6
	MMC	10	5	3.25 ± 1.29***	1.90 - 4.75	55.6 ± 2.5	52.6 - 58.5

MNPCE micronucleated polychromatic erythrocyte; PCE polychromatic erythrocyte; NCE normochromatic erythrocyte;
*** $p < 0.001$

Flonicamid technical is not clastogenic at dose levels approaching the MTD in the *in vivo* micronucleus assay in ICR mice.

Reference: Mehmood Z. (2003)
GLP: Yes
Guidelines: 2003/32/EC Annex 4G-B.39; OECD 486; US-EPA OPPTS 870.5550

DNA repair was assessed in hepatocytes of 5 males Sprague-Dawley-derived rats (6 w old; bw = 172-213g) which were given by gavage a single dose of 0 (vehicle), 600 or 2000 mg/kg bw flonicamid[23] technical suspended in 1% (w/v) aqueous methylcellulose[24] (dose volume = 10 mL/kg bw) and then sacrificed 2h and 14h after dosing (the 2 expression times were used to allow for variations in the rate of absorption, metabolism and accumulation of DNA damage).

[23] batch n° 9809 ; purity = 98.7%

[24] analysis of stability and homogeneity of test substance in vehicle and of achieved concentrations were not performed

Dose levels were selected from the results of a preliminary toxixicty test in which groups of 4 of 4 male rats were given by gavage, a single dose of 500; 1000; 1500 or 2000mg/kg flonicamid technical.

Concurrent positive control groups of 3 rats were similarly given by gavage a single dose of 4mg/kg bw dimethylnitrosamine in water (DMN) and killed after a 2h expression time or 50mg/kg bw 2-acetylaminofluorene (2-AAF) in 1% (w/v) methylcellulose and killed after a 14h expression time .

After sacrifice, perfusions were done to deplete the liver of Ca^{++} ions and to reduce cellular adhesion and to isolate hepatocytes with collagenase; hepatocytes suspensions were treated using procedures to remove as much debris as possible. Prior to the establishment of the cultures, a viable cell count was performed by trypan blue dye exclusion. Cells from 4 rats per treated and vehicle control group, and from 2 rats per positive control group, were selected for plating (suspensions of 0.2 x 10^6 cells/mL). Twelve replicate cultures/rat were initiated in multi-well culture dishes and incubated for 90 min. at 37°C to allow attachment; each culture was exposed to 10µCi/mL (methyl-^{3}H)-thymidine (specific activity 87Ci/mmole) for 4h. After labelling, the medium was replaced with medium containing unlabelled thymidine and the cultures incubated for a further 20h. Autoradiographs were prepared from 6 cultures/rat. Grain counting on the randomized and encoded slides was performed blindly by microscopy using a dedicated image analysis system via a solid-state video camera. Prior to grain counting, the slides were examined for signs of toxicity (pyknosis, reduced levels of labelling). Fifty hepatocytes/slide, 3 slides/rat, from several widely separated, randomly chosen fields were counted for the vehicle control and flonicamid-treated groups. Since the positive control substances produced a strong positive response, only 75 cells/rat were counted. Only results from cells not in S-phase with normal morphology and without staining artifacts were recorded. The number of silver grains overlying the nucleus in each cell was estimated using the image analysis system, then the number of grains in an equivalent and most heavily grained adjacent area of cytoplasm was estimated. The cytoplasmic grain count was subtracted from the gross nuclear grain count (GNG) to give the net nuclear grain count (NNG). Mean grain counts were calculated for each slide examined. For slides showing a strong response (i.e. mean NNG > 10) only 25 cells were examined. The number of cells in repair, with a NNG of ≥ 5, was also recorded.

Preliminary toxicity test: No deaths occurred at any dose level of IKI-200, but clinical signs were seen in all groups including underactivity, overactivity, head shaking, excessive grooming, flattened and hunched posture, abnormal gait, fast and irregular breathing, reduced body tone, prominent eyes, partially closed eyes, and reddened skin at 2000 mg/kg bw. The MTD flonicamid was considered to be approximately 2000 mg/kg bw.

Main study: All rats given at 600 or 2000mg/kg bw flonicamid technical survived to the scheduled sacrifice, but clinical signs occurred at both dose levels, including underactivity, flattened posture, abnormal gait, irregular breathing, reduced body tone and reddened skin, and additionally, prominent and partially closed eyes at 2000mg/kg bw. No deaths or clinical signs occurred in the vehicle and positive control groups.

There were no obvious signs of cytotoxicity in any of the slides in response to treatment with flonicamid at either dose level or to the positive control substances.

Hepatocytes from animals given flonicamid did not show any statistically significant increase in the gross or net nuclear grain counts at either dose level at either the 2 or 14-h expression times. In contrast, DMN and 2-AAF produced marked, statistically significant increases in both the gross and net nuclear grain counts. Both the vehicle and positive control groups showed net nuclear grain counts in the laboratory historical control ranges.

Table 45. Group mean grain counts

Expression	Treatment	Dose level	Group mean grain counts (and range of means[a]):

time (hrs)		(mg/kg)	Gross nuclear	Cytoplasmic	Net nuclear
2	Vehicle	0	12.2 (11.1 - 13.4)	16.5 (14.3 - 18.3)	- 4.3 (-2.7 - -6.6)
	flonicamid	600	12.2 (10.6 - 13.8)	16.7 (14.2 - 19.6)	- 4.5 (-3.1 - -5.9)
	flonicamid	2000	12.9 (11.4 - 13.8)	17.2 (16.2 - 18.7)	- 4.3 (-3.6 - -4.9)
	DMN	4	52.4* (51.2 / 53.5)	23.4 (23.1 / 23.7)	28.9* (27.4 / 30.4)
14	Vehicle	0	10.0 (9.1 - 11.2)	14.3 (11.9 - 16.8)	- 4.2 (-2.7 - -5.6)
	flonicamid	600	11.3 (9.5 - 13.3)	16.0 (13.8 - 17.7)	- 4.7 (-4.2 - -5.7)
	flonicamid	2000	11.2 (10.0 - 12.4)	16.2 (14.2 - 19.0)	- 5.0 (-3.5 - -6.6)
	2-AAF	50	44.7* (43.0 / 46.4)	24.8 (21.8 / 27.8)	19.9* (18.6 / 21.2)

[a] mean of 3 replicates; * $p < 0.001$

Flonicamid does not induce unscheduled DNA synthesis in rat hepatocytes following *in vivo* exposure.

Reference: Sasaki Y. F. (2002)
GLP: Yes
Guidelines: No applicable EU guideline ; study performed according to the guideline established at the International Workshop on Genotoxicity Test Procedures, Washington, D.C., March 25 - 26, 1999

Groups of 8 ddY male mice (8w old) were given a single oral dose of of 0 (vehicle); 375; 750 and 1500mg/kg bw of Flonicamid[25] technical suspended in 0.5% aqueous carboxymethylcellulose. Doses were selected from the results of a preliminary acute toxicity test in which 4 groups of 3 mice were given once orally 0; 1000; 1500 or 2000mg/kg bw of Flonicamid and were observed for 24 h. No deaths occurred at the 2 lower dose levels, but 2 mice died within 3 h post dosing and all mice died within 24 h post dosing at 2000 mg/kg bw. Consequently, the 1500mg/kg bw dose level was selected as the highest dose level for use in the main assay. A concurrent positive control group of 8 male mice were given a single oral dose of 160mg/kg bw methylmethanesulphonate (MMS).

All mice were observed following treatment for clinical signs of toxicity. Four mice/group were killed 3 and 24 h after dosing and the colon, liver and the lungs were removed at necropsy. The liver and lungs were separately homogenized, and the colon was opened, rinsed and the mucosa scraped into homogenisation buffer prior to homogenisation. Nuclei suspensions were prepared and electrophoresis was performed at 0°C in the dark for 15 min. Fifty nuclei per slide were examined at 200x magnification using a fluorescence microscope. The overall length and head diameter were measured for 50 nuclei per organ per mouse. The mean migration of 50 nuclei from each organ and animal was calculated as the difference between length and diameter. The differences in the means of 4 mice/group/time point between treated and vehicle controls were analysed statistically using one-way ANOVA followed by Dunnett's test. The induction of DNA damage was evaluated by an increase in the electrophoretic migration of DNA, but the assay acceptability and evaluation criteria were not specified in the report.

All mice survived to the scheduled sacrifice and there were no clinical signs in any group at any dose level.

The group mean migration of DNA from colon, liver and lung tissue from mice given Flonicamid technical, at all dose levels at both expression times, were comparable to the vehicle control values. There were no

[25] Batch n° 9809 ; purity=98.7%

statistically significant differences between the treated and vehicle control groups. In contrast, colon, liver and lung tissue DNA migration in MMS-treated animals was markedly and significantly greater than the vehicle control migration at both the 3 and 24-h expression times.

Table 46. Group mean colon, liver and lung DNA migration data

Expression time (h)	Treatment	Dose level (mg/kg)	Mean migration (μm) ± SEM		
			Colon	Liver	Lung
3	Vehicle	0	6.61 ± 0.92	1.37 ± 0.31	1.79 ± 1.39
	Flonicamid	375	7.02 ± 1.65	1.21 ± 0.64	2.17 ± 0.33
	Flonicamid	750	6.40 ± 0.67	1.43 ±0.40	2.18 ± 0.39
	Flonicamid	1500	7.26 ± 0.84	2.53 ± 0.78	0.72 ± 0.28
	MMS	160	38.0 ± 1.30*	38.4 ± 4.30*	35.7 ± 1.81*
24	Vehicle	0	6.60 ± 1.60	1.62 ± 0.59	1.60 ± 0.26
	Flonicamid	375	6.24 ± 1.67	2.00 ± 0.10	2.11 ± 0.63
	Flonicamid	750	8.05 ± 0.91	1.54 ± 0.23	2.16 ± 0.46
	Flonicamid	1500	7.82 ± 0.85	0.70 ± 0.27	1.85 ± 0.41
	MMS	160	38.5 ± 4.80*	39.5 ± 4.69*	23.7 ± 1.41*
* $p < 0.05$; vehicle= 0.5% CMC at 10 mL/kg bw					

Flonicamid technical does not induce DNA damage in mouse colon, liver and lung tissue DNA, as assessed by electrophoretic migration. Therefore, Flonicamid technical is considered not to be genotoxic in these tissues in the comet assay.

4.9.2 Human information

No data available

4.9.3 Other relevant information

No data available

4.9.4 Summary and discussion of mutagenicity

The potential of flonicamid technical 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, 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 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, inasmuch 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 that flonicamid does not exhibit primary genotoxic properties at the DNA, gene and chromosome levels of genetic organisation. Therefore no classification is required.

4.9.5 Comparison with criteria

1) Criteria for classification under Directive 67/548/EEC:

A substance shall be classified in category 3 for mutagenic endpoint if the substance causes concern for man owing to possible mutagenic effects. There would be evidence from appropriate mutagenicity studies, but this is insufficient to place the substance in category 2. Positive results are needed in assays showing mutagenic effects or other cellular interaction relevant to mutagenicity, in somatic cells in mammals *in vivo*. The latter especially would normally be supported by positive results from *in vitro* mutagenicity assays.
The following methods are considered as appropriate:

- In vivo somatic cell mutagenicity assays:
  - Bone marrow micronucleus test or metaphase analysis
  - Metaphase analysis of peripheral lymphocytes,
  - Mouse coat colour spot test
- In vivo somatic cell DNA interaction assays:
  - Test for SCEs in somatic cells,
  - Test for UDS in somatic cells,
  - Assay for the (covalent) binding of mutagen to somatic cell DNA,
  - Assay for DNA damage, e.g. by alkaline elution, in somatic cells.

2) Criteria in the CLP classification:

A substance shall be classified in category 2 for germ cell mutagenicity endpoint if the substance causes concern for humans owing to the possibility that they may induce heritable mutation in the germ cells of humans. This classification is based on:

- Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
  - Somatic cell mutagenicity tests in vivo, in mammals (mammalian bone marrow chromosome aberration test, mouse spot test or mammalian erythrocyte micronucleus test); or
  - Other in vivo somatic cell genotoxicity test (UDS or SCE assay) which are supported by positive results from in vitro mutagenicity assays (in vitro mammalian chromosome aberration test, in vitro mammalian cell gene mutation test or bacterial reverse mutation test).

3) 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 any criterion for classification.

4.9.6 Conclusions on classification and labelling

4.10 No classification is required for flonicamid under either Directive 67/548/EEC or the CLP Regulation.

Carcinogenicity

Table 47. Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
87/302/EC; US-EPA Guideline OPPTS 870.4300 **Dietary chronic toxicity and carcinogenicity Rat Wistar** 1.84; 3.68; 7.32 and 36.5mg/kg bw/d in males from the 50; 100; 200 and 1000 ppm groups, respectively and 8.92; 44.1 and 219mg/kg bw/d in females from the 200; 1000 and 5000 ppm groups, respectively	No carcinogenic effects α-2microglogulin nephropathy in males at 1000ppm Mild anemia (F) Liver: hypertrophy, ↑ weight, functional changes (M+F), Kidney: hyaline droplets, tubular vacuolation, chronic nephropathy, Accelerated age-related eye/muscle lesions in F at 52000ppm	-	Kuwahara (2002c)
OECD 451; US-EPA OPPTS 870.4200 **Dietary oncogenicity** **Mouse CD-1** 29, 88 and 261mg/kg bw/d in males and 38; 112 and 334mg/kg bw/d in females from the 250; 750 and 2250 ppm groups, respectively	↑ incidence of lung adenomas and carcinomas ↑ incidences of hyperplasia / hypertrophy in lung, hepatic hypertrophy, extramedullary hematopoiesis in spleen, and ↓ bone marrow cellularity	-	Ridder (2003a)
OECD 451; US-EPA OPPTS 870.4200 **78-w dietary oncogenicity** **Mouse CD-1** 0, 10, 25, 80, 250ppm 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	↑ incidences of lung epithelial hyperplasia/hypertrophy in both sexes at 250ppm ↑ incidence of pulmonary adenoma in males at 250ppm	A detailed weekly examination was performed only from w41 of the study; blood smears were not taken after 12 months; 78w blood smears were not examined for differential leucocyte count; adrenals, heart and reproductive organs were not weighed at necropsy; only liver, lung, spleen and femoral and sternal bone marrow from terminal kill animals were examined histopathologically. The deviations from the guideline are considered not to affect the validity of the study which was only designed to elucidate a NOAEL for a specific lesion identified in the previous oncogenicity study.	Nagaoka (2004)
Dietary cell proliferation	↑ cell proliferation in terminal		Nomura (2003a)

Method	Results	Remarks	Reference
(BrdU labelling) study **Male mouse CD-1** 0, 80, 250, 750, 2250ppm equivalent to 0; 12.3; 40.9; 129.6 and 339.3mg/kg bw/d	bronchiolar region of lung		
Dietary cell proliferation (BrdU labelling) study **Female mouse CD1 and rat Wistar** 0, 2250ppm (mouse) equivalent to 0, 374 mg/kg bw/d 0, 5000ppm (rat) equivalent to 0, 392 mg/kg bw/d	↑ cell proliferation in terminal bronchiolar region of lung in mice at 380mg/kg bw/day. No effect on cell proliferation in rats at 398mg/kg bw/day.	Comparative effect of flonicamid on BrdU uptake by lung epithelial cells in mice and rats	Nomura (2003b)
Dietary cell proliferation (BrdU labelling) study **Male mouse CD-1** 0, 2250ppm (0, 303 mg/kg bw/d)	↑ cell proliferation in terminal bronchiolar region of lung. flonicamid produces Clara cell hypertrophy/hyperplasia and morphological alterations. Both effects readily and fully reversible within 7 days.	Reversibility of flonicamid-induced effects on lung cell proliferation and specific effects on Clara cell morphology by EM	Nomura (2003c)
Dietary cell proliferation (BrdU labelling) study **Male mouse CD-1** 0, 2250ppm (0, 389 mg/kg bw/d)	↑ cell proliferation in terminal bronchiolar region of lung by flonicamid but no effect on cell proliferation by TFNG, TFNA, TFNA-AM	Effect of flonicamid and metabolites, TFNG, TFNA and TFNA-AM, on BrdU uptake by lung epithelial cells	Nomura (2003d)
Dietary cell proliferation (BrdU labelling) study Male mouse 0, 2250ppm (299; 316 and 306mg/kg bw/d for flonicamid, and for isoniazid, 298; 325 and 290mg/kg bw/d, for the CD-1, B6C3F1 and C57/6J strains, respectively)	↑ cell proliferation in terminal bronchiolar region of lung by flonicamid in CD-1 strain mice, but no effect on cell proliferation in B6C3F1 and C57/6J mouse strains. % of Clara cells similar in 3 mouse strains, but markedly < in the rat.	Effect of flonicamid on BrdU uptake by lung epithelial cells in 3 mouse strains, CD-1, B6C3F1 and C57/6J. Deterimnation of % Clara cells in target region of mouse lung, and comparison with the rat	Nomura (2003e)

4.10.1 Non-human information

4.10.1.1 *Carcinogenicity: oral*

The oral long-term toxicity of flonicamid was investigated via dietary administration in a combined chronic toxicity and carcinogenicity study in rats and oncogenicity studies in the CD-1 mouse. Based on the findings in the mouse study, a series of investigative studies was performed to elucidate the cause of an increased incidence of lung tumours.

Rat study

Reference: Kuwahara, 2002c
GLP: Yes
Guidelines: 87/302/EEC; US-EPA Guideline OPPTS 870.4300; JMAFF 59-Nousan-n° 4200

Groups of male and female Wistar rats (5-6 w old at start of dosing) were administered 0; 50 (males only); 100 (males only); 200; 1000 and 5000 ppm (females only) flonicamid technical in the diet for 104 w (main group: 52 rats/sex; initial bw ranges = 126-145 g in males and 116-140 g in females) or for 52w (satellite group 1: 14 rats/sex) or for 26w (satellite group 2: 10 rats/sex). Equivalence: 1.84; 3.68; 7.32 and 36.5mg/kg bw/d in males from the 50; 100; 200 and 1000 ppm groups, respectively and 8.92; 44.1 and 219mg/kg bw/d in females from the 200; 1000 and 5000 ppm groups, respectively.
The dose levels were selected from the results of 28-day range finding study and 90-day oral toxicity studies (Kuwahara, 2002a; 2002b) using dietary concentrations of flonicamid up to 5000 ppm in males and 10 000 ppm in females. In these studies, tubular basophilic changes and/or granular casts in dilated tubules in the kidneys were seen in males at concentrations ≥ 1000ppm; in the females, there were cytoplasmic vacuolation of the proximal renal tubular cells in all rats at 5000 ppm in the 90 days study and severe bw and food consumption reductions at 10 000 ppm in the 28 days study; these findings supported the selection of 1000 ppm and 5000 ppm as the top doses for chronic experiments on males and females, respectively.

Tests diets were prepared every 4w throughout the study period. The stability of flonicamid in 50 and 10 000ppm test diets after refrigerated storage for 35 d followed by storage at room temperature for 18 d was confirmed prior to the study. Homogeneity and achieved concentration analyses were performed on all formulations on 5 occasions i.e. at 1st preparations, every 6 months thereafter and at last preparation. In addition, samples were taken from tests diets including control diet of each batch after use and concentrations of test substance were determined every 6 months on samples of 2 – 3 randomly selected dose levels to ensure that proper storage was made.

All rats were observed for morbidity/mortality and clinical signs at least once daily; a detailed examination including palpation for masses was performed at least once a week. In addition, all rats of the main group and of satellite group 1 (52w) were examined weekly, within and outside the home cage, for abnormal clinical signs which were graded on a semi-quantitative subjective scale; functional examinations [motor activity, grip strength and sensorimotor responses (approach-, auditory-, touch- responses and aerial righting reflex)] were also carried out in random order on w49 on 10 rats/sex/dose level of the satellite group 1. Ophthalmoscopy was performed at w104 on all rats pre-dose and on all surviving control, 1000 ppm males and 5000 ppm females.

Bw were recorded pre-dose, weekly in w1- 13, every 4w thereafter and prior to necropsy. Food consumption was measured over a 3-d period weekly for w1 - w13 and every 4 w thereafter. Food efficiency was calculated for the main groups.

Urinalysis (specific gravity; glucose; bilirubin; ketones; occult blood; pH; proteins; urobilinogen; volume; appearance; sediment) and hematological (Ht; Hb; RBC; MCV; MCH; MCHC; PTL; WBC; differential leucocytes count; reticulocytes) and clinical chemistry (ALP; GOT; GPT; GGTP; CPK; creatinin; BUN; total proteins; albumin; globulins; A/G ratio; glucose; total cholesterol; trgilycerids; total bilirubin; calcium; inorganic phosphorous; sodium; potassium; chloride) investigations were performed on 10 animals/sex/group in w13, w26, w-52, w77/78 and w103/104.

The survivors were killed at the scheduled intervals and necropsied, as were rats found dead and those killed *in extremis*. Organ weights were recorded, a whole-body gross *post mortem* examination was performed and a full range[26] of tissues preserved from all rats except from rats of the satellite group 2 from which liver and kidneys only were preserved and from rats of the satellite group 1 from which tissues were preserved from

[26] Brain* (cerebrum, cerebellum, pons/medulla), spinal cord (cervical, thoracic and lumbar), sciatic nerve, pituitary gland, thymus, thyroid and parathyroid glands, adrenals*, spleen*, bone and bone marrow, lymph nodes (cervical and mesenteric), heart*, aorta, salivary glands (submandibular and sublingual) esophagus, stomach, liver*, pancreas, duodenum, jejunum, ileum, colon, rectum, head, pharynx, larynx, trachea, lung, kidneys*, urinary bladder, testes*, epididymes*, prostate, seminal vesicles, coagulating glands, ovaries*, uterus*, vagina, eyes, Harderian gland, skeletal muscle, skin, mammary gland, masses and other gross lesions.(* weighed)

only 10 rats/sex/dose level. Histopathological examination of tissues was performed on all tissues from all survivors treated at 0; 1000 or 5000 ppm designated to be killed after 52w or 104 w, and decedents of all treatment groups from all scheduled kills. Liver, lung, kidneys and all gross lesions from males treated at 50; 100 and 200 ppm, and females treated at 200 and 1000 ppm were also examined, together with liver and kidneys from all survivors killed at 26 w. In addition, the eyes and skeletal muscle of females treated at 200 and 1000 ppm and killed after 104w were examined.

Chemical analyses of test substance in diets: The stability of 50 and 10 000 ppm diet formulations was confirmed after refrigerated storage for 35 d followed by storage at room temperature for 18 d, at which time 90 and 93% initial concentrations, respectively, remained. All diets analysed for homogeneity showed a coefficient of variation of no more than 4.3% at every analysis and overall mean achieved concentrations were within the range 98 - 103% nominal concentrations for all diets, equivalent to 49; 98; 204; 1032 and 5075 ppm in the samples taken from the 50; 100; 200; 1000 and 2000 ppm diets, respectively. In addition, concentrations of test substance in samples of diets taken after use and determined for each dose level at 6 months intervals, were within 95 – 104 % of the target values showing that appropriate storage conditions were achieved.

Mortality: In the main study, increased mortality was seen in the 100; 200 and 1000 ppm males, the differences being statistically significant at w88, w103 and w104. Such excess did not appear to be dose-related and to be corroborated by specific lesions and should be considered as not related to treatment. Mortality in males at 50 ppm and in all female treated groups was comparable to control mortality. No premature deaths occurred at any dose level in either of the satellite groups.

Table 48. Cumulative mortality (main groups)

Table 49.	**Males**					**Females**			
Dose level (ppm)	**0**	**50**	**100**	**200**	**1000**	**0**	**200**	**1000**	**5000**
w1	0/52	0/52	0/52	0/52	0/52	0/52	0/52	0/52	0/52
w26	0/52	1/52	0/52	0/52	0/52	0/52	0/52	0/52	0/52
w52	1/52	1/52	3/52	0/52	0/52	0/52	1/52	0/52	0/52
w88	3/52	5/51	11/51*	5/52	12/52**	9/52	10/52	10/52	8/52
w104	9/52	11/51	24/51**	17/52	20/52*	20/52	20/52	22/52	21/52
* p< 0.05; ** p<0.01									

Clinical examinations:

In the main study, non specific or incidental changes were observed mainly in high dose rats: there were significantly increased incidences of red adhesive substance on the skin in females at 1000 and 5000 ppm ; there were also bradypnea and reduced spontaneous activity in males at 100 and 1000 ppm, but decreased activity was mostly observed in moribund rats and there were no changes on semi-quantitative open-field respiration and spontaneous activity in males at 1000 ppm; in addition no such changes were seen in the 200 ppm males. No treatment-related clinical signs occurred in satellite groups.
Semi-quantitative (scored) clinical examinations showed treatment-related effects in males at 1000 and females at 5000 ppm and included decreased frequency of rearing, especially in early phases of treatment period (w5 to w12 in females and w10 to w30 in males); significantly increased frequency of grooming were also occasionally seen (at 4 time points) in males at 1000 ppm and are not therefore likely to be related to treatment. Other significant fluctuations in the scored clinical observations occurred in both sexes at all dose levels, but only once of twice during the treatment period or the direction of the fluctuation was not constant and are therefore not considered to be related to treatment. There were no treatment-related effects at any dose level on the quantitative functional examinations, carried out at w49, on sensorimotor responses,

motor activity and grip strength All quantitative measurements in the treated groups were not significantly different from control values.

Table 50. Statistically significant changes in scored clinical signs (main group and satellite group 1)

Treatment week at which changes occurred								
		Males				**Females**		
Dose level (ppm)		**50**	**100**	**200**	**1000**	**200**	**1000**	**5000**
Main group								
Grooming	Increase	91	40	91	49, 65, 81, 89		32, 35	59
	decrease		73	73		67, 81	67, 81	67, 81
Rearing	Increase	10	9, 22, 26, 36, 47, 79			94	75	
	decrease	53, 57, 72, 86, 104	104	24, 45, 46, 68, 72	10, 12, 15, 16, 18, 20, 25, 27, 45, 53, 57, 59, 61, 104	55, 66, 71, 73, 77	66, 71, 5, 6, 8, 12, 71, 77, 82	
Satellite group 1								
Rearing	Increase		16,22, 23		8	38	38	
	decrease	41		7, 12, 14				

There were no treatment-related ophthalmological findings in males at 1000 ppm and females at 5000 ppm after 104 w of treatment

Bw and food consumption: Significant changes were only seen in high dose groups (changes at lower dose level were seen at only 1 or 2 time points): a treatment-related decrease in the overall bw gain occurred in males at 1000 ppm and females at 5000 ppm (8 and 11% lower than the controls values, respectively) and the group mean bw were reduced by 6 and 8%, respectively, after 104 w of treatment. Bw gain was also reduced from w9 to w28 in the 5000 ppm females of the satellite group 1. The food consumption of females at 5000 ppm was significantly reduced during the first 48 w of treatment, leading to a 6.3% decrease in overall food consumption throughout the treatment period. There were no effect of treatment in either sex on food consumption at lower dose levels. The average test substance intake was 1.84; 3.68; 7.32 and 36.5mg/kg bw/d in males from the 50; 100; 200 and 1000 ppm groups, respectively and 8.92; 44.1 and 219mg/kg bw/d in females from the 200; 1000 and 5000 ppm groups, respectively.

Table 51. Overall food consumption, bw and bw gain at w104, and test substance intake (main groups)

Parameter	**Mean value in males at (ppm):**					**Mean value in females at (ppm):**			
	0	**50**	**100**	**200**	**1000**	**0**	**200**	**1000**	**5000**
Mean bw (g)	605	604	591	593	566*	436	440	436	401
% of control value	100	100	98	98	94	100	101	100	92
Mean bw gain (g)	470	469	456	458	431	309	313	309	274
% of control value	100	100	97	97	92	100	101	100	89
Mean food consumption (g/d)	18.7	18.7	18.5	18.4	18.2	14.3	14.5	14.3	13.4

Average test substance intake (mg/kg bw/d)	-	1.84	3.68	7.32	36.5	-	8.92	44.1	219
* p < 0.05									

Laboratory investigations:

Urinalysis: Significant treatment related changes occurred only in high dose groups, including a decrease in specific gravity in females at 5000 ppm (in w13) and in males at 1000 ppm (in w13 and w26) and a increased urinary protein concentration in males at 1000 ppm (in w52). Other statistically significant differences in urinary parameters (increased WBC and/or dark colored urine) did not show dose-relationship and were therefore considered not to be treatment-related.

Table 52. Summary of selected urinalysis findings at selected time intervals

	Time (w)	Males					Females			
		0 ppm	50 ppm	100 ppm	200 ppm	1000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
Specific gravity [a]	13	1.072	1.078	1.065	1.069	1.049*	1.056	1.049	1.049	1.029*
	26	1.070	1.079	1.066	1.070	1.057*	1.060	1.053	1.064	1.053
	52	1.073	1.067	1.070	1.064	1.066	1.064	1.058	1.050	1.051
	77	1.065	1.064	1.068	1.074	1.062	1.049	1.060	1.041	1.059
	103	1.061	1.058	1.055	1.061	1.050	1.037	1.047	1.044	1.057
Proteins										
++	26	7	8	3	5	5	1	-	-	1
+++		1	2	1	1		-	-	-	1
++++		-	-	-	-	-	-	-	-	1
++	52	4	5	5	8	1	4	5	2	6
+++		4	3	4	2	-	1	1	3	2
++++		2	2	1	-	9*	1	1	1	2
++	103	2	-	1	-	-	3	3	4	-
+++		8	10	-	10	10	7	7	6	10
++++		-	-	-	-	-	-	-	-	-

[a] mean values; * p < 0.05 ** p < 0.01
Satellite group 1 (N= 10; w13 &26); satellite group 2 (N = 10 ; w52) ; Main group (N= 10; w78 & 104)

Hematology: Mild anemia indicated by significantly reduced Ht, reduced Hb and reduced RBC was evident in females at 5000 ppm after 104 w of treatment There was no consistent effect in these high dose females at earlier sampling intervals, and no effect at any sampling interval in females at lower dose levels and in males at all dose levels. Other minor statistical significances in hematological parameters between treated and control groups occurred at isolated intervals or did not show a dose-relationship and were considered not to be related to treatment.

Table 53. Summary of selected hematological at selected time points (mean values)

		Males					Females			
	Time (w)	**0 ppm**	**50 ppm**	**100 ppm**	**200 ppm**	**1000 ppm**	**0 ppm**	**200 ppm**	**1000 ppm**	**5000 ppm**
Ht (%)	26	38.6	38.4	38.2	38.6	37.3	38.4	37.8	37.1	36.7*
	52	41.9	40.6	41.0	41.4	40.4	39.7	37.6	39.5	38.3
	104	40.2	41.3	40.6	40.6	38.5	41.3	40.5	40.0	36.4**
Hb (g/dL)	26	14.3	14.3	14.3	14.5	14.1	14.5	14.3	14.3	14.3
	52	14.8	14.5	14.6	14.8	14.6	14.2	13.5	14.4	14.2
	104	14.3	14.6	14.3	14.3	13.9	14.7	14.6	14.4	13.3*
MCHC[a] (g/dl)	26	37.2	37.3	37.3	37.6	37.8	37.8	38.0	38.5	38.9
	52	35.4	35.7	35.7	35.7	36.2	35.8	35.9	36.4	37.0**
	104	35.6	35.3	35.3	35.3	36.1	35.6	36.0	35.8	36.5
RBC (10^6/mm^3)	26	7.69	7.68	7.69	7.76	7.56	7.13	7.13	7.00	6.79
	52	8.11	8.10	7.97	8.22	8.03	7.40	6.88	7.29	7.07
	104	7.68	7.89	7.88	7.75	7.52	7.43	7.60	7.15	6.50*

[a] mean corpuscular hemoglobin concentration * $p < 0.05$ ** $p < 0.01$

Satellite group 1 (N= 10; w13 &26); satellite group 2 (N = 10 ; w52) ; Main group (N= 10; w78 & 104)

Clinical chemistry: significant changes of the clinical chemistry profile were seen in the females at 5000 ppm, including increased serum GGT activity and total cholesterol concentration from w-52 and reduced TG concentrations from w13 indicative of hepatic dysfunction; such changes were not evident at lower dose levels in females or in males at any dose level or were seen at only one time point. Electrolyte and total and specific protein concentrations in females at 5000 ppm also differed significantly from control values but the differences were generally inconsistent and / or minimal and / or transient in nature.

Table 54. Summary of statistically significant clinical chemistry findings (mean values)

	Time (w)	Males					Females			
Dose (ppm)		**0 ppm**	**50 ppm**	**100 ppm**	**200 ppm**	**1000 ppm**	**0 ppm**	**200 ppm**	**1000 ppm**	**5000 ppm**
AlP (U/L)	13	78	86	79	71	73	60	51	62	45**
	26	72	79	67	75	72	51	45	49	40*
γ-GTP (U/L)	52	2	2	2	2	2	1	1	1	1*0
	78	1	2	1	2	2	1	1	1	2*
	104	2	2	2	2	2	1	1	1	2**
BUN (mg/dL)	13	14.7	14.7	14.2	14.6	15.7	17.1	18.0	19.3	19.4*
Total protein (g/dL)	13	6.22	6.14	6.15	5.99	6.07	6.20	6.23	6.28	6.41*
Albumin (g/dL)	13	2.98	2.93	2.95	2.84	2.83	3.18	3.25	3.08	3.41*
	52	2.74	2.65	2.75	2.72	2.66	3.63	3.39*	3.54	3.34**
Globulin (g/dL)	78	3.52	3.56	3.45	3.54	3.48	3.25	3.23	3.33	3.50*
A/G ratio	52	0.78	0.73	0.79	0.77	0.76	1.15	1.09	1.12	1.04**
Glucose (mg/dL)	52	129	127	125	128	127	113	116	117	127**
Total cholesterol (mg/dL)	52	83	75	76	78	89	66	57	69	93**
	78	98	88	93	99	100	80	75	93	117**
	104	117	136	136	123	121	98	122	102	144**
Triglyceride (mg/dL)	13	158	161	177	165	144	105	110	70	51**
	26	149	168	155	173	160	100	84	72	52**
	52	209	177	185	163	188	226	202	169*	124**
	78	252	193	232	249	206	309	300	304	139*
Total bilirubin (mg/dL)	52	0.12	0.17	0.13	0.13	0.14	0.22	0.23	0.21	0.18**
Calcium (mg/dL)	13	10.2	10.0	10.0	9.8*	9.9	9.9	9.9	9.7	10.0
	52	10.3	10.1	10.1	10.0*	10.0*	10.2	10.2	10.1	10.0
Inorganic phosphorus (mg/dL)	52	4.9	4.9	4.9	4.8	5.0	3.5	4.1*	3.5	4.0*
Sodium (mEq/L)	52	144.3	144.5	144.6	144.4	144.2	143.8	143.4	143.5	142.6**
Potassium (mEq/L)	52	3.49	3.47	3.42	3.38	3.46	2.71	2.99**	2.94*	3.24**
	78	3.45	3.35	3.62	3.42	3.54	2.96	3.06	2.80	3.31*
Chloride (mEq/L)	26	107.2	107.9	107.8	107.3	107.7	108.5	110.0*	110.2**	109.8*
	52	105.7	106.3	106.3	106.3	106.0	105.8	107.3*	107.0*	106.7
	78	106.7	106.9	106.6	106.9	107.0	103.1	103.9	103.8	105.7*

* $p < 0.05$ ** $p < 0.01$

Organ weights: Absolute and / or relative liver weights were slightly, but significantly increased in w26, w52 and w104 in females at 5000 ppm (corroborated by histopathological changes), but not at lower dose levels; in males, absolute and relative liver weights were also slightly, but significantly increased in w26 only, at 200 and 1000 ppm and relative liver weights were increased at 100 ppm, but no histopathological

alterations were seen at either dose level. Relative kidney weights were increased only in in females at 5000 ppm in w26, w52 and w104. Absolute and /or relative adrenal weights (without histopathological changes) were reduced in females at 5000 ppm in w52 and w104.

Table 55. Significant organ weights changes – Group mean values in rats killed after 26, 52 and 104 w

	Time (w)	Males					Females			
		0 ppm	50 ppm	100 ppm	200 ppm	1000 ppm	0 ppm	200 ppm	1000 ppm	5000 ppm
Liver weights										
Absolute (g)	26	11.36	11.71	12.28	12.61*	12.62	6.14	5.98	6.15	6.61
	52	13.03	13.36	12.58	12.76	13.72	6.87	7.79	6.74	8.45**
	104	13.35	14.14	13.86	14.07	13.46	9.81	11.27	9.80	11.19
Relative	26	2.46	2.53	2.63*	2.68*	2.67*	2.24	2.18	2.27	2.51**
	52	2.47	2.54	2.41	2.48	2.61	2.17	2.28	2.17	2.64**
	104	2.34	2.42	2.55	2.42	2.54	2.49	2.63	2.45	3.14**
Kidneys weights										
Absolute (mg)	26	2651	2615	2776	2703	2740	1551	1587	1605	1657
	52	2909	2920	2838	2961	3174	1838	1959	1953	2129**
	104	3443	3408	4451	3530	3782	2408	2531	2354	2510
Relative	26	058	0.57	0.59	0.58	0.58	0.57	0.58	0.59	0.63**
	52	0.55	0.56	0.55	0.58	0.60*	0.58	0.59	0.63	0.67**
	104	0.61	0.59	0.84	0.61	0.73	0.61	0.60	0.60	0.70*
Adrenal weights										
Absolute (mg)	52	51.2	52.2	48.9	48.1	50.8	69.9	71.6	68.5	58.8*
	104	73.9	241.5	66.6	109.9	77.1	96.1	79.7	76.3	58.0**[a]
Relative	52	0.010	0.010	0.009	0.009	0.010	0.022	0.022	0.022	0.019*
	104	0.013	0.042	0.012	0.019	0.015	0.025	0.019	0.020	0.017

* $p < 0.05$; ** $p < 0.01$: [a] data excludes 1 female control with high adrenal weight due to cortical adenoma

Necropsy findings: There were no treatment-related gross lesions at any dose level in the satellite groups. In the main study, increased incidences of dark coloration and accentuated lobular pattern in the liver and luminal dilatation of the common bile duct were seen in females at 5000 ppm at termination (104 w) and also in some female decedents at this dose level. The incidence of discharge from the eye(s) was significantly increased in females at 5000 ppm. Increased incidences of renal pelvic dilatation observed in males at 200 and 1000 ppm and lung masses seen in males at 1000 ppm were considered as incidental in the absence of histopathological correlates. Other significant differences between control and treated groups in the incidences of gross lesions were considered incidental (no dose-response pattern).

Table 56. Selected necropsy findings in decedents rats and at terminal sacrifice (104w)

Organ / finding	Fate[a]	No. of males at (ppm):					No. of females at (ppm):			
		0	50	100	200	1000	0	200	1000	5000
Liver (no. examined)	TK	43	40	25	34	32	31	32	30	31
- spot(s)		1	1	1	2	1	1	2	7*	5
- accentuated lobes		0	0	1	0	0	0	1	2	6*

Liver (no. examined)	TK+ID	52	51	51	52	52	52	52	52	52
- dark color		0	0	2	1	0	0	1	0	5*
- spot(s)		2	2	4	2	2	3	2	10*	5
- accentuated lobes		0	0	2	1	1	0	3	2	8**
Bile duct (no. examined)	TK	43	40	25	34	32	31	32	30	31
- luminal dilatation		15	5*	5	6	7	0	3	1	5*
Bile duct (no. examined)	TK+ID	52	51	51	52	52	52	52	52	52
- luminal dilatation		15	6*	6*	8	8	2	5	1	7
- eye discharge		9	13	12	9	14	4	9	10	13*
Lungs - spot(s)		5	0*	4	1	2	522	2	521	0
- mass(es)		0	3	0	2	5*	3	2	3	3
Kidneys (		1	3	3	5	7*	3	13*	9	3
- pelvic dilatation										

[a] TK survivors at 104 weeks only, TK+ID decedents + survivors killed after 104 weeks

Histopathological examination:

Non neoplastic findings:

In Wistar rats, the liver and the kidneys are the specific target organs after long-term administration of flonicamid and lesions are similar to those seen in the 13 w study; in the liver, centrilobular hepatic hypertrophy occurs with signs of liver dysfunction, but is evident only in female rats at the highest dose level. Effects on the kidneys occur in both sexes, but there is a clear difference between the sexes in lesion morphology; in males, the nephrotoxicity closely resembles α2-microglobulin nephropathy and is characterized by tubular epithelial hyaline droplet deposition occurring in parallel with an increased incidence of tubular basophilia up to 52 w of treatment; hyaline degeneration is accompanied by an increased incidence of chronic nephropathy after 104 w treatment; in females, the morphological alterations are confined to proximal tubular vacuolation, chronic nephropathy and increased incidence of brown pigment deposition; female rats also develop signs of mild anemia and accelerated expression of the common age-related lesions, such as cataract, retinal atrophy and striated muscle fibre atrophy. The incidence of lesions in the nerves supplying the muscles are unaffected by treatment. The lowest NOAEL value established in the rat for non-neoplastic effects is 200 ppm in males and 1000 ppm in females i.e.7.3 and 44.1 mg/kg bw/d, respectively.

Table 57. Incidence of selected non-neoplastic findings in satellite groups 2 (26 w) and 1 (52w)

Organ / finding	kill	**No. of males at (ppm)**					**No. of females at (ppm)**			
		0	**50**	**100**	**200**	**1000**	**0**	**200**	**1000**	**5000**
No of rats examined		10	10	10	10	10	10	10	10	10
Liver										
centrilobular hypertrophy	26w	0	0	0	0	0	0	0	0	10**
	52w	0	0	0	0	0	0	0	0	9**
Kidney										
- hyaline droplet deposition[a]	26w	0	0	2	8**	10**	0	0	0	0
	52w	0	0	0	0	10**	0	0	0	0
- tubular basophilia	26w	3	5	4	5	8*	0	0	0	0
	52w	4	6	6	8	10**	3	5	4	6
- granular casts in dilated tubules	26w	0	0	0	0	5*	0	0	0	0
	52w	-	-	-	-	-	-	-	-	-
- vacuolation[a]	26w	0	0	0	0	0	0	0	0	10**
	52w	0	0	0	0	0	0	0	0	10**

[a] proximal tubular cells; * $p < 0.05$; ** $p < 0.01$

Table 58. Incidence of selected non-neoplastic findings – main group (104 w terminal kill and decedents)

Organ / finding	No. of males at (ppm):					No. of females at (ppm):			
	0	**50**	**100**	**200**	**1000**	**0**	**200**	**1000**	**5000**
Decedents only (n° examined)	9	11	26	18	20	21	20	22	21
Kidneys - hyaline droplet deposition[a]	4	3	3*	5	11	5	4	4	5
- vacuolation[a]	0	0	0	0	0	0	0	0	6*
- pyelitis / pyelonephritis	1	0	3	2	4	2	2	8*	2
Eye - cataract	2	3	10	5	6	5	2	5	12*
- retinal atrophy	2	1	6	4	5	1	1	3	10**
Muscle[b] - atrophy of striated muscle	3	6	18	13	11	5	4	7	21**
Forestomach - erosion / ulcer	2	2	9	7	13*	2	5	6	10**
Liver - periportal fatty change	0	0	3	0	1	1	6*	4	5
Killed after 104 weeks (incidence/N° examined rats)									
Muscle[b] - atrophy of striated muscle fiber	41/43	-	-	-	32/32	13/31	17/32	18/30	31/31**
Liver - centrilobular hypertrophy	0/43	0/40	0/25	0/34	0/32	0/31	0/32	0/30	20/31**
- eosinophilic cell foci	15/43	9/40	12/25	17/34	12/32	5/31	5/32	8/30	14/31*
Kidneys - tubular basophilic change	25/43	16/40	11/25	25/34	9/32**	20/31	18/32	18/30	15/31
- chronic nephropathy	18/43	24/40	13/25		23/32**		14/32	10/30	14/31
- vacuolation[a]	0/43	0/40	0/25	9/34		8/31	0/32	0/30	31/31**
				0/34	0/32	0/31			
Eye - retinal atrophy	12/43	13/18	10/11	8/12	14/32	8/31	4/32	8/30	20/31**
All rats (incidence/N° examined rats)									
Muscle[b] - atrophy of striated muscle fiber	44/52	6/11	18/26	13/18	43/52	18/52	21/52	25/52	52/52**
Forestomach - erosion / ulcer	4/52	2/11	9/26	7/18	15/52**	3/52	5/20	6/22	12/52*
Liver - periportal fatty change	8/52	7/51	6/51	6/52	5/52	5/52	13/52	7/52	8/52
- centrilobular hypertrophy	0/52	0/51	0/51	0/52	0/52	0/52	*	0/52	20/52**
- eosinophilic cell foci	15/52	9/51	13/51	17/52	13/52	6/52	0/52	10/52	15/52*
							7/52		
Kidneys - tubular basophilic change	30/52	21/51	24/51	34/52	20/52**	31/52	29/52	29/52	28/52
- chronic nephropathy	18/52	26/51	18/51			11/52	17/52	11/52	21/52*
- hyaline droplet deposition[a]	11/52	8/51	4/51*	14/52	25/52	7/52	5/52	6/52	5/52
- vacuolation[a]	0/52	0/51	0/51		19/52	0/52	0/52	0/52	37/52**
- brown pigment deposits[a]	0/52	0/51	0/51	8/52	0/52	6/52	7/52	5/52	14/52*
				0/52	0/52				
				0/52					
Eye - cataract	34/52	19/29	20/37	15/30	28/52	24/52	16/52	22/52	34/52*
- retinal atrophy	14/52	14/29	16/37		19/52	9/52	5/52	11/52	30/52**
				12/30					

[a] proximal tubular cells; [b] *m. triceps surae*; * $p < 0.05$; ** $p < 0.01$

Neoplastic findings:

No consistent cause of death was evident in decedents. The nature and incidence of all tumor types were similar in all groups of animals scheduled to be killed after 104 w, 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 5000ppm. Treatment-related rare tumor types did not occur in either sex and the multiplicity of tumors and latencies did not indicate a treatment effect in either sex at any dose level.

Table 59. Incidences of neoplastic findings in rats scheduled to be killed after 104 w

Parameter	**No. of males at (ppm):**					**No. of females at (ppm):**			
	0	**50**	**100**	**200**	**1000**	**0**	**200**	**1000**	**5000**
No. rats killed after 104 w:	43	40	25	34	32	31	32	30	31
- no. benign tumors	53	45	14	35	32	62	48	46	52
- no. malignant tumors	13	13	3	5	8	5	7	4	12
- no. benign + malignant tumors	66	58	17	40	40	67	55	50	64
- no. animals with benign tumors	31	25	11	24	24	29	28	25	28
- no. animals with malignant tumors	10	13	3	5	8	5	6	4	10
- no. animals with any tumor	33	30	12	25	27	29	28	26	30
No. rats dead before 104 w:	9	11	26	18	20	21	20	22	21
- no. benign tumors	3	6	21	14	14	30	34	31	23
- no. malignant tumors	6	8	17	14	16	7	4	5	12
- no. benign + malignant tumors	9	14	38	28	30	37	38	36	35
- no. animals with benign tumors	3	5	15	10	9	19	18	18	17
- no. animals with malignant tumors	6	8	16	12	15	7	4	5	11
- no. animals with any tumor	8	10	24	15	18	20	18	18	20
Total scheduled 104 w rats:	**52**	**51**	**51**	**52**	**52**	**52**	**52**	**52**	**52**
- no. benign tumors	56	51	35	48	46	94	82	77	75
- no. malignant tumors	19	21	20	20	24	12	11	9	24
- no. benign + malignant tumors	75	72	55	68	70	106	93	86	99
- no. animals with benign tumors	**34**	**30**	**26**	**34**	**33**	**48**	**46**	**43**	**45**
- no. animals with malignant tumors	**16**	**21**	**19**	**17**	**23**	**12**	**10**	**9**	**21**
- no. animals with any tumor	41	40	36	40	45	49	46	44	50

The nature and incidence of all tumour types were similar in all groups of animals scheduled to be killed after 104 w, including the decedents. Statistically significant changes in tumour 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 tumour types did not occur in either sex and the multiplicity of tumours and latencies did not indicate a treatment effect in either sex at any dose level. However in order to support this conclusion, additional details about rare tumours formation and a discussion were presented in the addendum 3 to volume 3 B.6 (France, 2006); these include pulmonary masses, squamous cell carcinomas in the nasal cavity and cerebellum granular cell tumors.

Pulmonary masses

Table 60. Histopathological findings of pulmonary mass(es) noted at necropsy in male decedent and terminally killed rats

Group (ppm)	Animal number	Fate * (week)	Macroscopic finding	Histopathology
0	35	tk (104)	nothing	Adenoma (B)
	43	tk (104)	nothing	Adenoma (B)
50	66	tk (104)	1 mm in diameter	Hyperplasia, alveolar epithelial cells++ Adenoma (B)

Group (ppm)	Animal number	Fate * (week)	Macroscopic finding	Histopathology
	78	tk (104)	10 mm in diameter	Adenoma (B)
	98	tk (104)	nothing	Adenoma (B)
	104	tk (104)	4 mm in diameter	Adenoma (B) In other site: Hyperplasia, alveolar epithelial cells +
100	112	ke (104)	nothing	Adenoma (B)
200	170	tk (104)	5 mm in diameter	Adenoma (B)
	171	tk (104)	6 mm in diameter	Adenoma (B)
			2 mm in diameter	Hyperplasia, alveolar epithelial cells +
1000	212	tk (104)	5 mm in diameter	Hyperplasia, alveolar epithelial cells ++
	221	tk (104)	3 mm in diameter	Adenoma (B)
	240	ke (79)	30 x 30 x 50 mm	Adenocarcinoma (M)
	241	fd (62)	5 mm in diameter	Malignant methothelioma (M) In other site:Adenoma (B)
	253	fd (100)	7 mm in diameter	Histiocytic sarcoma (M)
			5 mm in diameter	Histiocytic sarcoma (M)
			5 mm in diameter	Histiocytic sarcoma (M)
			5 mm in diameter (Multiples masses)	Histiocytic sarcoma (M)

*: tk, terminal kill; fd, found dead; ke, killed *in extremis*.
Grade: +, slight; ++, moderate.
B, benign; M, malignant.

The lung masses in males at the high dose were described as tumours of various histopathological types and were concluded to be not treatment related.

The **squamous cell carcinomas in the nasal cavity** were neither dose-related nor with statistical significance and were observed unilaterally and without preneoplasic lesions; these were re-evaluated as occurring in the nasolacrimal duct. Further information was submitted to support the non relevance of squamous cell carcinomas of the nasal cavity.
Since squamous cell carcinoma of the epithelium lining the nasal cavity is generally considered to occur infrequently in the nose of rats, the number of tumors reported in the study was considered to be relatively high. The diagnosis of "Nasal Cavity – Squamous Cell Carcinoma" reported in the study was reviewed and further investigated to clarify their possible significance. Part of this investigation involved sectioning of the remaining noses from the intermediate dose groups so a complete data set was available for interpretation. The results of these additional rat nasal cavity evaluations are reported in Table 61. No additional squamous

cell carcinomas were observed during the examination of these additional sections; a unilateral benign squamous cell papilloma was observed in one male rat in the 200 ppm group.

Table 61. Incidence of nasal cavity tumours in rats

Males

Finding	**Dose (ppm)**					**Historical Control, Mean (Range)**
	0	**50**	**100**	**200**	**1000**	**IET**
No. examined	52	51	51	52	52	205
Squamous cell carcinoma	2 3.8%	3	4	2	6 11.5%	9[a] 4.32%[a] (0-10%)
Unilateral	2	2	4	2	6	
Bilateral	0	1	0	0	0	
Squamous cell papilloma	0	0	0	1*	0	

* unilateral

Females

Finding	**Dose (ppm)**				**Historical Control, Mean (Range)**
	0	**200**	**1000**	**5000**	**IET**
No. examined	52	52	52	52	204
Squamous cell carcinoma	0	0	0	3* 5.8%	2[a] 0.98% (0-4%)

[a] Four control studies: 0/56, 0/49, 4/50, 5/50 for males and 0/56, 0/49, 2/49, 0/50 for females; * unilateral

A review of the macroscopic findings that corresponded to these lesions diagnosed as "Nasal Cavity – Squamous Cell Carcinoma" indicated that these lesions had a wide variety of descriptions ranging from swellings to masses. There was some concern that the topography (nasal cavity) that was used to describe the tumors was driven by the computer software program and that they may not be arising from the nasal cavity but from other tissues in the region. The gross observation and association with overgrown incisor teeth of some of the gross lesions are tabulated in Table 62.

Table 62. Tumors recorded as nasal cavity, squamous cell carcinoma or adenoma

Dose Group	**Animal No.**	**Sex**	**Gross Observation**	**Malocclusion Syndrome Effect**
0 ppm	42	Male	Skin (Dorsal): Subcutaneous mass	

Dose Group	Animal No.	Sex	Gross Observation	Malocclusion Syndrome Effect
			(R), 10 mm in diameter	
	47	Male	Skin (Buccal): Mass, 10 mm in diameter	
50 ppm	55	Male	Nasal Cavity: Mass	
	57	Male	Nasal Cavity: Mass, 10 mm in diameter	Elongation of Upper and Lower Incisors
	88	Male	Skin (Head): Swelling	Elongation of Lower Incisor
100 ppm	126	Male	-	
	129	Male	Skin (Head): Swelling	
	130	Male	Skin (Head): Swelling	
	137	Male	Nasal Cavity: Mass, 10 mm in diameter	
	145	Male	Skin (Periocular): Subcutaneous mass (L), 20 mm in diameter	
200 ppm	185	Male	Skin (Periocular): Erosion/Ulcer (L)/Swelling (L)	
	207	Male	Skin (Periocular): Subcutaneous mass (L), 30 mm in diameter	
1000 ppm	210	Male	Skin (Periocular): Swelling	Incisor: Malocclusion
	222	Male	Skin (Nasorostral): Enlargement	Incisor: Malocclusion
	224	Male	Skin (Buccal): Subcutaneous mass (R), 20 mm in diameter	
	230	Male	Skin (Buccal): Subcutaneous mass, 20 mm in diameter	Incisor: Malocclusion
	238	Male	Nasal Cavity: Mass, 10 mm in diameter	
	260	Male	-	Elongation of Lower Incisor
5000 ppm	573	Female	Skin (Head): Elevated area	
	581	Female	Skin (Buccal): Subcutaneous mass (R), 20 mm in diameter	Elongation of Lower Incisor
	582	Female	Skin (Buccal): Subcutaneous mass, 20 mm in diameter	Elongation of Lower Incisor

The slides were re-examined for non-neoplastic lesions of the nasolacrimal duct. All the tumors diagnosed as “Nasal Cavity – Squamous Cell Carcinoma” were confirmed to be tumors arising from the nasolacrimal duct

and not arising from the epithelium lining the nasal cavity. With a single exception, all tumors were unilateral and infiltrated the surrounding soft tissue medial and lateral to the incisor tooth that was also present in the section.

Although tumors of the nasolacrimal duct are not frequently reported in carcinogenesis bioassays, spontaneous squamous cell carcinomas arising from the nasolacrimal duct have been reported in the literature by Schoevers *et al.*[27] (1994) for the Wistar rat; these tumours prevail in males and are primarily associated with chronic inflammation. The chronic inflammatory changes were present with and without squamous metaplasia and/or hyperplasia of lining epithelium. Malocclusion of incisor teeth was considered a contributing factor when present. In the combined chronic toxicity/carcinogenicity study of flonicamid in rats, 50% of rats with the diagnosis of "Nasal Cavity – Squamous Cell Carcinoma" also had malocclusion due to overgrowth of the incisors described clinically. The incidence of chronic inflammation, squamous metaplasia and squamous cell hyperplasia was similar in control and treated groups of male and female rats. Additionally, the majority of the changes were present unilaterally, further supporting the spontaneous nature of these changes. If these changes were associated with systemic toxicity of the tested substance, then the changes should have occurred bilaterally in paired organs rather than unilaterally and at a greater frequency.

Historical control data from the testing facility (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 0-10% (mean 4.32%) in male rats and ranged from 0-4.1% (mean 0.98%) in females. The slight non-statistically significant increase in the incidence of squamous cell carcinomas was similar to the reported historical control data with a preponderance of tumors being observed in male rats. In the female rats the incidence observed in the 5000 ppm dose group is slightly above the historical control mean and range; unlike the male rats, it could not be clearly correlated with the inflammatory response due to the low incidence of both neoplastic and non-neoplastic lesions.

Conclusion: there was no dose-related or statistically significant increase in the incidence of the tumours in male or female rats. No treatment-related occurrences of inflammation, metaplasia or hyperplasia which can be an origin of the tumours were observed in the nasolacrimal duct of male or female rats. Taking these facts into consideration, the squamous cell tumours in the nasolacrimal duct which were observed in this study were considered to be unrelated to the treatment with the test substance

The incidence of **cerebellum granular cell tumors** was 3/52 (6%) in the high dose group of female rats. These tumours occurred late in the study (> 94 weeks). Granular cell tumours involving the meninges are the most frequently reported benign neoplasms of the brain. Since they are often only observed microscopically, they may be included in the section examined microscopically purely by chance. This makes the true incidence of these small benign tumors difficult to determine and these are probably underreported. Poteracki and Walsh[28] (1998) reported incidences of benign granular cell meningiomas, the most common tumour of the nervous system in Wistar rats, of 2.4 % in males and 1.5 % in females. In historical control data from 39 two-year bioassays at RCC Ltd, Itingen, Switzerland[29], the mean incidence was 2.12 % (range 0-7.14 %) and 1.01 % (0-4.35 %) in males and females respectively. They have not been reported to metastasize and generally grow slowly by expansion. It is concluded that the occurrence of 3 cases in the high dose female rats in this study can be considered incidental.

Conclusion

No carcinogenic potential was found after dietary administration of flonicamid for 104 w at dose levels up to to 36.5 and 219 mg/kg bw/d in male and female rats, respectively. Consequently, the NOAEL for all effects was 200 ppm in males i.e. 7.32 mg/kg bw/d (based on the occurrence of reduced bw and nephropathy at

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27 Schoevers EJ *et al*. (1994). Spontaneous squamous cell carcinoma of nasal and paranasal structures in the Cpb:WU (Wistar random) rat: nasolachrymal duct as major site of origin. J Environ Pathol Toxicol Oncol 1994; 13(1):49-57

28 Poteracki J, Walsh KM. Spontaneous neoplasms in control Wistar rats: a comparison of reviews. Toxicol Sci 45:1-8, 1998

29 RCC Ltd. (2003) Historical control data on neoplastic findings in Wistar rats (Planned sacrifice after 103 Week), Compiled from 2-year bioassays, Itingen/Switzerland, 2003(EPA MRID Number 46323701)

1000 ppm) and 1000 ppm in females i.e. 44.1 mg/kg bw/d (based on the occurrence of reduced bw gain, mild anemia, hepatic hypertrophy, hepatic dysfunction, renal tubular vacuolation, chronic nephropathy and accelerated age-related eye and muscle lesions at 5000 ppm).

First mouse study
Reference: Ridder, 2003a
GLP: Yes
Guidelines: **O**ECD 451; US-EPA OPPTS 870.4200

Groups of 60/sex Swiss mice (Crl:CD-1® (ICR) BR VAF/Plus strain; 40 d old at start of dosing; mean bw = 23.5 - 28.6g) were administered 0; 250; 750 and 2250 ppm of flonicamid technical in the diet for 78 w; satellite control and high dose groups of 10 mice/sex were sacrificed at 26w or 52w. The dose levels were selected from the results of the 13w feeding study in mice (Ridder W.E., 2001) with 0; 100; 1000 and 7000 ppm of flonicamid in the diet, in which the NOEL was 100 ppm in both sexes (i.e. 15.25 mg/kg bw and 20.1 mg/kg bw in males and females, respectively); the LOAEL was 1000 ppm (hepatocellular hypertrophy in males and increased splenic extramedullary hematopoiesis in both sexes). The average test substance intake was 29, 88 and 261mg/kg bw/d in males and 38; 112 and 334mg/kg bw/d in females from the 250; 750 and 2250 ppm groups, respectively.

Tests diets were prepared at approx. weekly intervals throughout the study period. The stability of flonicamid in test diets was confirmed in the preliminary 13w study; test substance was shown to be stable in the diet for at least 14 d under both and refrigerated and ambient temperature conditions. Diet homogeneity was confirmed prior to the initiation of the study on batches from the 250 and 2250 ppm levels. Achieved concentration analyses were performed on all formulations in w1 and every 4 w.

All mice were observed at least once daily for morbidity / mortality throughout the study and physical examination was performed weekly until termination. Individual bw and food consumption were recorded weekly for the first 13 w and every 4 w thereafter.

A blood smear (for differential leucocyte count) was prepared in w26 and w52 from all mice from satellite groups and after w52 and 73 w from all surviving mice of the main study; a blood smear was also prepared from mice killed prematurely, where possible. Initially, smears were read from mice of the control and high dose groups.

Mice from satellite groups (killed after w26 or w52) were necropsied, and major organs and tissues were examined, and the liver, kidneys and spleen were weighed. In the main study, necropsy and *post mortem* examination of major organs and tissues were carried out on all mice surviving at 18 months and on all mice found dead or killed *in extremis*. Organ weights[30] were recorded from 10 mice/sex/group and liver, kidneys and spleen were weighed from the remaining mice. All tissues[31] and gross lesions were preserved from survivors at 18 months treated at 0 or 2250 ppm and from all mice dying or killed prematurely and were examined microscopically. The lungs, liver, kidneys and gross lesions from all survivors at 18 months treated at 250 or 750 ppm were also examined. Slides of lung tissue were peer reviewed, and a Pathology Working Group was constituted to review study findings.

[30] adrenals, brain, heart, kidneys, liver, testes, epididymes, ovaries, uterus, spleen

[31] Adrenals, bone, bone marrow, brain, esophagus, eye, duodenum, jejunum, ileum, colon, cecum, rectum, head (nasal cavity & pharynx), heart with thoracic aorta, kidneys, liver with gallbladder, lungs, lymph nodes (cervical & mesenteric), pancreas, pituitary gland, testes, epididymes, seminal vesicles, prostate, ovaries, uterus, cervix, vagina, salivary glands (both submandibular), skeletal muscle with peripheral nerve, skin, spinal cord (cervical, midthoracic & lumbar), spleen, stomach, tail, thymus, thyroid with parathyroid glands, tongue, trachea, urinary bladder, all gross lesions.

Chemical analyses of test substance in diets : The homogeneity of diet mixing was acceptable as shown by the results of 5 analyses of the low and high dosed test diets (248.95 ± 4.204 ppm and 2260.36 ± 21.926 ppm, respectively) which were within 98.2 - 102.3% of nominal concentration. Achieved concentrations throughout the study were acceptable, since all diets on all occasions were within the range 89.5 – 111.6% nominal concentration.

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

Table 63. Deaths and survival incidences in main groups (60 mice/sex/dose level)

Treatment group (ppm)	Number of death (% survival) :			
	Males at:		Females at:	
	w52	Termination	w52	Termination
0	4 (*93*)	13 (*78*)	4 (*93*)	14 (*77*)
250	1 (*98*)	11 (*82*)	3 (*95*)	10 (*83*)
750	2 (*97*)	6 (*90*)	2 (*97*)	13 (*78*)
2250	4 (*93*)	14 (*77*)	4 (*93*)	14 (*77*)

Clinical examinations: There were no treatment-related clinical findings: the most common findings included hair loss and rough hair coat in all groups; higher incidence of ear lesions and hair loss in males referred as the progressive necrosing dermatitis which in some cases resulted to sacrifice of the mice; occasional mass development in the preputial area detected as early as 10-12 w; abdominal mass development in females mostly associated with ovarian cysts development or uterine horn enlargement.

Bw and food consumption: There was no treatment-related effect on bw gain in either sex at any dose level, although bw gains were significantly higher in all treated females throughout the study period; this resulted from the significantly heavier bw at w0 in control females, any of which lost weight during study w1. There were no treatment related effects on food consumption in either sex at any dose level; occasional statistically significant values seen in the treated groups were minor, transient and represented both higher and low values, and were therefore considered as incidental. The average test substance intake was 29, 88 and 261mg/kg bw/d in males and 38; 112 and 334mg/kg bw/d in females from the 250; 750 and 2250 ppm groups, respectively.

Table 64. Summary of bw and bw gain

Group mean values ± SD								
	Males				**Females**			
	0 ppm	**250 ppm**	**750 ppm**	**2250 ppm**	**0 ppm**	**250 ppm**	**750 ppm**	**2250 ppm**
Mean bw (g)								
Pretest	28.4±2.04	28.2±2.22	28.6±2.25	28.4±2.12	23.3±1.90	22.2±2.28**	21.7±1.84**	22.3±1.86**
w1	29.7±2.30	29.5±2.22	30.7±2.50	30.1±2.22	23.5±1.94	24.5±2.05*	24.6±1.76**	24.1±2.12
w53	41.4±4.40	41.7±3.69	41.8±3.75	42.2±4.56	34.0±3.52	35.3±4.35	35.7±3.68	35.2±3.42
w77	42.2±4.32	42.4±4.92	42.1±3.91	42.6±5.28	36.9±3.87	36.9±4.26	38.6±4.45	36.8±4.08
Bwg (g)								

w1	1.3±0.84	1.3±0.87	2.1±0.96**	1.7±0.92*	0.2±0.91	2.2±1.48**	2.9±0.95**	1.8±1.07**
w53	12.9±3.89	13.5±2.80	13.2±2.76	13.5±3.36	10.8±2.88	13.1±3.56**	14.0±2.83**	12.7±2.48**
w77	13.9±3.90	14.3±3.86	13.6±3.17	13.9±4.52	13.8±3.13	14.9±3.63	16.9±3.75**	14.3±3.33

Laboratory investigations: There were no treatment related effects in the 2250 ppm mice with respect to leucocyte differential counts at w26, w52 and w73; statistically significant changes were seen at w52 in males (reduced mean number of monocytes in males and increased mean number of eosinophils in females) but these changes were minor and not seen at other intervals. All other values were not significantly different from control values.

Necropsy findings: Treatment-related gross findings at necropsy in main study groups were confined to increased incidences of tissue masses in the lungs of both sexes at 750 and 2250 ppm and males at 250 ppm.

Organs weights: Liver weights changes were the only treatment-related effects which was seen in high dose mice only at w26, w52 and w78: slightly increased absolute and relative liver weights occurred in both sexes in w26 and w52, but not all values were statistically significant, and these liver weight changes persisted only in females at w78. There were no other treatment-related effects on organ weights at any dose level.

Table 65. Summary incidence of lung masses/nodules and of selected organ weights – main study mice (60/sex)

Group mean values ± SD								
	Males				**Females**			
	0 ppm	**250 ppm**	**750 ppm**	**2250 ppm**	**0 ppm**	**250 ppm**	**750 ppm**	**2250 ppm**
Incidence of lung masses								
	2/60	7/60	15/60	15/60	3/60	4/60	7/60	7/60
Group mean liver absolute weight (g)								
w26	1.967± 0.253	-	-	2.160 ± 0.160	1.599 ± 0.284	-	-	1.689 ± 0.14
w52	2.013 ± 0.229	-	-	2.251 ± 0.205*	1.717 ± 0.393	-	-	1.932 ± 0.242

w78	2.3041 ±0.6199	2.4204 ± 0.7441	2.4298 ± 0.6559	2.4143 ± 0.4033	1.930 ± 0.4430	1.8712 ± 0.3412	2.1057 ± 0.6321	2.1330 ± 0.3328*
Group mean liver relative weight (g / g x 100)								
w26	4.883 ± 0.347	-	-	5.255 ± .347*	±4.605 ± 0.458	-	-	±5.145 ± 0.444*
w52	5.016 ± 0.646	-	-	5.453 ± 0.524	4.825 ± 0.679	-	-	5.396 ± 0.549
w78	5.4234 ± 1.4121	5.6871 ± 1.9822	5.7101 ± 1.5730	5.6587 ± 0.8762	5.2561 ± 1.0033	5.1977 ± 0.8710	5.5094 ± 1.2635	5.7581 ±0.6648**
* $p < 0.05$ ** $p < 0.01$								

- **Histopathological examination**:

- Non neoplastic changes: Treatment-related non-neoplastic changes were seen in the liver, spleen and bone marrow:

  - *Liver*: In satellite groups, minimal to moderate centrilobular hepatocyte hypertrophy occurred only in males at 2250 ppm; in the main group, this finding was found in males at all dose levels in which it exhibited dose dependent severity and in females at 2250 ppm.

  - *Spleen*: In satellite groups, increased splenic extramedullary hematopoiesis and pigment deposition (both graded as minimal to moderate) occurred in both sexes at 2250 ppm at both interim kills. In the main group, this finding was seen in males at all dose levels and in females at the 2 highest dose levels and increased pigment deposition (golden brown granular material consistent with hemosiderin) occurred in both sexes at 2250 ppm.

  - Bone marrow: In satellite groups, an increased incidence of minimal to moderate bone marrow hypocellularity was evident at low incidence in both sexes after 26 w and in males only after 52 w and 2 males also showed pigment deposition after 26 w. In the main groups, increased incidence of minimal bone marrow hypocellularity accompanied by pigment deposition was evident in both sexes at 750 or 2250 ppm, suggesting a treatment-related effect on erythrogenesis.

  - Other changes: Increased incidences of enlargement and increased eosinophilia of the epithelial cells lining the terminal bronchioles occurred in a large proportion of mice in all treated groups, but there was

no clear dose-response relationship. This treatment-related finding was attributed to an increased size and granularity of the cytoplasm of these epithelial cells (presumably Clara cells) and was considered as suggestive of an induction of cytochrome enzymes. Although hypertrophy localized within airway cells appeared to be the principal change, increased cell numbers could not be precluded. Therefore, the alteration was referred to as hypertrophy/hyperplasia. There were no other treatment-related non-neoplastic alterations.

Table 66. Selected non-neoplastic histopathological findings in stallie and main groups

SATELLITE GROUPS	**Males**				**Females**			
Terminal kill	**26 w**		**52 w**		**26 w**		**52 w**	
Dose levels (ppm)	**0**	**2250**	**0**	**2250**	**0**	**2250**	**0**	**2250**
Liver - centrilobular hypertrophy	0/10	6/10	0/10	7/10	0/10	0/10	0/10	0/10
Spleen								
- extramedullary hematopoiesis	0/10	6/10	4/10	9/10	6/10	8/10	7/10	10/10
- pigment deposition	0/10	1/10	0/10	3/10	4/10	10/10	0/10	3/10
Bone marrow								
- hypocellularity	0/9	2/10	0/10	2/10	0/10	2/10	0/10	0/10
- pigment deposition	0/9	2/10	0/10	0/10	0/10	0/10	0/10	0/10
MAIN GROUP (78 w kill)	**Males**				**Females**			
Dose levels (pm)	**0**	**250**	**750**	**2250**	**0**	**250**	**750**	**2250**
Liver - centrilobular	4/60	14/60	36/60	40/60	0/60	0/60	1/60	11/60
hypertrophy	4	14	29	15	0	0	1	8
- minimal	0	0	6	19	0	0	0	3
- slight	0	0	1	6	0	0	0	0
- moderate								
Spleen								
- extramedullary hematopoiesis	17/60	30/60	28/60	45/60	33/60	34/60	43/60	46/60
- minimal	7	17	14	19	14	21	20	16
- slight	5	9	12	13	7	8	16	20
- moderate	4	3	2	8	8	4	5	8
- moderately severe	1	0	0	5	4	1	2	2
- severe	0	1	0	0	0	0	0	0
- pigment deposition	1/60	0/60	2/60	8/60	15/60	9/60	15/60	28/60
- minimal	1	0	2	8	11	8	14	25
- slight	0	0	0	0	4	1	1	2
- moderate	0	0	0	0	0	0	0	1

Bone marrow								
- hypocellularity (femoral)	0/60	1/60	7/60	24/60	1/60	7/60	11/60	22/60
- minimal	0	1	7	16	0	4	7	15
- slight	0	0	0	6	1	3	4	4
- moderate	0	0	0	2	0	0	0	3
- pigment deposition	0/60	4/60	12/60	32/60	4/60	2/60	7/60	25/60
- minimal	0	2	6	12	4	2	2	10
- slight	0	2	6	13	0	0	5	15
- moderate	0	0	0	7	0	0	0	0
- hypocellularity (sternal)	0/60	1/60	5/60	22/60	1/59	3/60	6/60	12/60
- minimal	0	1	5	18	0	2	4	10
- slight	0	0	0	3	1	1	1	1
- moderate	0	0	0	1	0	0	1	1
- pigment deposition	0/60	7/60	15/60	31/60	5/59	6/60	10/60	24/60
- minimal	0	3	9	13	4	6	5	12
- slight	0	4	6	17	1	0	5	11
- moderate	0	0	0	1	0	0	0	1

- Neoplastic changes:

Lung: Treatment-related statistically significant increased incidences of primary lung tumors occurred in both sexes at all dose levels. Initial histopathological examination showed that the overall incidences of treated mice with any primary lung tumor (including alveolar/bronchiolar adenoma and carcinoma) were in the range 36.7 - 60.0% compared with control incidences of 16.7 and 15.0% in males and females, respectively; there were also increased incidences of focal alveolar/bronchiolar hyperplasia of epithelial cells (most likely Clara cells) in terminal bronchioles in both sexes at all dose levels. The diagnoses were confirmed by a pathology working group. Although a very small number of diagnoses were modified on peer review, the group incidences of both benign and malignant tumors were comparable to the original incidences. There were clear dose related increased incidences of malignant tumors and of the number of mice with multiple tumors.

Only a small proportion of incidental deaths in all treatment groups had a primary lung tumor and most were diagnosed in animals killed at termination in w78, indicating that the lung tumors were generally not life-threatening.

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

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

Table 67. Incidence of pulmonary neoplastic lesions, and focal hyperplasia – main group (78-w necropsy)

Organ / finding	Incidence at 78 w:							
	In males at (ppm):				In females at (ppm):			
	0	250	750	2250	0	250	750	2250
Initial histopathological evaluation								
No. lungs examined	60	60	60	60	60	60	60	60

Alveolar/bronchiolar adenoma	9	26**	24**	32**	9	21*	30**	25**
Alveolar/bronchiolar carcinoma	2	4	9*	10*	0	3	4	5*
No. animals with 1° tumor[a]	10	27	29	36	9	22	32	26
No. with multiple adenomas	2	8	12	17	1	4	12	12
No. with multiple carcinomas	0	1	3	5	0	2	2	3
No. with focal hyperplasia[b]	2	22	46	46	4	20	41	42
Pathology working group								
No. lungs examined	10	27	29	36	9	22	32	26
Alveolar/bronchiolar adenoma	7	25	25	33	9	20	30	24
Alveolar/bronchiolar carcinoma	4	6	12	12	0	3	3	7
No. animals with 1° tumor[a]	10	27	29	36	9	22	32	25

[a] some animals had both adenoma and carcinoma; [b] alveolar/bronchiolar ; * $p < 0.05$; ** $p < 0.01$

Most of the tumours were diagnosed in mice killed at termination in w78, indicating that the lung tumours were generally not life-threatening. There was no excess incidence of tumours in any other organ or tissue.

In CD-1 mice, the liver, spleen, bone marrow and lung were identified as target organs for non-neoplastic effects: increased incidence of liver hypertrophy, splenic extramedullary hematopoiesis with pigment deposition, lung hyperplasia/hypertrophy and reduced bone marrow cellularity were observed. Liver hypertrophy, splenic extramedullary hematopoiesis in males and lung hyperplasia / hypertrophy in both sexes were observed at the lowest dose level and no NOAEL was set.
Increased incidence of primary lung tumours (alveolar/bronchiolar adenomas and carcinomas) was observed in both genders at all diet concentrations in the range 250 – 2250 ppm, equivalent to 29 – 334 mg/kg bw/d and no NOAEL could be set.

Second mouse study
Reference: Nagaoka, 2004
GLP: Yes
Guidelines: OECD 451; US-EPA OPPTS 870.4200; JMAFF 12 NouSan n° 8147
Deviations: a detailed weekly examination was performed only from w41 of the study; blood smears were not taken after 12 months; 78w blood smears were not examined for differential leucocyte count; adrenals, heart and reproductive organs were not weighed at necropsy; only liver, lung, spleen and femoral and sternal bone marrow from terminal kill animals were examined histopathologically. The deviations from the guideline are considered not to affect the validity of the study which was only designed to elucidate a NOAEL for a specific lesion identified in the previous oncogenicity study.

This additional mouse oncogenicity study 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; 5w old; mean bw at start of 7d quarantine: 18.9-25.0 g in males and 17.2-23.0 g in females) were administered 0; 10; 25; 80 and 250 ppm of flonicamid technical in the diet for 78 w (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).
The stability of a 10 ppm diet formulation was evaluated after 28-day storage in the cold and dark room followed by storage during 1 day in the dark and 7 days at room temperature. The homogeneity and achieved concentrations of all diet formulations were investigated pre-dose and once in w13, w26, w52 and w78.

All mice were observed once daily for morbidity / mortality and for clinical signs of toxicity throughout the study period; a palpation was performed weekly from w41. Individual bw and food consumption were recorded once pre-dose, weekly for 13 w and once every 4 w thereafter.

A blood smear was prepared from all moribund animals, where possible, and from all survivors killed at termination. The smears were retained for possible differential leucocyte counting.

All animals dying or killed *in extremis* during the study, and survivors at 78w were subjected to necropsy and full *post mortem* examination of major organs and tissues. The weights of the brain, liver, spleen and kidneys were recorded from all animals killed after 78w. A full range of tissues[32] including left and right lung and bronchus (infused with formalin) and all gross lesions was preserved from all animals. Histopathological examinations were performed on the liver, lung, spleen and femoral and sternal bone marrow from all mice killed after 78w and on all organs and tissues (except lacrimal gland and carcass) retained from animals that died or were killed prematurely; in addition, gross lesions recorded at necropsy from all study mice were subjected to histopathological examination where the study pathologist judged the lesions to be toxicologically relevant.

Chemical analyses of test substance in diets: Homogeneity of test substance in the diets was acceptable since all preparations were within 10% of the target values.

Mortality: Although cumulative mortality was significantly increased in females at 25 and 80 ppm, death rates did not appear to be treatment related because of the lack of dose dependency.

Table 68. Survival incidences

Fate	Number of mice									
	Males					**Females**				
Dose (ppm	**0**	**10**	**25**	**80**	**250**	**0**	**10**	**25**	**80**	**250**
KE/FD	16	13	17	21	16	6	6	13*	14*	13
TK	34	37	33	29	34	44	44	37	36	37
Total	50	50	50	50	50	50	50	50	50	50
KE/FD killed *in extremis*/found dead; TK killed at termination after 78 weeks treatment										

Clinical examinations: There were no treatment-related effects at any dose level on the nature and incidence of clinical findings which were all considered as incidental because occurring only in 1 group and/or without dose dependency.

Bw and food consumption: There were no treatment-related effects at any dose level on bw gain and food consumption (changes were transient and not dose dependent). The overall mean achieved dose levels were 1.203 ± 0.098; 3.136 ± 0.298; 9.995 ± 0.917 and 30.266 ± 2.834 mg/kg bw/d in males and 1.419 ± 0.135; 3.664 ± 0.330; 11.849 ± 1.091 and 36.335 ± 2.953 mg/kg bw/d in females

[32] Adrenals, bone, bone marrow, brain (cerebrum, cerebellum), esophagus, eyeballs, duodenum, jejunum, ileum, colon, cecum, rectum, heart, aorta, kidneys, lacrymal gland, larynx, liver with gallbladder, lungs, lymph nodes (submandibular & mesenteric), mammary gland, nasal cavity, pancreas, pituitary gland, testes, epididymes, seminal vesicles, prostate, ovaries, uterus, cervix, vagina, salivary glands, skeletal muscle, peripheral nerve, skin, spinal cord, spleen, stomach, thymus, thyroid with parathyroid glands, tongue, trachea, urinary bladder, all gross lesions.

Table 69. Mean bw gain and food consumption

	Males					**Females**				
	0 ppm	**10 ppm**	**25 ppm**	**80 ppm**	**250ppm**	**0 ppm**	**10 ppm**	**25 ppm**	**80 ppm**	**250ppm**
Bw (g)										
w1	27.81	27.81	27.80	27.82	27.82	24.19	24.17	24.03	24.26	24.34
w11	39.91	40.44	40.09	40.83	40.37	34.24	33.92	31.92**	31.45**	32.23*
w45	46.30	49.18*	46.91	48.95*	48.75	45.45	47.71	44.72	46.21	46.46
w61	47.10	50.82**	48.94	50.13*	49.13	48.36	50.14	47.35	47.70	48.21
w69	45.93	49.12*	47.93	49.72*	47.17	46.72	48.35	45.64	45.23	47.42
w77	46.64	49.45	47.88	49.61	48.03	47.57	48.68	47.51	46.25	48.20
Food consumption (g/mouse/d)										
w1	5.55	5.71	5.59	5.86	5.56	4.67	4.58	4.74	4.74	4.74
w11	4.86	4.97	5.22*	5.48**	5.19*	5.25	5.04	4.88	5.41	5.29
w45	5.11	5.27	5.37	5.44	5.15	5.48	5.76	5.22	5.25	5.32
w61	4.91	4.82	5.10	5.28	4.99	4.97	5.21	5.43	5.78*	5.69*
w69	5.31	4.90	5.15	5.61	4.92	5.53	5.06	4.98	5.22	5.19
w77	4.92	4.96	5.29	5.17	4.98	5.16	5.00	5.41	5.19	5.18

Necropsy: Gross pathological examination showed a stastically significantly increased incidence of lung masses only in males at 250 ppm (overall incidence rates were 10/50; 14/50; 13/50; 10/50 and 22/50 in males and 12/50; 13/50; 11/50; 14/50 and 17/50 in females from the controls, 10; 25; 80 and 250 ppm groups, respectively). The only significant finding in females was the increased incidence of thickened wall of the uterus at 250 ppm, which was considered as incidental in the absence of histological correlate.

Organ weights: There were no treatment related significant changes in the brain, liver, spleen and kidney absolute and relative weights in any group of either sex, except a significant reduction of absolute kidney weight in females at 250 ppm which is to be considered as incidental in the absence of histological correlate.

Histopathological examination:

- Non neoplastic changes:

➢ *Lung*: Treatment-related effects on the incidence of non-neoplastic lesions were confined to statistically significant increases in the incidences of pulmonary hyperplasia/hypertrophy of the terminal bronchiolar epithelium in both sexes treated at 250 ppm which were only seen in mice killed at termination (after 78w). At the lower dose levels, the incidences of these changes were not statistically significantly different from that of controls in both sexes. These lesions were graded as slight in all groups.

- *Other tissues*: A slight, but significantly increased incidence of centrilobular hepatocellular fatty change in the liver was seen in the 250 ppm females which were subjected to unscheduled necropsy. This findings was considered as incidental. Other statistically significant differences between the control and treated groups in the incidences of other non-neoplastic histopathological alterations were not considered to be treatment-related because no dose-relationship was apparent and/or a similar effect of treatment had not been observed in the dose range 250 - 2250ppm in the first oncogenicity study (Ridder W.E., 2003a).

Table 70. Incidences of selected non neoplastic changes

	Fate										
		Males					**Females**				
Group (ppm)		**0**	**10**	**25**	**80**	**250**	**0**	**10**	**25**	**80**	**250**
Lung											
Hyperplasia	tk	4/34	4/37	4/33	2/29	11/34 *	3/44	5/44	2/37	3/36	11/37 *
	ke/fd	1/16	1/13	1/17	1/21	3/16	0/6	0/6	0/13	1/14	0/13
	total	5/50	5/50	5/50	3/50	14/50 *	3/50	5/50	2/50	4/403	11/50 *
Liver											
Fatty change	tk	10/34	13/37	7/33	10/29	10/34	0/44	0/44	0/37	0/36	0/37
	ke/fd	0/16	1/13	0/17	1/21	0/16	0/6	0/6	0/13	3/14	5/13
	total	10/50	14/50	7/50	11/50	10/50	0/50	0/50	0/50	3/50	5/50*
Kidney											
Tubule dilatation	tk	1/9	1/11	6/10	0/4	0/6	3/44	5/44	1/37	2/36	3/37
	ke/fd	0/16	2/13	3/17	5/21*	0/16	0/16	1/6	1/13	1/14	1/13
	total	1/25	3/24	9/27	5/25	0/22	0/50	6/50	2/50	3/50	4/50
Dilated pelvis	tk	0/9	0/11	2/10	0/4	1/6	0/44	1/44	0/37	0/36	0/37
	ke/fd	2/16	3/13	6/17	13/21**	6/16	0/6	0/6	0/13	0/14	0/13
	total	2/25	3/24	8/27	13/25	7/22	0/50	1/50	0/50	0/50	0/50
ke/fd killed *in extremis*/found dead; tk killed at termination after 78 weeks treatment; * $p < 0.05$											

- Neoplastic changes:

Lung: Single or multiple alveolar/bronchiolar adenomas of the lungs were statistically significantly increased in males at 250 ppm.; the total number of males at 250 ppm bearing either type of pulmonary tumor was significantly higher than the controls. 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 56w of treatment in the control, 10 ppm; 25 ppm; 80 ppm and 250 ppm groups, respectively. The incidences of pulmonary epithelial adenoma and carcinoma in males treated at up to 80 ppm, and in females at all dose levels up to 250 ppm, were not significantly different from the control incidences. The NOAEL for lung lesions was 10.0 mg/kg bw/day.

Table 71. Incidence of pulmonary neoplastic lesions

	Fate										
		Males					**Females**				
Group		**0**	**10**	**25**	**80**	**250**	**0**	**10**	**25**	**80**	**250**

(ppm)											
Lung Alveolar / bronchiolar adenoma											
	tk	8/34	11/37	8/33	7/29	15/34	10/44	8/44	11/37	14/36	12/37
	ke/fd	0/16	0/13	4/17	4/21	6/16**	0/6	0/6	0/13	0/14	1/13
	total	8/50	11/50	12/50	11/50	21/50**	10/50	8/50	11/50	14/50	13/50
Nature of pulmonary alveolar / bronchiolar lesions											
Total examined	50	50	50	50	50	50	50	50	50	50	50
Adenomas											
Single		7	8	9	11	16	9	6	9	9	10
Multiple		1	3	3	0	5	1	2	2	5	3
Total		8	11	12	11	21**	10	8	11	14	13
Carcinomas											
Single		3	6	3	4	7	1	2	2	3	3
Multiple		0	0	0	0	2	0	2	0	0	0
Total		3	6	3	4	9	1	4	2	3	3
Total n° of mice with primary lung tumour		11	16	15	14	27**	10	12	12	16	16

A series of experiments designed to investigate the cause of lung tumour formation in CD-1 mice was performed, based on the measurement of cell proliferation rates in the terminal bronchiolar region of the lung:

- lung cell cycle analysis using BrdU in the mouse
- a comparative study of lung cell cycle analysis in rats and mice
- a reversibility study of the lung effect in the mouse
- lung cell cycle analysis using BrdU in the mouse following dietary exposure to flonicamid and its metabolites
- a comparison of cell cycle analysis in the mouse lung following dietary exposure to flonicamid or isoniazid in 3 mouse strains.

4.10.1.2 *Carcinogenicity: inhalation*

No data

4.10.1.3 *Carcinogenicity: dermal*

No data

4.10.2 Human information

No data available

4.10.3 Other relevant information

Lung cell cycle analysis (using BrdU) in the mouse (Nomura M., 2003a)

Groups of 5 males[33] mice (Crj:CD-1(ICR) strain, 7-8 w old, 37-33 g at start of study) were administered dietary concentrations of 0 (diet only); 80; 250; 750 and 2250 ppm of flonicamid technical for 3 consecutive days.
Mice were observed at least twice daily for mortality / morbidity and clinical signs of toxicity; the food consumption was recorded daily and individual bw were recorded pre-test and at termination.

The aim of this study was to investigate the etiology of the increased incidence of primary lung tumors seen in the mouse oncogenicity studies; accordingly, the terminal bronchiolar region of the lung, was selected for investigation because increased incidences of lung tumors occurred in this region in the mouse long term studies.

All mice were given intraperitoneal injections of 100 mg/kg bw of Bromodeoxyuridine (BrdU) at 14 and 2 h prior to the termination of treatment; the terminal kill was performed at 72h after initiation of feeding administration and all mice were subjected to necropsy; the lungs were removed with the trachea and infused directly with 4% paraformaldehyde solution to fix the organ *in toto*. A sample of small intestine was also taken from each animal and similarly fixed to confirm BrdU reaction. The preserved organs were subjected to immunohistopathological examination. Initially, tissue samples were taken to 4μm paraffin sections and then subjected to the strepto-avidin-biotin method using an antibody against BrdU as the primary antibody. The slides were successively treated with primary antibody for 30 minutes at room temperature. Biotinylated anti-mouse IgG and streptoavidin conjugated with horseradish peroxidase were applied to the slides for 10 and 4 minutes, respectively. Slides were counterstained with hematoxylin. One thousand BrdU-negative and positive nuclei in epithelial cells of the terminal bronchiolar region of the lung were scored for BrdU uptake. Counting was performed in duplicate by each of 2 operators under blind conditions. The final results were the mean of 4 sets of counts. The validity of the immunochemical technique was verified by the positive reaction in the G zone (cell reniewal area) of the small intestine. The BrdU labelling index was expressed as the number of positive nuclei per 1000 nuclei counted.

Results

There was a dose-related increase in the BrdU-labelling indices in lung epithelial cells of the groups treated at 250; 750 and 2250 ppm, with statistical significance ($p<0.01$) at the 750 and 2250 ppm levels. The mean, SD and range of BrdU-labelling indices were 7.8 ± 2.4 (range 5 - 11); 7.8 ± 2.2 (5 - 10); 16.0 ± 8.9 (5 - 28); 25.2 ± 6.9** (17 - 33) and 28.4 ± 5.3** (21 - 34) in the groups treated at 0; 80; 250; 750 and 2250 ppm, respectively. Although not statistically significant, the approximate doubling of the labelling index at 250 ppm is to be considered as treatment related. These results indicate that there was an increase in the incidence of cells in the DNA synthesis phase of the cell cycle i.e. increased cell at 250 – 2250 ppm proliferation. There was no effect of treatment on the BrdU-labelling index at 80 ppm.

Conclusion

Flonicamid elicits a dose-related increase in cell proliferation in the epithelial cells of the terminal bronchiolar region of the lung in 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. This study suggests that a 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.

Comparative study of lung cell cycle analysis in rats and mice (Nomura M., 2003b)

[33] Only males were studied since they were more sensitive for lung tumors induction than females

Groups of 5 females mice (Crj:CD-1(ICR) strain; 7-8 w old; bw = 27-33 g before arrival) were given dietary concentrations of 0 (diet only) or 2250 ppm flonicamid technical for 3 or 7 consecutive days and groups of 5 females Wistar rats (7-8 w old; bw before arrival = 120-126 g) were similarly given dietary concentrations of 0 (diet only) or 5000 ppm of the test substance for 3 or 7 consecutive days. The dose levels used in this experiment for this study were the highest dose levels used in the oncogenicity studies in rats. and mice.

The animals were observed at least twice daily for mortality / morbidity and clinical signs of toxicity; the food consumption was recorded for 3 and 7 days and individual bw were recorded pre-test and at termination i.e. on d3 or d7).

All animals were given intraperitoneal injections of 100 mg/kg bw of Bromodeoxyuridine (BrdU) in saline at 2 h prior to the termination of treatment; the terminal kills were performed at 3 or 7 days after initiation of feeding administration and all animals were subjected to necropsy; the lungs were removed with the trachea and infused directly with 4% paraformaldehyde solution to fix the organ *in toto*. A sample of small intestine was also taken from each animal and similarly fixed to confirm BrdU reaction. The preserved organs were subjected to immunohistopathological examination. Initially, tissue samples were taken to 4μm paraffin sections and then subjected to the strepto-avidin-biotin method using an antibody against BrdU as the primary antibody. The slides were successively treated with primary antibody for 30 minutes at room temperature. Biotinylated anti-mouse IgG and streptoavidin conjugated with horseradish peroxidase were applied to the slides for 10 and 4 minutes, respectively. Slides were counterstained with hematoxylin. One thousand BrdU-negative and positive nuclei in epithelial cells of the terminal bronchiolar region of the lung were scored for BrdU uptake. Counting was performed in duplicate by each of 2 operators under blind conditions. The final results were the mean of 4 sets of counts. The validity of the immunochemical technique was verified by the positive reaction in the G zone (cell reniewal area) of the small intestine. The BrdU labelling index was expressed as the number of positive nuclei per 1000 nuclei counted.

Results

The group mean BrdU labelling indices of mice treated at 2250 ppm for 3 or 7 days were significantly higher than the control values and the index was higher after 3 days than after 7 days. On the other hand, the group mean BrdU labelling indices of rats treated at 5000 ppm for 3 or 7 days were comparable to the control values (Table 72)

Table 72. Group mean BrdU labelling indices in epithelial cells of the terminal bronchiolar region of lung

Groups	**Dose (ppm)**	**BrdU labelling index (no./10^3) after (days of treatment)**			
		3 d		**7 d**	
		Mean ± SD	Range	Mean ± SD	Range
Mouse	0	3.6 ± 1.1	2 - 5	3.8 ± 1.3	2 - 5
	2250	13.6** ± 3.6	9 - 18	10.4** ± 2.1	8 - 13
Rat	0	3.8 ± 0.8	3 - 5	3.2 ± 1.5	1 - 5
	5000	3.6 ± 1.1	2 - 5	3.8 ± 1.3	2 - 5
** $p < 0.01$					

Conclusion

Flonicamid elicits an increase in cell proliferation in epithelial cells of the terminal bronchiolar region of the lung in female mice after 3 or 7 days of treatment at an average dose level of 380 mg/kg bw/day, but not in female rats at an average dose level of 398 mg/kg bw/day.

Toxicological effects on mouse lung: Reversibility study (Nomura M., 2003c)

Groups of 20 male CD-1 mice (Crj:CD-1(ICR) strain; 34-36 d old; bw = 28 - 31g) were administered dietary concentrations of 0 (diet only) or 2250 ppm flonicamid technical for 28 consecutive days (the dose level selected for this study was the highest dose level used in the oncogenicity study in mice); 5 mice / group were killed at 0; 7; 14 or 28 days after the cessation of treatment.

The mice were observed at least twice daily for mortality / morbidity and clinical signs of toxicity; the food consumption was recorded every 3 and 4 days in a week throughout the treatment and recovery periods; individual bw were recorded pre-test and weekly thereafter and at terminal kill.

All mice were given intraperitoneal injections of 100 mg/kg bw of Bromodeoxyuridine (BrdU) in saline at 2h prior to the terminal scheduled kill (i.e 5 mice/group at 0; 7; 14 or 28 d after the cessation of treatment); the lungs[34] were removed with the trachea and infused directly with 4% paraformaldehyde solution to fix the organ *in toto*. A sample of small intestine was also taken from each animal and similarly fixed to confirm BrdU reaction.

Before fixation in paraformaldehyde, a small piece of lung tissue from the first 2 mice in each group at each sacrifice was preserved in 2.5% glutaraldehyde for electron microscopy.The preserved organs were subjected to immunohistopathological examination. Initially, tissue samples were taken to 4μm paraffin sections and then subjected to the strepto-avidin-biotin method using an antibody against BrdU as the primary antibody. The slides were successively treated with primary antibody for 30 minutes at room temperature. Biotinylated anti-mouse IgG and streptoavidin conjugated with horseradish peroxidase were applied to the slides for 10 and 4 minutes, respectively. Slides were counterstained with hematoxylin.

An additional section of lung tissue from each animal was similarly treated but using polyclonal antibody against Clara cells as the primary antibody (CC-10 staining) and immunohistochemically stained for Clara cells

One thousand BrdU-negative and positive nuclei in epithelial cells of the terminal bronchiolar region of the lung were scored for BrdU uptake. Counting was performed in duplicate by each of 2 operators under blind conditions. The final results were the mean of 4 sets of counts. The validity of the immunochemical technique was verified by the positive reaction in the G zone (cell reniewal area) of the small intestine. The BrdU labelling index was expressed as the number of positive nuclei per 1000 nuclei counted.

Sections of lung tissue from each animal were processed for electron microscopy as ultra-thin, resin-embedded, toluidine blue-stained sections. Representative photographs of the target tissue cells were taken. Hematoxylin-stained sections of lung were also examined by light microscopy.

Results

CC-10 staining in the lung: The reaction to anti-Clara cell antibody was increased in the terminal bronchioles of the lung in all mice killed at the end of treatment without a recovery period. Electron microscopic examination revealed longitudinal elongation and hyperplasia/hypertrophy of the Clara cells, graded trace (grade 1 of 4) in one mouse, minimal (grade 2 of 4) in 3 mice, and trace elongation and minimal hyperplasia/hypertrophy in the other animal. The reaction to treatment was readily reversible within 7 days since all control and treated mice killed after a 7d; 14d or 28d recovery period showed no significant lesions on electron microscopic examination.

BrdU labelling: The group mean BrdU labelling index of mice treated at 2250 ppm for 28 days and killed without a recovery period was significantly higher than the control values. In contrast, the group mean BrdU

[34] The tissue and specific region of the tissue for examination in this experiment, the terminal bronchiolar region of the lung, was selected because increased incidences of lung tumors occurred in this region in the mouse oncogenicity study.

labelling indices of animals treated at 2250 ppm for 28 days and subjected to a recovery period of 7, 14 or 28 days before necropsy were not significantly different from the control values. Thus, cell proliferation in response to flonicamid was readily reversible within 7 days of the cessation of treatment.

Electron microscopy of the lungs: The increased numbers of Clara cells in the terminal bronchiolar region of the lung at the end of 28 days treatment at 2250 ppm was confirmed by electron microscopy. In addition, the cells from these mice exhibited elongation, protruding cytoplasm and a greater degree of cell compaction. The secretory granules in the cytoplasm of the activated Clara cells were also slightly enlarged. However, there was no evidence of secondary deleterious reactions, such as necrosis or inflammation, around the activated Clara cells, suggesting a mitogenic effect rather than a cytotoxicity-mediated response. These ultrastructural changes were readily reversible within 7 days of the cessation of treatment since they were not apparent in animals allowed a 7d; 14d or 28d recovery period after treatment.

Table 73. Group mean BrdU labelling indices in epithelial cells of the terminal bronchiolar region of lung

Recovery period (d)	**Mean BrdU labelling index (no./10^3) in animals treated at:**			
	0 ppm		**2250 ppm**	
	Mean ± SD	Range	Mean ± SD	Range
0	3.4 ± 1.1	2 - 5	9.8** ± 2.8	7 - 14
7	3.8 ± 1.3	2 - 5	3.6 ± 1.3	2 - 5
14	3.2 ± 1.9	1 - 6	3.6 ± 0.9	3 - 5
28	3.6 ± 1.5	2 - 6	3.4 ± 1.1	2 - 5
** $p < 0.01$				

Conclusion

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 303mg/kg bw/d, which is readily and fully reversible within 7 days of the cessation of treatment. Specifically, flonicamid produces a readily and fully reversible elongation and hypertrophy/hyperplasia of the Clara cells in the terminal bronchiolar region of the lung, but does not exert a cytotoxic effect on the activated Clara cell. Therefore, it should be anticipated that pathological progressive effects including BrdU labeling and changes in the number and size of Clara cells originated from mitogenic effects of flonicamid which were rapidly and completely reversible.

Cell cycle analysis in mouse lung following dietary exposure to flonicamid and its metabolites (Nomura M., 2003d)

Groups of 10 male CD-1 mice (Crj:CD-1(ICR) strain; 41-43 d old; bw= 27-33 g) were administered dietary concentrations of 0 (control diet); 2250 ppm of flonicamid technical or 2250 ppm of TFNG, or 2250 ppm of TFNA or 2250 ppm of TFNA-AM; 5 mice/group were killed after either 3 or 7 days of treatment.
The mice were observed at least twice daily for mortality / morbidity and clinical signs of toxicity; the food consumption was recorded for the treatment periods and individual bw were recorded pre-test and at scheduled termination i.e. on d3 or d7.

All mice were given intraperitoneal injection of 100 mg/kg bw of Bromodeoxyuridine (BrdU) at 2 h prior to the scheduled sacrifice i.e. after 3 or 7 days of treatment; the lungs were removed with the trachea and infused directly with 4% paraformaldehyde solution to fix the organ *in toto*. A sample of small intestine was also taken from each animal and similarly fixed to confirm BrdU reaction. The preserved organs were subjected to immunohistopathological examination. Initially, tissue samples were taken to 4μm paraffin sections and then subjected to the strepto-avidin-biotin method using an antibody against BrdU as the primary antibody. The slides were successively treated with primary antibody for 30 minutes at room temperature. Biotinylated anti-mouse IgG and streptoavidin conjugated with horseradish peroxidase were

applied to the slides for 10 and 4 minutes, respectively. Slides were counterstained with hematoxylin. One thousand BrdU-negative and positive nuclei in epithelial cells of the terminal bronchiolar region of the lung were scored for BrdU uptake. Counting was performed in duplicate by each of 2 operators under blind conditions. The final results were the mean of 4 sets of counts. The validity of the immunochemical technique was verified by the positive reaction in the G zone (cell reniewal area) of the small intestine. The BrdU labelling index was expressed as the number of positive nuclei per 1000 nuclei counted.

Results

BrdU labelling: The group mean BrdU-labelling indices in lung epithelial cells in the terminal bronchiolar region of mice given flonicamid were significantly increased, relative to the controls, after both 3 or 7 days of treatment and the increase was greater after 3 days of treatment. In contrast, the BrdU-labelling indices in animals treated with the metabolites TFNG, TFNA and TFNA-AM were not significantly different from control values after 3 or 7 days treatment. Since increased BrdU uptake indicates an increase in the incidence of cells in the DNA synthesis phase of the cell cycle, the increased BrdU labelling indices in mice treated at 2250 ppm flonicamid indicate increased cell proliferation (Table 74)

Table 74. Group mean BrdU labelling indices in epithelial cells of the terminal bronchiolar region of lung

Treatment group (test material / ppm)	**BrdU labelling index (no./10^3) after (days of treatment):**			
	3 days		**7 days**	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
0ppm	3.6 ± 1.1	2 - 4	3.2 ± 1.1	2 - 4
flonicamid / 2250ppm	12.6** ± 3.6	8 - 17	9.4** ± 4.5	3 - 14
TFNG / 2250ppm	3.0 ± 0.7	2 - 4	3.4 ± 1.5	2 - 5
TFNA / 2250ppm	3.4 ± 1.5	1 - 5	3.4 ± 1.1	2 - 5
TFNA-AM / 2250ppm	3.8 ± 0.8	3 - 5	3.6 ± 1.5	2 - 5
** $p < 0.01$				

Conclusion

Flonicamid at 2250 ppm, equivalent to a dose of 389 mg/kg bw/day, elicits an increase in cell proliferation in the epithelial cells of the terminal bronchiolar region of the lung in male mice. Since the 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.

Comparison of cell cycle analysis in mouse lung following dietary exposure to flonicamid or Isoniazid in 3 mouse strains (Nomura M., 2003e)

Groups of 5 male mice of 3 different strains [Crj:CD-1 ICR strain (50-52 d old; bw = 28-35g); Jcl:B6C3F1 strain (50-52 d old; bw= 22-27g) and C57/6J strain (47-52 d old; bw = 20-22g)] were administered dietary concentrations of 0 (control diet) or 2250 ppm of flonicamid technical or 2250 ppm of isoniazid for 3 consecutive days.

The mice were observed at least twice daily for mortality / morbidity and clinical signs of toxicity; the food consumption was recorded for the 3-d treatment periods and individual bw were recorded pre-test and at scheduled termination.

All mice were given intraperitoneal injection of 100 mg/kg bw of Bromodeoxyuridine (BrdU) at 2 h prior to the scheduled sacrifice i.e. after 3 days of treatment; the lungs were removed with the trachea and infused directly with 4% paraformaldehyde solution to fix the organ *in toto*. A sample of small intestine was also taken from each animal and similarly fixed to confirm BrdU reaction. The preserved organs were subjected

to immunohistopathological examination. Initially, tissue samples were taken to 4μm paraffin sections and then subjected to the strepto-avidin-biotin method using an antibody against BrdU as the primary antibody. The slides were successively treated with primary antibody for 30 minutes at room temperature. Biotinylated anti-mouse IgG and streptoavidin conjugated with horseradish peroxidase were applied to the slides for 10 and 4 minutes, respectively. Slides were counterstained with hematoxylin.

An additional section of lung tissue from each mouse was similarly treated but using polyclonal antibody against Clara cells as the primary antibody (CC-10 staining) and immunohistochemically stained for Clara cells.

One thousand BrdU-negative and positive nuclei in epithelial cells of the terminal bronchiolar region of the lung were scored for BrdU uptake. The number of CC-10 positive cells in the control groups of each mouse strain was also recorded and expressed as a percentage of the cells scored. Counting was performed in duplicate by each of 2 operators under blind conditions. The final results were the mean of 4 sets of counts. The validity of the immunochemical technique was verified by the positive reaction in the G zone (cell reniewal area) of the small intestine. The BrdU labelling index was expressed as the number of positive nuclei per 1000 nuclei counted. The proportion of Clara cells in control mouse lung was compared with that in Wistar rat lung. The samples of control rat lung were obtained as paraffin blocks from a previous study (Nomura, 2003b).

Results

BrdU labelling: In the CD-1 mice, the BrdU-labelling indices in lung epithelial cells in the terminal bronchiolar region were significantly increased, relative to the controls, after 3 days of treatment with flonicamid or isoniazid and the greatest increase was seen in isoniazid-treated mice. On the other hand, flonicamid treatment at 2250 ppm did not affect the BrdU labelling index in the B6C3F1 and C57/6J strain mice, but isoniazid treatment produced statistically significant increases in the BrdU labelling index in these 2 strains.

CC-10 staining in the lung: The mean proportion of Clara cells in the terminal bronchiolar region in the lung of control mice of all 3 strains was remarkably consistent with mean values of 79, 81 and 80% in CD-1, B6C3F1 and C57/6J strains, respectively. On the other hand, control Wistar rat lung (study number AN-2130) had a mean of 35% Clara cells. This species difference between mice and rats for the number of Clara cells in the terminal bronchioles of the lung had been reported in the literature[35].

Table 75. Group mean BrdU labelling indices and proportion of Clara cells in the epithelium of the terminal bronchiolar region of lung in CD-1, B6C3F1 and C57/6J mice

Species / strain	Treatment group (test material / ppm)	BrdU labelling index (no./10^3)		% Clara cells	
		Mean ± SD	Range	Mean ± SD	Range
Mouse / CD-1	0ppm	3.4 ± 1.3	2 - 5	79 ± 3.2	75 - 83
	flonicamid / 2250ppm	10.6** ± 3.6	5 - 15	-	-
	Isoniazid / 2250ppm	23.6** ± 2.7	22 - 27	-	-
Mouse / B6C3F1	0ppm	3.0 ± 0.5	2 - 5	81 ± 3.2	77 - 85

[35] Mohr U. et al, Pathobiology of the aging mouse ; vol.1 ; respiratory system, development, growth and aging of the lungs, ILSI press, 1996
Boorman G.A. et al., Pathobiology of the Fisher rat; Academic press, 21, Lung, 1990

	flonicamid / 2250ppm	3.0 ± 1.4	3 - 5	-	-
	Isoniazid / 2250ppm	12.0** ± 3.3	7 - 16	-	-
Mouse / C57/6J	0ppm	2.0 ± 0.8	1 - 3	80 ± 3.4	75 - 83
	flonicamid / 2250ppm	2.2 ± 0.7	1 - 3	-	-
	Isoniazid / 2250ppm	4.0* ± 1.6	2 - 6	-	-
Rat / Wistar[a]	0ppm	NA	NA	35 ± 5.1	29 - 42
* $p < 0.05$; ** $p < 0.01$; [a] from study no. AN-2130 (Nomura, 2003b); - not examined; NA not applicable					

Conclusion

Cell proliferation of the lung terminal bronchiolar epithelial cells (as indicated by BrdU index) was significantly increased after 3-day dietary administration of 2250 ppm flonicamid technical (equivalent to 299 mg/kg bw/d) in CD-1 mice only, but not in B6C3F1 nor in C57 mice; on the other hand, the 3-day dietary administration of 2250 ppm isoniazid (equivalent to 298 mg/kg bw/d) induced proliferation of lung terminal bronchiolar epithelial cells which was more pronounced than with flonicamid in CD-1 mice, and less marked in B6C3F1 and C57 mice. Contrary to the differential response seen among the 3 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 3 strains employed, namely 80% of the terminal bronchiolar cells were Clara cells.

Therefore, it appears that the CD-1 mouse exhibited strain-specificity to the cell proliferating effect of flonicamid, conversely to the effect of isoniazid which was not strain-specific. In Wistar rats, the proportion of Clara cells in the terminal bronchiolar region of the lung was low relative to the mouse.

Overall conclusion

There is unequivocal evidence that flonicamid elicits a dose-related increase in cell proliferation in the epithelial cells in this region of the lung in CD-1 mice at dietary concentrations in the range 250 – 2250 ppm, equivalent to 40.9 - 339.3 mg/kg bw/d. The effect is obvious after 3 or 7 d of treatment, as assessed by increased BrdU labelling indices and a NOEL for the effect was established as 80 ppm, equivalent to 12.3 mg/kg bw/d (Nomura, 2003a).

In another experiment (Nomura, 2003c), the induction of cell proliferation in the mouse lung was shown to be readily and fully reversible within 7 days of the cessation of treatment and ultrastructural examination of the terminal bronchiolar region of mouse lung demonstrated that the Clara cell was the specific target for the effects of flonicamid since this cell type undergoes a reversible hypertrophy/hyperplasia and morphological elongation in response to the administration of flonicamid. Furthermore, the area surrounding activated Clara cells shows no evidence of secondary tissue responses such as necrosis or inflammatory changes. Thus, the pattern of effects suggests a mitogenic effect of flonicamid rather than cytotoxicity.

Cell proliferating activity is restricted to flonicamid since none of the 3 major metabolites found in mammals, i.e. TFNG, TFNA and TFNA-AM, causes any lung cell proliferation in the mouse (Nomura, 2003d).

In 2 further experiments (Nomura, 2003b; Nomura, 2003e), it was demonstrated that flonicamid does not elicit a lung epithelial cell proliferation effect in female rats exposed to diet concentrations of 5000 ppm (equivalent to *ca.* 397 mg/kg bw/d), or in B6C3F1 and C57/6J strain mice exposed to 2250 ppm (equivalent

to 316 and 306 mg/kg bw/d). Thus, the response to flonicamid exhibits both species-specificity and mouse strain-specificity. Isonicotinic acid hydrazide (Isoniazid), which is structurally similar to flonicamid, was also shown to have a species-specific effect on lung epithelial cell proliferation in mice. Although the response was not strain-specific, strain-sensitivity was evident because the effect in the CD-1 mouse was more marked than in B6C3F1 and C57/6J strain mice. Furthermore, the effect of Isoniazid in the CD-1 mouse was more marked than that of flonicamid. Investigation of the Clara cell complement of mouse lung in the terminal bronchiolar region demonstrated that approximately 80% are Clara cells and that the proportion is very similar in all 3 mouse strains examined. In contrast, the proportion of Clara cells in Wistar rat lung is only approximately 35%.

Control CD-1, B6C3F1 and C57/6J strain mice show reducing spontaneous epithelial cell proliferation rates in the order CD-1 > B6C3F1 > C57/6J, which correlates with published spontaneous incidences of bronchiolo-alveolar tumour incidences (Maita, *et al*., 1988, Dixon & Maronpot, Rao, *et al*., 1988)[36]. Unciliated Clara cells are widely regarded as progenitor cells that undergo further differentiation, and as such reflect the degree of maturity of lung epithelial cell differentiation (Boers, *et al*., 1999, Wuenschell, *et al*., 1996, Plopper, *et al*., 1992)[37]. The Clara cell complement of mouse lung in the terminal bronchiolar region is relatively constant at approximately 80% in CD-1, B6C3F1 and C57/6J strain mice. In contrast, the proportion of Clara cells in Wistar rat lung is only approximately 35%. (Kato, *et al*., 2002)[38]. The aging CD-1 mouse has a high incidence of spontaneous bronchioloalveolar 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.

- The results of the investigative studies strongly support the contention that flonicamid induces cell proliferation, by a mitogenic mechanism, in the Clara cells of bronchiolar epithelium of the lung, which is species-specific, strain-specific and rapidly and fully reversible on cessation of treatment. The threshold dose for the mitogenic effect lies between 12.3 and 40.9 mg/kg bw/d, and there is a NOEL of 12.3 mg/kg bw/d, as measured by the BrdU labelling index. The NOEL established in the 2nd mouse oncogenicity study is in agreement with this mechanism-based NOEL. The putative mechanism is consistent with the findings in the comprehensive genotoxicity testing battery, including a Comet assay in mouse lung tissue, which demonstrate that flonicamid is not mutagenic or genotoxic at the DNA, gene and chromosome levels of organisation. Thus, there is a robust science-based rationale for the contention that the potential of flonicamid to induce bronchioloalveolar tumours is unique to the mouse, and specifically to the CD-1 strain.

- The human lung can be regarded as the most mature with respect to epithelial cell differentiation because the Clara cell complement is very low at 22 and 11% in respiratory and terminal bronchioles, respectively (Kato, *et al*., 2002). Furthermore, epidemiological studies on Isoniazid have revealed no relationship between its therapeutic use and the occurrence of human lung tumours (IARC, 1987)[39].

It is therefore concluded that the mechanism of lung tumours formation in mice is not relevant to humans.

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38 Kato, M. *et al*., (2002): New Histology, Nihon Iji Shinpo-sha, p 95.

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

4.10.4 Summary and discussion of carcinogenicity

Flonicamid has no carcinogenic potential in rats.
In mice, it was concluded from the studies investigating the mechanism of lung tumour induction that flonicamid exhibited a species-specific cell proliferating effect. In addition this effect was strain-specific as the response was more pronounced in the CD-1 mouse, conversely to isoniazid whose effect does not exhibit strain-specificity. Moreover the CD-1 mouse has a considerably higher spontaneous rate of lung lesions than does the B6C3F1 mouse, which has a higher incidence than the C57BL mouse. In these studies, increased mitogenesis has been demonstrated as the most plausible mode of action of flonicamid and the sequence of events for mouse lung tumours has clearly been delineated as predominantly arising from bronchiolar Clara cells and undergoing the sequence of increased proliferation followed by hyperplasia, leading to adenomas and ultimately carcinomas. More importantly, there is also evidence that in absence of early changes of increased proliferation and hyperplasia, adenomas and carcinomas do not develop later. This sequence of events and increased susceptibility have been further explored at the molecular level with recent cloning and identification of a specific gene, the pulmonary adenoma susceptibility 1 locus (Pas 1) identifying the increased susceptibility of the CD-1 strain compared to other strains[40] .
Consequently no classification is proposed.

Note 1:
The EFSA conclusion of the peer review of flonicamid has come to a different opinion as the experts could not conclude about classification Carc. cat.3, R40 and decided to highlight this issue to EChA.
Note 2:
In 2005 the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs evaluated the carcinogenic potential of flonicamid and they classified flonicamid into the category "suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential".

4.10.5 Comparison with criteria

Rationale for classification as a Carcinogen:

The CLP criteria for classification as a category 2 Carcinogen (category 3 carcinogen according to Directive 67/548/EEC) are as follow :
"Substances are classified as a category 2 Carcinogen when evidence is 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 effects observed in mice are considered to be species and strain specific and not relevant to humans; these do not fulfil any criterion for carc cat 3 R40 or CLP cat 2 H351 classification.

4.10.6 Conclusions on classification and labelling

No classification is required for flonicamid under either Directive 67/548/EEC or the CLP Regulation.

[40] Alvin M. Malkinson: Genetic Studies of Lung Tumor Susceptibility and Histogenesis in Mice, Environmental Health Perspective, Vol. 93, page 149 – 159, 1991

4.11 Toxicity for reproduction

Table 76. Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Preliminary reproductive (dietary) study **Rat Wistar** 0, 50, 200, 1000, 2000ppm equivalent to 0; 2.86; 11.49; 57.7 and 114.2mg/kg bw/d for the males and 5.28; 20.8; 103.7 and 214mg/kg bw/d for the females, respectively	Renal tubular basophilia and hyaline droplet deposition	-	Takahashi (2002a)
OECD 416 **Dietary 2-generation reproductive study** **Rat Wistar** 0, 50, 300, 1800ppm	No reproductive effects NOAEL > 1800 ppm (109 mg/kg bw/d) Male rat-specific renal alterations only. NOAEL females 300 ppm (28 mg/kg bw/d) ↓ ovary/adrenal weights (P); ↓ uterus weights (F1 / F2); delayed vaginal opening (F1); Renal tubular vacuolation (P / F1); ↑ gonadotrophins (F1); ↓ 17β-estradiol (F1);	-	Takahashi(2002b)
Preliminary teratogenicity (oral) study **Rat Wistar** 0, 30, 100, 300, 1000 mg/kg bw/d	Maternal deaths and ↓ bw gain at 1000 mg/kg bw/d (>MTD) ; no effects in fetus	-	Hojo (2002a)
Preliminary teratogenicity (oral) study **Rabbit Japanese White** 0, 3, 10, 30 mg/kg bw/d	Bw loss, ↓ food consumption; ↓ live litter size at 30 mg/kg bw/d	-	Takahashi (2002c)
OECD 414 **Oral teratogenicity study** **Rat Wistar** 0, 20, 100, 500 mg/kg bw/d	NOAEL maternal & developmental 100 mg/kg bw/d ↑ placental weight; ↑ weight and liver, hypertrophy. Kidney : tubular vacuolation ↑ incidence of cervical rib	Treatment was administered from d6 through d19 *post coitum*, rather than from d6 through d15, as specified in 88/302/EEC. The deviation does not adversely affect the validity of the study	Hojo (2002b)
OECD 414 **Oral teratogenicity study** **Rabbit Japanese White** 0, 2.5, 7.5, 25 mg/kg bw/d	↓ food consumption / weight gain at 25 mg/kg bw/d NOAEL developmental 25 mg/kg bw/d	-	Takahashi (2002d)
IKI-220 (Flonicamid): effects on hormonal levels in 28-day and 90-day feeding studies	Investigative study	-	Nomura (2006)

Method	Results	Remarks	Reference
Hormonal examination in female Jcl: Wistar rats at pro-estrous	Investigative study	-	Inui (2006)
AN-1666 (Flonicamid): a preliminary teratogenicity study in rats 0, 10, 100, 500 mg/kg bw/d gavage	No effects in fetus: no pre-implantation loss, no extra cervical rib	Preliminary study	Takahashi (2006)

4.11.1 Effects on fertility

4.11.1.1 *Non-human information*

Reference: Takahashi (2002a)
GLP: Yes
Guidelines: not applicable (dose range finding study)

Groups of 8/sex Wistar rats (8 w old at start of treatment; bw = 243-260 g for males and 159-175 g for females) were administered dietary concentrations of 0; 50; 1000 and 2000 ppm of flonicamid[41] technical for approximately 10w i.e. from 3w prior to mating, 1w of mating and continuing through 3w of gestation and 3w of lactation periods.

Test diets were prepared monthly (stability was demonstrated for 53 days after preparation in the 28-day dose range finding study; Kuwahara M., 2002a); homogeneity and concentration of test substance in each test diet were analysed.

Cohabitation of females with males was initiated after completion of 3 w of treatment on a 1:1 basis and the day on which sperm was observed in a vaginal smear or the presence of a vaginal plug, was designated d0 of gestation; duration of mating did not exceed 2w and females which did not show evidence of copulation during the mating period were housed individually until necropsy. Each litter was reduced to 8 pups (4 pups/sex where possible) on d4 of lactation. Culled pups were killed and subjected to a gross necropsy examination. Pups were weaned on d 21 of lactation. After weaning of the pups, all parents and weanings were terminated.

Parental rats were examined at least once daily for mortality and clinical signs and a detailed clinical examination was performed on weighing days (males were weighed on d-1, at weekly intervals during premating and breeding periods and at necropsy; females were weighed at weekly intervals during pre-mating period, on d0; d7; d14 and d20 of gestation, on d0; d7; d14 and d21 of lactation and at necropsy. Food consumption was measured on weighing days, except during the mating period.

Daily vaginal smears were examined from all females to detect the stage of oestrous for 1w prior to mating. The mating index, the fertility index and the duration of gestation were calculated and live and dead pups were counted and their sexes determined on d0 of lactation.

All parental rats were killed and subjected to necropsy after weaning of the litters. The numbers of implantation sites in the uterine horns were counted. Gross pathological lesions were recorded in survivors

[41] Batch n° 9809 ; purity = 98.7%

and decedents. Liver and kidney weights were recorded in rats surviving to terminal necropsy and samples from all parental rats were processed for histopathological examination.

Litters were examined daily for clinical signs or mortality during the lactation period and a detailed external examination was performed on weighing days. Pups found dead were immediately subjected to necropsy. Pups were sexed externally on d0; pup bw were recorded on d0; d4; d7; d14 and d21 of lactation in each litter. All weaned pups were killed and subjected to gross necropsy shortly after weaning (d22 - d26 of age); no organs and tissues were retained.

Parental generation:

Mortality, morbidity, bw and food consumption**:** There were no deaths, nor treatment-related clinical signs at any dose level throughout the study. Bw, bw gain and food consumption of both sexes were comparable between all treated groups and not significantly different from control values. The overall mean achieved dose levels for the entire treatment period were 2.86; 11.49; 57.7 and 114.2mg/kg bw/d for the males and 5.28; 20.8; 103.7 and 214mg/kg bw/d for the females of the 50; 200; 1000 and 2000 ppm groups, respectively.

Table 77. Group mean test substance intake (mg/kg bw/d)

Group mean test substance intake [range] (mg/kg bw/d)		
Group	**Males**	**Females**
50 ppm	2.86 [2.51 - 3.37]	5.28 [3.11 - 10.78]
200 ppm	11.49 [9.77 - 13.75]	20.8 [12.4 - 42.3]
1000 ppm	57.7 [48.7 - 69.9]	103.7 [59.9 - 208.5]
2000 ppm	114.2 [97.0 - 136.8]	214 [126 - 442]

Reproductive performance: There were no treatment-related effects on reproductive performance indicators at any dose level (Table 6.6.1.1-2). There were no significant differences between the control and treated groups with respect to incidence of females with normal oestrous cycles, the mating, fertility and gestation indices, duration of gestation and the mean number of implantation sites. Although the fertility indices at 1000 ppm (75.0%) and 2000 ppm (62.5%) were lower than the control value (87.5%), the differences were considered not to be treatment-related because there was no such effect at 1800 ppm in the main study (Takahashi, K., 2002b)

Table 78. Group mean reproductive indices and selected reproductive parameters

Group (ppm)	**0**	**50**	**200**	**1000**	**2000**
Mating index[a] (%)	100	100	87.5	100	100
Fertility index[b] (%)	87.5	87.5	100	75.0	62.5
Gestation index[c] (%)	100	85.7	100	100	100
Duration of gestation (d)	22.0	22.2	22.4	22.0	22.2
N° of implantation sites	16.3 ± 1.3	14.6 ± 6.1	15.9 ± 1.9	16.5 ± 0.8	15.4 ± 1.3

N° of pups born	15.4 ± 1.0	14.8 ± 1.3	12.7 ± 3.3	15.5 ± 1.8	14.0 ± 1.6
Sex ratio	0.533	0.529	0.644	0.582	0.514
Viability index (%)					
Lactation d-0	90.5	89.8	91.9	78.7	92.4
Lactation d-4					
Lactation d-21	98.3	94.5	95.5	98.7	98.8
	100.0	100	100	100	100
[a] no. mated / no. paired for mating x 100; [b] no. pregnant / no. mated x 100; [c] no. normal parturitions / no. pregnancies x 100					

Pathological examination:

Gross necropsy findings: Significantly increased incidences of pale coloured kidneys were seen in males from the 1000 and 2000 ppm groups only. There were no other treatment-related gross lesions at necropsy (few cases of pelvic dilatation of the kidney and/or reddish tear in both sexes, of atrophy of the testes and luminal dilatation of the uterus occurred at similar incidences in all groups including controls).

Organ weights: The absolute and relative kidney weights were significantly increased only in males of the 2000 ppm group). The liver weight of both sexes was unaffected by treatment at all dose levels.

Histopathological examination: The only significant change was the increased incidences of hyaline droplet deposition in the proximal tubular epithelium of the kidneys in males of the 200, 1000 and 2000 ppm groups which was accompanied by tubular basophilia at 1000 and 2000 ppm and granular casts in dilated tubules at 2000 ppm. The observed renal tubular lesions in males are indicative of a male rat-specific phenomenon, α2-microglobulin-mediated nephrotoxicity.

Table 79. Gross necropsy changes, group mean organ weights and histological findings in parental rats

	Males					Females				
Dose (ppm)	**0**	**50**	**200**	**1000**	**2000**	**0**	**50**	**200**	**1000**	**2000**
Gross necropsy findings										
Kidney										
Pale in color	0/8	0/8	0/8	4/8*	7/8***	0/8	0/8	0/8	0/8	0/8
Pelvic dilatation	1/8	3/8	1/8	0/8	0/8	-	-	-	-	-
Testes atrophy	0/8	0/8	0/0	0/8	1/8	-	-	-	-	-
Uterus luminal dilatation	-	-	-	-	-	0/8	0/8	0/8	1/8	0/8
Organ Weight										
Liver										
Absolute (g)	16.97	16.61	17.22	17.06	17.46	15.42	15.58	15.92	16.85	16.62
Relative[a] (%)	3.89	3.81	3.84	3.86	3.91	5.86	5.89	5.94	6.14	6.15
Kidney										
Absolute (mg)	1.366	1.421	1.407	1.499	1.633***	0.989	1.011	1.038	1.078	1.071
Relative[a] (%)	0.313	0.327	0.314	0.339	0.366***	0.376	0.383	0.389	0.393	0.397
Histopathological findingsin the kidney										
Pelvic dilatation	1/8	3/8	1/8	0/8	0/8	3/8	2/8	2/8	0/8	0/8
Hyaline droplets[b]	0/8	0/8	5/8*	8/8***	8/8***	0/8	0/8	0/8	0/8	0/8
Tubular basophilia	0/8	0/8	0/8	8/8***	8/8***	0/8	0/8	0/8	0/8	0/8
Granular casts[c]	0/8	0/8	0/8	1/8	8/8***	0/8	0/8	0/8	0/8	0/8

* $p < 0.05$; *** $p < 0.001$; [a] relative to bw; [b] deposition in proximal tubules; [c] in dilated tubules.

Offspring:

Clinical signs and viability: There were no treatment-related clinical signs in the pups at any dose level and pre-weaning mortality was comparable between control and treated groups. There were no treatment-related effects or statistically significant differences between the control and treated groups in the mean litter number at birth, sex ratios, and the viability indices on d0; d4 and d21 of lactation. All viability indices on d0; d4 and d21 of lactation were comparable to control values, with the exception of the d0 index at 1000 ppm (78.7%) which was lower than the control value of 90.5%. However, the d0 index at 2000 ppm was 92.4% and a treatment-related effect was not inferred. There were no treatment-related adverse clinical findings in F1 progeny at any dose level and pup weight gains during lactation were unaffected by treatment at all dose levels

Pathological examination:. No treatment-related gross lesions were evident at necropsy at any dose level in the pups culled on lactation d4, those dying before weaning or in those killed shortly after weaning.

Although dilatation of the renal pelvis occurred only in male pups from the treated groups (2/46 at 50ppm, 3/51 at 200 ppm, 2/44 at 1000 ppm and 3/40 at 2000 ppm) and not in control male pups, an effect of treatment was not inferred because this gross lesion occurred in control male pups of both generations in the main study (Takahashi, K., 2002b) at incidences of up to 7.9%.

The NOAEL **in the dose range finding reproductive toxicity study** in the Wistar rat was 200 ppm (equivalent to 11.49mg/kg bw/d) in parental males based on the occurrence of histopathological findings in the kidneys indicative of a male rat-specific phenomenon, α2-microglobulin-mediated nephrotoxicity at dose levels ≥ 1000 ppm. The NOEL in female parental animals and F1 generation progeny was 2000 ppm (equivalent to 214mg/kg bw/d) i.e. the highest dose used in the study and the NOEL in males was 50 ppm (equivalent to 2.86 mg/kg bw/d), based on renal effects.

Reference: Takahashi (2002b)
GLP: Yes
Guidelines: OECD 416; EPA OPPTS 870.3800; JMAFF, 12-Nousan-n° 8147

In the main multigeneration study, groups of 24/sex Wistar rats (Jcl:Wistar strain; 5 w old at start of study; bw: 124-137 g in males and 99-111 g in females) were administered dietary concentrations of 0 (diet only); 50; 300 and 1800 ppm of flonicamid technical through 2 generations; P and F1 generation animals (24 /sex /generation) were treated over a 10w pre-mating period or following weaning, throughout 1-2w mating, 3w gestation and 3w lactation periods (i.e. for 17- 18w in total).
Tests diets were prepared 1 or 2 times / month; stability was demonstrated for 53 days after preparation in the 28-day dose range finding study (Kuwahara M, 2002a); homogeneity was determined on the first test diets and concentration of test substance in each test diet was analysed at approx. monthly intervals.

• Parental generations:
Mating strategy: P and F1 parental generations were mated on a 1:1 basis within the same treatment groups, following 2 w of daily estrous cycle monitoring of females. During pairing, the females were examined daily for the presence of a vaginal plug and sperm in vaginal smears. The day of observation of sperm in a vaginal smear or of the presence of a vaginal plug, was designated d0 of gestation. Females not mating with the first male pair within 2 w were paired with another male of the same group which had already mated. The day of completion of parturition was designated d0 of lactation. On d4 of lactation, each litter was reduced by random selection to 8 pups (4 pups/sex) where possible; culled pups were killed and remaining pups were weaned on d21 of lactation. P and F1 generation parental rats of both sexes were killed and subjected to necropsy after weaning of the litters. Groups of 24 /sex weaned F1 progeny were randomly selected to produce the F2 generation (1 or 2/sex/litter) from among litters born during the 4 or 5 d in which the largest number of parturitions occurred. The unselected F1 weanlings were killed and subjected to necropsy. The F1 progeny selected to produce the F2 generation were treated and mated (avoiding sibling matings) in a similar manner to the P generation. At weaning of the F2 generation, 24 F2 females/group were selected and grown on until vaginal opening had occurred, after which they were killed. Unselected F2 weanlings were killed at weaning.
Follow up of adult generations: P and F1 generation parental rats were examined at least once daily for morbidity/mortality and clinical signs and a detailed clinical examination was performed on weighing days. Animals found dead were necropsied immediately. Parental males were weighed on d1, at weekly intervals during premating and breeding periods and at necropsy; parental females were weighed on d1, at weekly intervals during premating period, on d0; d7; d14 and d20 of gestation and on d0; d7; d14 and d21 of lactation and at necropsy; females not producing a litter were weighed on the presumed d25 of gestation, weekly thereafter and at necropsy. Food consumption was measured on weighing days, except during the mating period. Achieved dose levels were calculated for weekly intervals.

Mating, fertility and gestation indices were calculated. The duration of gestation was calculated and live and dead pups were counted and their sexes determined on d0 of lactation.

All P and F1 parental animals were killed and subjected to necropsy after weaning of the litters. The P generation females were killed in confirmed diestrous or metestrous, and F1 females were killed in confirmed proestrous. The numbers of implantation sites in the uterine horns were counted and gross pathological lesions were recorded.

Serum samples from 8 F1 parental rats/sex/group, confirmed to have been fertile, were selected to investigate possible effects of flonicamid on serum gonadotrophin and sex hormone levels and the binding affinity to α- and β-estrogen receptors. The hormone assays included measurements of follicle stimulating hormone and luteinising hormone in both sexes, testosterone in males and progesterone and 17β-estradiol in females.

Sperm heads and sperm were collected from the right testis and caudal epididymis from all P and F1 male parental rats; the number of testicular sperm was determined by hemocytometer; the number and motility of caudal epididymal sperm were analysed by a computerised videomicrographic system. The numbers were expressed per tissue and per gram tissue and the motility was recorded as % motile. The morphology of 200 caudal epididymal sperm/rat was examined microscopically after fixation.

Liver, kidney, brain, thyroid, adrenal, pituitary, spleen, ovary, uterus (including cervix and oviduct), testis, epididymis, seminal vesicle including coagulating glands and prostate weights were recorded in surviving P and F1 parental animals, and samples of liver and kidney from all parental animals were processed for histopathological examination. Ten animals/sex/group of both generations from the groups treated at 0 or 1800 ppm were selected for histological examination of the reproductive organs and pituitary, thyroids and adrenals. The testes were closely examined for abnormalities of spermatogenesis and the numbers of primordial follicles in the ovaries were recorded. In addition, the reproductive organs and pituitary, thyroids and adrenals of all pairs failing to mate or produce a litter and all females showing abnormalities of parturition, or exhibiting total litter loss were examined histologically. The thyroids of all P generation males, ovaries and adrenals of P generation females, seminal vesicles and coagulating glands of F1 generation males and the spleen of F1 generation females treated at 0 or 1800 ppm were also examined histologically because of significant weight differences at 1800 ppm.

• Offsprings:
F1 and F2 generation litters were examined daily during lactation for clinical signs or mortality and a detailed external examination was performed on weighing days. Each pup was sexed externally on lactation d0. The viability index was calculated on d0; d4 and d21 of lactation. Dead pups were subjected to necropsy. Pup bw were recorded on d0; d4; d7; d14 and d21 of lactation. After weaning, selected progeny were examined daily for sexual maturation, from d35 in males for balano-preputial separation and from d27 in females for vaginal patency, until completion, at which time bw was recorded. Anogenital distances were measured by calliper in F2 progeny on lactation d4, and expressed as absolute distances and to the cube root of bw.

All culled pups on d-4 were killed and subjected to gross necropsy, and weanlings not selected to form the next generation were killed and subjected to necropsy shortly after weaning on d25 - d27. One weanling/sex/litter from the F1 and F2 generations was selected for brain, spleen, thymus and uterus (including cervix and oviduct) weight recording. F2 females selected for recording of vaginal patency were killed and subjected to necropsy after occurrence.

Parental generations:

There were no treatment-related deaths or clinical signs at any dose level in the P and F1 parental generations; a single F1 female at 300ppm was found dead during the pre-mating treatment period and exhibited a mass in the thoracic cavity at necropsy.

There was no effect of treatment on bw at any dose level in either sex or parental generation. The group mean bw gain were significantly lower than control values (approx. 6%), during the first w of treatment only,

in P males and F1 females at 1800 ppm; such changes were considered likely to reflect diet palatability. Treatment-related effects on food consumption were confined to slightly, but significantly reduced consumption (up to 10.9%) during the first 2 w of gestation and the first w of lactation in P generation females at 1800 ppm; there were no other effects on food consumption.

Table 80. Bw gains (selected results) and overall mean achieved dose level

Dose levels	**0 ppm**		**50 ppm**		**300 ppm**		**1800 ppm**	
	P	**F1**	**P**	**F1**	**P**	**F1**	**P**	**F1**
BODY WEIGHT GAIN (g)								
Males								
Premating								
w 0-1	51±3	43±3	50±2	42±4	49±4	42±4	48±4**	41±4
w0-10	263±22	339±24	266±23	336±25	257±26	334±23	262±23	325±22
Treatment w 0-18	318±27	394±29	313±26	388±39	311±28	392±29	316±25	382±28
Females								
Premating								
w 0-1	27±5	34±2	36±3	34±2	26±4	33±2	37±3	32±2***
w0-10	138±14	184±11	136±11	189±13	136±12	187±19	132±10	180±14
Gestation d 0-20	107±9	104±17	103±20	107±13	105±14	111±15	101±10	113±11
Lactation d 0-21	18±11	16±14	23±14	8±16	28±15*	13±20	36±15***	25±13
Treatment w 0-18	170±13	222±12	169±10	228±14	167±13	226±20	171±16	222±16
OVERALL TEST SUBSTANCE INTAKE : MEAN [RANGE] (mg/kg bw/d								
Males			3.07 [2.3-5.00]	3.39 [2.3-5.4]	18.3 [13.5-30.3]	20.7 [13.9-32.8]	109.1 [82.8-179.0]	124.8 [85.9-197.3]
Females			4.67 [2.71-10.2]	4.95 [2.8-10.9]	28.2 [16.3-62.8]	30.5 [17.5-67.0]	163.8 [94.2-367.9]	176.8 [96.1-402.6]

There was no effect of treatment at any dose level in either generation, on the duration and cyclicity of estrous, the proportion of animals mating or the average time to mating, the fertility and gestation indices and the duration of gestation. Although the mean duration of gestation in P generation females at 1800 ppm was slightly, but significantly longer than the control value, the difference of 0.4 d is within the limits of accuracy

of measurement[42]; in addition, a more accurate determination of the completion of parturition in F1 females, by 3 times daily observation, did not reveal an effect of treatment and therefore, the difference in the P generation is considered not to be an effect of treatment.
There was no effect of treatment at any dose level in either generation on the mean numbers of implantation sites and pups born and sex ratios; all values were not significantly different from the controls with the exception of the sex ratio of F2 progeny at 50 ppm which is considered fortuitous based on the absence of a dose-relationship and a slightly high control value.

Table 81. Summary of estrous cycle data,mating behaviour, group mean fertility, gestation indices, duration of gestation and pregnancy outcoume-parental rats

Dose levels	**0 ppm**		**50 ppm**		**300 ppm**		**1800 ppm**	
	P	**F1**	**P**	**F1**	**P**	**F1**	**P**	**F1**
N° examined	24	24	24	24	24	23	24	24
ESTROUS CYCLE DATA AND MATING BEHAVIOUR								
Estrous cycle length (d)	4.0	4.1	4.0	40	4.0	4.0	4.0	4.0
Normal cycling[a] (%)	100	100	100	100	100	100	100	100
% mating								
Males	100	100	95.8	100	100	100	100	95.8
Females	100	100	100	100	100	100	100	100
N° of d until mating	1.0	1.0	1.6	1.0	1.0	1.0	1.2	1.6
GROUP MEAN FERTILITY AND GESTATION INDICES AND DURATION OF GESTATION								
Fertility index[b] (%)	91.7	87.5	100	91.7	95.8	91.3	91.7	100
Gestation index[c] (%)	100	100	91.7	100	100	100	100	100
Duration of gestation (d)	22.1	22.0	22.3	22.2	22.3	22.2	22.5*	22.1
PREGNANCY OUTCOME								
N° implants (± SD)	15.0±1.4	13.2±3.4	13.9±4.1	13.9±3.2	13.9±3.1	14.5±3.0	13.5±2.4	14.5±1.3
N° pups (± SD)	13.5±1.3	12.5±3.4	13.8±2.0	12.4±3.2	12.7±3.0	13.2±3.2	12.6±2.4	14.0±1.3

[42] in P generation parental females, both the time of copulation and the time of completion of parturition were determined at a single time each day even though these events could have occurred any time during the previous 24h.

Sex ratio	54.7	57.9	0.8	48.9*	52.2	54.3	48.2	53.1

[a] proportion of females showing normal estrous cyclicity; [b] no. pregnant / no. mated x 100; [c] no. normal parturitions / no. pregnancies x 100; * $p < 0.05$

There was no effect of treatment at any dose level in the P and F1 generation males on the group mean testicular sperm head counts and epididymal sperm counts expressed either as per gram testis or epididymis or as per testis or epididymis. Sperm motility and morphology were also unaffected by treatment at all dose levels. The group mean number of ovarian primordial follicles in 10 selected F1 females at 1800 ppm (144 ± 29) was comparable to the control group value (153 ± 41) and was unaffected by treatment.

Table 82. Summary of group mean sperm analysis data

Dose levels	**0 ppm**		**50 ppm**		**300 ppm**		**1800 ppm**	
	P	**F1**	**P**	**F1**	**P**	**F1**	**P**	**F1**
N° examined	24	24	24	24	24	24[a]	24	24
Sperm (head) count (x 10^6/g)								
Testis	130 ± 14	130 ± 25	131 ± 16	144 ± 11	128 ± 16	138 ± 29	129 ± 15	141 ± 15
Epididymes	718 ± 214	618 ± 152	763 ± 138	719 ± 131	813 ± 163	647 ± 168	793 ± 194	727 ± 175
Motility (% motile)								
	76.2 ± 5.8	77.3± 4.6	76.3 ± 7.0	81.7±5.6	77.2 ± 6.5	79.4±6.7	75.0 ± 7.1	80.6±7.3
Morphology (% normal)								
	96.4 ± 2.6	93.5± 6.8	93.5 ± 8.2	97.0±2.2	97.6 ± 1.5	91.4±20.7	95.2 ± 3.9	95.4±2.8

[a] 23 males examined for sperm motility

Pathological/histopathological examination

Treatment-related gross pathological changes were confined to pale kidneys in P and F1 generation parental males at 1800 ppm, which occurred at incidences of 24 / 24 and 10 / 24, respectively, compared with zero incidences in all other treated and control groups. There were no other treatment-related findings in males and none at any dose level in the P and F1 generation females.

Absolute and relative kidney weights were statistically significantly increased in both P and F1 parental males at 1800 ppm; a minimal, but significantly increased relative kidney weight was also seen in the F1 males at 300 ppm and was not considered biologically relevant because the absolute weights were not significantly altered and no histopathological changes were seen at this dose level. Absolute and relative thyroid weights were significantly increased in P generation males, but not in F1 males at 1800 ppm and therefore this finding was also considered not to be treatment related, inasmuch as no histopathological alterations were seen. Significantly decreased absolute testis weight in F1 males at 1800 ppm was not

considered to be an effect of treatment because relative weight was comparable to the control value; there were also increased relative liver weight and decreased seminal vesicle weights in F1 males at 1800 ppm.

In the P generation females, the absolute and relative ovary weights and the relative adrenals weight were significantly decreased at 1800 ppm, but not at lower dose levels. In the F1 females, the relative weights of the liver, the kidneys and the spleen were significantly increased at 1800 ppm, but not at lower dose levels, but these differences were not considered as adverse findings because there were no histopathological alterations identified. Occasional other statistically significant organ weight differences occurred (increased absolute pituitary and kidney weight in F1 females), but none showed a relationship to dose and were therefore considered unrelated to treatment.

Table 83. Summary of selected absolute and relative (to bw) organ weights - parental animals

Groups	0 ppm		50 ppm		300 ppm		1800 ppm	
	P	F1	P	F1	P	F1	P	F1
	MALES							
Kidney	1374	1358	1392	1349	1409	1406	1601***	1588***
Abs. (mg)	0.307	0.296	0.314	0.298	0.320	0.307*	0.329***	0.355***
Rel. (%)								
Liver								
Abs. (mg)	16914	15828	16950	16101	16516	15916	17180	16103
Rel. (%)	3.77	3.44	3.82	3.55	3.74	3.48	3.85	3.59**
Thyroid								
Abs. (mg)	22.3	19.6	22.0	19.9	24.1	22.1	25.3**	20.5
Rel. (%)	0.0050	0.0043	0.0050	0.0044	0.0055	0.0048*	0.0057**	0.0046
Testes								
Abs. (mg)	1618	1648	1589	1635	1599	1653	1615	1600**
Rel. (%)	0.362	0.360	0.360	0.363	0.364	0.362	0.363	0.359
	FEMALES							
Kidney								
Abs. (mg)	1000	976	1017	1020	1004	1033*	1030	1033
Rel. (%)	0.366	0.345	0.370	0.352	0.368	0.359	0.373	0.364**
Liver								
Abs. (mg)	11889	10856	12081	11489	11965	11463	12512	11883
Rel. (%)	4.34	3.83	4.39	3.97	4.38	3.97	4.51	4.18**
Ovaries								
Abs. (mg)	68.4	62.1	64.7	60.6	65.5	60.9	60.1***	57.8
Rel. (%)	0.025	0.022	0.024	0.021	0.024	0.021	0.022	0.020
Adrenals								
Abs. (mg)	42.8	37.6	41.2	38.5	42.7	39.7	39.8	37.2
Rel. (%)	0.0156	0.0133	0.0150	0.0133	0.0156	0.0138	0.0144*	0.0131
Spleen								
Abs. (mg)	644	633	635	662	654	670	652	684
Rel. (%)	0.235	0.224	0.231	0.229	0.239	0.232	0.236	0.240*
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$								

There were no treatment-related histopathological alterations at any dose level in either generation in the tissues of the male or female reproductive tract, endocrine organs and the liver. Specifically, no histopathological changes were seen in testes and ovaries in animals at 1800 ppm that showed organ weight differences from the controls.

There were, however, treatment-related histopathological alterations in the kidneys of both sexes in both generations, but the morphology of the alterations differed between the sexes: in male P and F1 animals at 1800 ppm, hyaline droplet deposition in the proximal tubules, tubular basophilic change and the presence of granular casts in dilated tubules were evident at very high incidences (data from the 28-d toxicity study indicate that the hyaline droplets and granular casts contain the male rat-specific protein α2μglobulin). Although hyaline droplet deposition was also evident in both male generations at 300 ppm, the alteration was not accompanied by the degenerative changes observed at the higher dose level, and was not, therefore, considered to be an adverse effect. In female parental animals, the renal alteration was characterized by vacuolation of proximal tubular cells at 1800 ppm only.

Table 84. Summary of incidences of selected histopathological findings in the kidneys - parental animals

Dose levels	**0 ppm**		**50 ppm**		**300 ppm**		**1800 ppm**	
	P	**F1**	**P**	**F1**	**P**	**F1**	**P**	**F1**
MALES (N° examined)	**24**	**24**	**24**	**24**	**24**	**24**	**24**	**24**
Hyaline droplets (proximal tubular cells)	0	0	0	0	23***	24***	24***	24***
Tubular basophilic change	0	0	0	0	0	0	24***	21***
Granular casts in dilated tubules	0	0	0	0	0	0	21***	23***
FEMALES (n° examined)	**24**	**24**	**24**	**24**	**24**	**23 + 1[a]**	**24**	**24**
Vacuolation of proximal tubular cells	0	0	0	0	0	0	22***	24***

[a] incidental death in pre-mating phase; *** $p < 0.001$

Offspring:

Daily clinical examination of F1 and F2 generation pups did not show any treatment-related clinical signs at any dose level during the lactation and post-weaning periods. Similarly, pup viability throughout lactation was unaffected by treatment (similar numbers of pups were lost due to maternal cannibalism in all groups including controls) and all viability indices were comparable to the control values, with the exception of the d4 viability index for F1 progeny at 50 ppm which was significantly greater than the control value (no pups were lost in this group). There was no effect of treatment at any dose level in either sex or generation on group mean pup bw during lactation.

Table 85. Viability index during lactation and group mean pup bw of F1 and F2 progeny

Groups	**0 ppm**	**50 ppm**	**300 ppm**	**1800 ppm**

	F1		F2		F1		F2		F1		F2		F1		F2	
VIABILITY INDEX[A] DURING LACTATION(%)																
d-0	91.5		91.3		88.2		95.2		85.8		92.6		85.9		89.9	
d-4	96.7		98.8		100*		99.7		95.9		97.4		97.1		96.9	
d-21	100		100		100		99.4		99.4		99.4		100		99.5	
GROUP MEAN PUP WEIGHT (G) ON LACTATION DAY																
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
d-0	5.8	5.5	5.9	5.5	5.8	5.5	6.0	5.7	6.0	5.6	6.1	5.7	6.1	5.6	5.9	5.7
d-4	10.3	9.9	10.8	10.2	10.3	909	11.0	10.6	10.8	10.3	11.2	10.7	10.8	10.6	10.7	10.3
d-7	17.0	16.3	17.4	16.5	17.1	16.5	17.5	16.7	17.9	16.9	17.8	17.1	17.7	17.2	17.7	16.8
d-14	35.8	34.5	37.1	35.5	36.1	35.1	37.2	36.1	36.5	35.1	37.9	36.7	36.2	35.3	37.9	36.6
d-21	55.5	53.4	57.3	54.3	55.9	54.2	58.1	56.0	56.3	54.0	58.7	56.4	55.7	54.2	57.4	55.7

[a] d-0 = pups alive on d 0 / pups born x 100, d-4 = pups alive on d-4 / pups alive on d-0 x 100, d-21 = pups alive on d-21 / pups alive on d- 4 x 100; * $p < 0.05$

Sexual maturation of male F1 progeny, as assessed by bw and age at the time of balano-preputial separation, was unaffected by treatment at all dose levels. However, vaginal opening was significantly delayed in F1 females at 1800 ppm only by a mean of 1.5 d, at which time their bw were slightly greater than the controls; although vaginal opening in F2 females was, on average, 0.8 d later and bw were slightly greater than the controls, the differences were not statistically significant. The group mean ano-genital distances in both sexes of F2 progeny on lactation d4 were not significantly different from control values at all dose levels. There were no treatment-related effects on the incidence of clinical signs, bw gains, food consumption and gross findings at necropsy of the F2 females selected for examination of vaginal opening.

Table 86. Group mean age and bw at completion of preputial separation or vaginal opening, and ano-genital distance – progeny

Generation	**Group (ppm)**	N° M / F examined	**Preputial separation complete**		**Vaginal opening complete**	
			Age (days)	**bw (g)**	**Age (days)**	**bw (g)**
F1	**0**	24 / 24	41.9 ± 1.1	187.5 ± 10.7	32.6 ± 1.3	106.7 ± 6.7
	50	24 / 24	41.6 ± 1.4	185.9 ± 10.8	32.5 ± 2.0	107.6 ± 13.1
	300	24 / 23	41.7 ± 1.3	187.6 ± 9.5	32.7 ± 2.2	107.2 ± 11.6
	1800	24 / 24	41.9 ± 1.7	186.0 ± 11.6	34.1 ± 2.0**	112.0 ± 11.8
F2	**0**	24 / 24	-	-	32.3 ± 1.5	101.8 ± 9.8
	50	24 / 24	-	-	32.7 ± 1.6	107.4 ± 8.5
	300	24 / 24	-	-	32.0 ± 1.5	104.6 ± 9.0
	1800	24 / 24	-	-	33.1 ± 1.6	107.5 ± 9.5
			Males		**Females**	
			Bw (g)	**Ano-genital distance**	**Bw (g)**	**Ano-genital distance**

				(mm)	(relative[a])		(mm)	(relative[a])
F2	**0**	24 / 24	10.6	6.06±0.88	0.276	10.1	2.86±0.39	0.133
	50	24 / 24	10.8	6.58±0.64	0.298	10.3	3.12±0.32	0.144
	300	24 / 23	10.9	6.41±0.68	0.289	10.5	3.10±0.32	0.142
	1800	24 / 24	10.5	6.33±0.54	0.289	10.2	3.12±0.30	0.144
- not measured; ** p < 0.01; M male; F female; [a] relative to the cube-root of bw								

No treatment-related gross lesions were evident at necropsy in F1 and F2 progeny, including pups dead on d0 of lactation, neonatal deaths, pups culled on d4 and pups dying or killed subsequently. There were no treatment-related effects at any dose level in progeny of either generation on the absolute and relative organ weights of brain, thymus and spleen; however, the absolute and relative uterus weights were significantly reduced in F1 progeny at 1800 ppm but this finding was not seen in F2 weanlings.

Table 87. Selected absolute and relative organ weights – progeny

Groups	**0 ppm**		**50 ppm**		**300 ppm**		**1800 ppm**	
	F1	**F2**	**F1**	**F2**	**F1**	**F2**	**F1**	**F2**
Group mean uterus weight								
Abs. (mg)	77.6 ± 14.1	69.2 ± 16.5	73.8 ± 19.2	66.7 ± 19.0	76.0 ± 14.6	64.1 ± 11.0	62.8 ± 14.5*	61.0 ± 11.6
Rel (%)	0.106 ± 0.0209	0.091 ± 0.0193	0.100 ± 0.0206	0.087 ± 0.0183	0.105 ± 0.0164	0.084 ± 0.0100	0.086 ± 0.0164*	0.081 ± 0.0129

Hormone measurements:

As effects suggestive of interference with the normal sexual maturation of female progeny were seen at 164 mg/kg bw/d (reduced uterus weights in F1 & F2 generation and slightly delayed vaginal opening in F1 progeny), further investigation regarding hormonal levels was conducted.
Serum samples from 8 F1 parental rats/sex/group, confirmed to have been fertile, were selected to investigate possible effects of flonicamid on serum gonadotrophin and sex hormone levels and the binding affinity to α- and β-estrogen receptors. The hormone assays included measurements of follicle stimulating hormone and luteinising hormone in both sexes, testosterone in males and progesterone and 17β-estradiol in females.

Serum levels:
There were no treatment-related effects at any dose level on serum gonadotrophin and testosterone concentrations in males. In females, a dose dependent increase of serum LH concentration was seen at 300 and 1800 ppm and the serum concentration of FSH was increased at 1800 ppm, but not at 300 ppm. LH and FSH concentrations at 50 ppm were unaffected by treatment. Progesterone concentrations were unaffected by treatment at all dose levels. The concentration of 17β-estradiol was lower than the control group by 26.7% in the 1800 ppm group, but the difference was not statistically significant. Nevertheless, it is considered to be a biologically relevant effect of treatment, based on the in-life observations of delayed vaginal opening, and reduced ovary and uterus weights. The absence of an effect on male serum gonadotrophin concentrations suggests that the primary effect of flonicamid in the female is one of reduction

in the concentration of circulating 17β-estradiol, rather than a direct stimulation of female gonadotrophin secretion.

Table 88. Summary of group mean serum hormone concentrations - F1 progeny

Groups	**0 ppm**		**50 ppm**		**300 ppm**		**1800 ppm**	
	Males	**Females**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Group mean serum concentration								
LH (ng/mL)	1.50	1.55	1.38	1.82	1.27	2.03*	1.47	2.33**
FSH(ng/mL)	7.23	5.09	6.65	5.74	7.11	5.56	6.96	8.06**
Testosterone (ng/mL)	1.27		1.55		0.93		1.65	
Progesterone (ng/ml)		14.5		16.6		18.2		14.1
17β-E (pg/mL)	-	57.6	-	63.6	-	56.5	-	42.2
* p < 0.05; ** p < 0.01								

Two additional studies (Inui, 2006; Nomura, 2006) were performed in the rat in order to clarify these findings (isolated increased LH at 300 ppm, increased LH and FSH and decreased 17β-estradiol at 1800 ppm).

As historical control data on the physiological LH and oestradiol levels in the Wistar rat from the same laboratory for a similar time period were not available, an additional study (Inui, 2006) was carried out to investigate normal fluctuation of LH, FSH and 17β-oestradiol levels in normal female Wistar rats of multiparous animals at pro-estrous. Forty five retired female breeders were sampled from the 1st to 5th pro-estrous day and at termination on the 6th pro-estrous day. Results are summarized in figure 1.

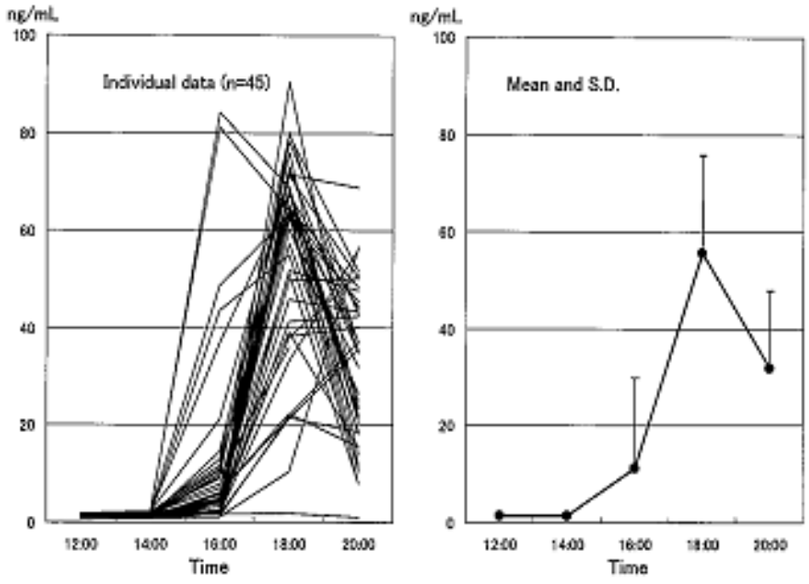

Fig. 1. The pattern of plasma LH level obtained from the tail vein at each sampling time

Since there were no data at 13h00 and 15h00 in the present study condition, estimated values were calculated from the individual data at 12h00, 14h00 and 16h00. Maximum and minimum value, mean, standard deviation and coefficient of variance at each sampling time are summarized in the table under.

(ng/mL)

Time	12:00	13:00	14:00	15:00	16:00	18:00	20:00
Min	0.82	0.80[a]	0.71	0.97[a]	1.00	1.91	1.08
Max	1.90	2.06[a]	2.27	42.72[a]	84.24	90.29	68.86
Mean	1.43	1.43[a]	1.43	6.30[a]	11.18	55.65	31.95
S.D.	0.32	0.31[a]	0.39	9.45[a]	18.83	20.12	15.99
C.V.(%)	22.8	21.4[a]	27.2	149.8[a]	168.5	36.2	50.1

[a] : Estimated value obtained from individual animals.

At 12h00 and 14h00 all animals showed base-line value around 1.43 ng/mL. However at 16h00, about 1/3 animals showed a rapid increase in LH level of 11.18 ± 18.83 ng/mL. Thereafter, mean value revealed a peak of 55.65 ± 20.12 ng/mL at 18h00. At 20h00, 37/45 individuals decreased their values when compared to previous time point, but 8/45 individuals still showed increasing pattern.
Hormonal levels at termination are summarized in figure 2:

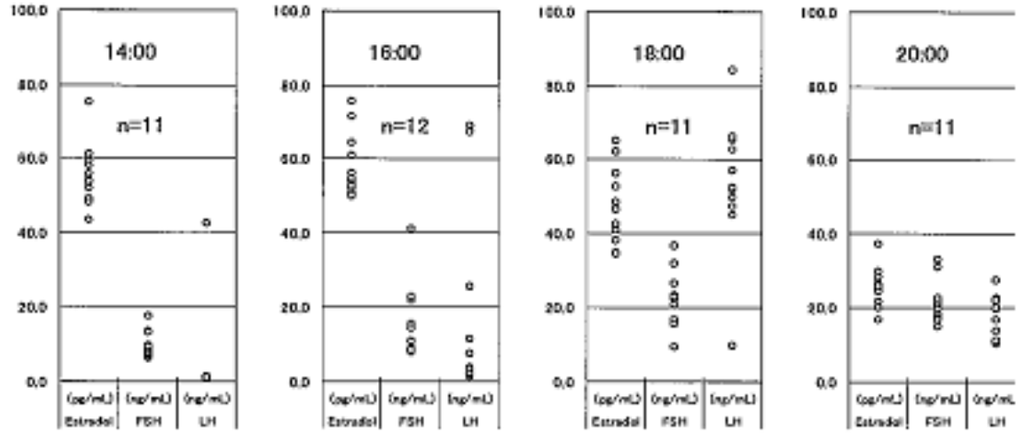

Fig. 2. Individual hormone levels at each sampling time.
Unit: pg/mL for estradiol and ng/mL for FSH and LH.

The plasma levels of 17β-estradiol seemed to be higher at 14h00-16h00 but gradually decreased at 18h00-20h00. On the other hand, LH levels revealed a clear surge pattern with a peak at 18h00. FSH showed the same pattern as LH, but less markedly.

Discussion:

The experimental conditions of this study, completed on September 13, 2006 are comparable to the ones of the reproduction study in the rat (study report 99-0085). In the reproduction study (n° 99-0085 and 01-8008), blood sampling was performed between 13h00 and 15h00 at pro-estrous and the sequence of blood sampling was: control group, low dose, middle dose and high dose groups. This means that sampling of the control animals was conducted early in the sampling time and that sampling of high dose groups was done late in the sampling time. It can be therefore concluded that the rats of higher dose groups were already in the early stage of LH surge, explaining the significant difference from control and very small standard deviation. Indeed, as shown in table 6, LH values during the 13h00-15h00 interval vary from 0.71 to 42.72 ng/mL. This report strongly indicates that the minor changes observed at 300 and 1800 ppm in LH levels are within the range of basal oscillation at pro-estrous in normal female Wistar rats. It is therefore concluded that the increase in LH levels observed in the 300 and 1800 ppm groups of the reproduction study was not a treatment-related effect but an observation induced by experimental bias.

An additional study (Nomura, 2006) was performed to assess the true effect of flonicamid on hormone levels in the Wistar rat treated at the same doses, during 28 and 90 days, and in the same conditions as in the reproduction study. Moreover, an additional dose of 100 ppm was set between 50 and 300 ppm to evaluate a possible threshold of the effect. The hormonal values measured in the two studies are summarized in the tables below:

28-day feeding study:

Dose level (ppm)	Luteinizing hormone (LH) (ng / ml)	Follicle stimulating hormone (FSH) (ng / ml)	17β-estradiole (pg / ml)
0	2.14 ± 0.27	7.71 ± 1.87	30.1 ± 17.4
50	2.22 ± 0.25	6.88 ± 0.60	26.9 ± 11.1
100	2.00 ± 0.26	6.34 ± 1.14	30.7 ± 5.02
300	2.10 ± 0.22	6.75 ± 0.97	39.9 ± 16.2
1800	2.34 ± 0.51	7.28 ± 0.54	33.7 ± 15.3

90-day feeding study:

Dose level (ppm)	Luteinizing hormone (LH) (ng / ml)	Follicle stimulating hormone (FSH) (ng / ml)	17β-estradiole (pg / ml)
0	2.19 ± 0.49	9.6 ± 4.9	52.2 ± 34.8
50	2.36 ± 0.47	10.5 ± 4.8	68.0 ± 50.4
100	2.31 ± 0.23	11.5 ± 6.1	43.0 ± 36.9
300	2.24 ± 0.51	9.4 ± 4.5	60.2 ± 49.8
1800	2.11 ± 0.26	10.1 ± 5.2	57.1 ± 39.6

There were no differences in the levels of LH, FSH and 17β-estradiol compared with controls after administration of flonicamid in the diet for 28 days or 90 days. This study clearly demonstrates that there is no treatment-related dose-response relationship on hormonal homeostasis and confirms again that the differences observed in hormonal levels in the rat reproduction study were due to a longer blood sampling time period of 2 hours compared with the present study where blood sampling was performed within ca. 30 minutes.

Regarding the slight decrease in 17β-oestradiol (not significant) observed in the 1800 ppm group in the reproduction study, this effect can be related to the renal findings (renal tubular vacuolation, kidneys are the target organs of flonicamid) at this dose level, inducing higher urine volume and higher excretion of 17β-oestradiol. In addition to the experimental bias, the slight increase in LH and FSH levels may also reflect a positive feed-back at this dose level, in response to decreased 17β-oestradiol levels.

Binding affinity for estrogens receptors:

α- and β-receptor estrogen binding by flonicamid was very low at all tested concentrations in the range 10^{-2} - 10^{-9}M. These data suggest that the treatment-related findings in the main study were not mediated by a receptor binding mechanism and were likely related to the reduced blood 17β-estradiol levels.

Table 89. estrogen α- and β-receptor inhibition by flonicamid - F1 progeny

Estrogen α- and β-receptor inhibition by flonicamid				
	Mean % inhibition of:			
Concentration (log M)	α-receptor		β-receptor	
	Standard	flonicamid	Standard	flonicamid
- 11.0	0.7	5.3	2.6	- 12.1
- 10.0	10.3	0.4	1.1	- 12.1
- 9.1	16.6	-	4.5	-
- 9.0	-	0.1	-	- 12.7
- 8.7	32.4	-	14.0	-
- 8.0	64.7	3.0	34.9	- 13.8
- 7.3	86.7	-	61.5	-
- 7.0	-	1.3	-	- 14.1
- 6.3	94.7	-	79.8	-
- 6.0	-	0.9	-	- 13.6
- 5.3	103.8	-	85.2	-
- 5.0	-	5.0	-	- 12.4
- 4.0	-	19.1	-	1.3
* $p < 0.05$; ** $p < 0.01$				

Conclusion on hormonal measurements:
In the rat reproduction study several findings including delayed vaginal opening, reduced absolute and relative uterus weight observed in the 1800 ppm group, along with equivocal results in some hormone levels (isolated increased LH at 300 ppm, increased LH and FSH and decreased 17β-oestradiol at 1800 ppm) raised some concern about potential anti-oestrogenic effects of flonicamid. Further investigation enable to conclude that these 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.

Conclusion of the multigeneration study
In the 2 generation reproductive toxicity study, flonicamid did not affect fertility and fecundity in both sexes and the NOAEL was 109 and 164 mg/kg bw/d in males and females, respectively.

4.11.2.1 *Human information*

No data available.

4.11.3 Developmental toxicity

4.11.3.1 *Non-human information*

Rat studies

Reference: Hojo (2002a)
GLP: Yes
Guidelines: not applicable (dose range finding study)

In the **preliminary developmental study in rats**, groups of 8 naturally-mated females Wistar rats (Jcl:Wistar strain; 13 w old; bw = 180-230 g) were given by gavage 0 (vehicule); 30; 100; 300 and 1000 mg/kg bw/d of flonicamid technical suspended in 1% carboxymethylcellulose (dose volume = 10 mL/ kg bw) from d6 through d19 of gestation.

Prior to initiation of the study, dosing suspensions containing 1 or 200 mg/mL flonicamid technical (i.e. below and above the low dose and the high dose of this study) were analysed for stability after storing in cold room (5°C) during 0; 7 and 14 d and for homogeneity.

All females were observed at least once daily for clinical signs and mortality, and a detailed physical examination was performed at weighing intervals. Individual bw and food consumption were recorded on d0; d6; d9; d12; d15; d18 and d20 of gestation. Animals found dead prior to termination were immediately necropsied. All surviving females were killed on d20 of gestation and subjected to necropsy and *post mortem* examination of major organs and tissues (gross lesions were recorded but maternal tissues were not retained). The ovaries and uterine contents of pregnant animals were examined. The gravid uterus weight, the numbers of corpora lutea and implantation sites, the numbers of live and dead fetuses was recorded. Resorbed embryos or dead fetuses were classified as implantation site, placental remnants or macerated fetuses. If no implantation sites were grossly apparent, the uterus was stained with 10% ammonium sulfide solution to detect early resorption. Live fetuses were sexed and the weights of individual placentae and live fetuses were recorded. The fetuses were examined to detect external abnormalities including those in the body orifices. All fetuses and placentae were discarded.

Test diet analysis: The 1 and 200mg/mL formulations were found to be homogeneous, with coefficients of variation of 1.3 and 1.1%, respectively, for 3 samples/concentration. The formulations were stable at ca. 5°C for 14 days at which time 101 and 100% nominal concentrations remained, respectively (therefore, dosing suspensions were prepared twice during the study at an interval of 12 days). Achieved concentrations in the first formulations prepared were in the range 93 - 100% nominal concentration.

Maternal findings:
6/8 females from the high dose group died during gestation d9-d13 from which, 3 exhibited decreased locomotor activity prior to death. There were no treatment related clinical signs in any group, except vaginal hemorrhage or a white discharge in 2 females from the high dose group. Bw loss occurred in the high dose females during d6 to d9 (significant) and during d6 - d12 (non significant); there was no effect on bw gain at dose levels up to and including 300 mg/kg bw/d. The food consumption at 1000 mg/kg bw/d was significantly reduced on d6 - d9 and d18 - d20 and no effect on food consumption occurred at lower dose levels.

Table 90. Group mean bw gain and food consumption

Dose (mg/kg bw/d)	0	30	100	300	1000
Mean bw gain (g)					
d6-9	7±4	6±3	7±4	9±4	-4±7**

d6-12	22±4	19±3	22±5	22±4	10±14
d6-20	96±17	93±6	99±11	91±32	93±4
Mean food consumption (g/d)					
d6-9	16.6±0.8	16.3±1.0	16.1±0.9	16.0±1.0	10.2±3.1*
d9-12	17.9±1.2	16.7±0.9	17.8±1.5	16.6±0.8	12.0±5.6
d18--20	18.8±1.0	17.9±1.0	19.4±1.5	18.9±1.3	15.3±1.8*
* p < 0.05; ** p < 0.01					

Necropsy findings:
There were no treatment-related gross necropsy findings in maternal rats at any dose level, although 1 decedent female from the 1000 mg/kg bw/d group exhibited showed red spots in the forestomach and another hemorrhage in the uterus.

Reproductive parameters:
All females surviving at termination of the study were proved to be pregnant except 2/8 in the control group and 1/8 in the 30 mg/kg bw group; in addition 1/8 female at 300 mg/kg group had resorptions only. There were no significant changes between the treated and control groups with respect to gravid uterine weights, numbers of corpora lutea and implantation sites, pre-implantation loss except for the 300 mg/kg bw/d group which exhibited reduced mean values for gravid uterus weight and implantation number and increased pre-implantation loss, but these findings originated from a single female which had 2 corpora lutea and one implantation site only.

Fetuses examination:
There were no statistically significant differences between the treated and control groups with respect to post-implantation losses, gravid uterus weight, number of live fetuses, fetal and placental weights, and sex ratio, except in the 300 mg/kg bw/d group which exhibited a lower mean value for the number of live fetuses and higher post-implantation loss (but these findings were due to a single female with 2 corpora lutea and one implantation site only). No external fetal abnormalities occurred in the groups treated at 0; 30; 100 and 1000 mg/kg bw/d; one fetus in the 300 mg/kg bw/d group had meningoencephalocele, microphthalmia, open eye and protruding tongue, which is a spontanous finding occasionally observed in this strain of rats.

Table 91. Group mean reproductive and fetal data

	Group mean values				
Dose level(mg/kg bw/d)	**0**	**30**	**100**	**300**	**1000**
No. pregnancies on day 20	6	7	8	8	2
Gravid uterus weight (g)	65	71	67	58	76
Number corpora lutea/dam	15.3 ± 1.8	15.9 ± 0.9	15.9 ± 0.9	16.1 ± 6.2	16.0 ± 0.0
Number implantations/dam	14.0 ± 2.9	15.6 ± 1.0	14.5 ± 2.2	12.6 ± 5.7	15.5 ± 0.7
Pre-implantation loss (%)	8.8	1.8	8.9	23.5	3.2
Number live fetuses/dam	13.2 ± 3.2	14.4 ± 1.7	13.0 ± 2.1	11.3 ± 5.8	15.0 ± 0.0
Post-implantation loss (%)	6.3	7.5	10.4	22.1	3.2
Male fetal weight (mg)	3172 ± 72	3165 ± 92	3251 ± 190	3232 ± 256	3094 ±139
Female fetal weight (g)	2944 ±172	2971 ± 121	3026 ± 156	2909 ± 165	2885 ± 23
Placental weight (mg)	412 ± 37	415 ± 48	435 ± 48	482 ± 161	420 ± 26
Sex ratio (% males)	46	43	49	46	47

Conclusion
The dose level of 1000 mg/kg bw/d clearly exceeded the maternal maximum tolerated dose level. The NOEL in this preliminary study was 300 mg/kg bw/d for maternal rats, based on the occurrence of maternal death and reduced bw gain at 1000 mg/kg bw/d and the NOEL for fetuses would be > 1000 mg/kg bw/d, based on the absence of fetal effects at this dose level, although only 2 litters were available for evaluation.

Reference: Hojo (2002b)
GLP: Yes
Guidelines: OECD 414; US-EPA OPPTS 870.3700; JMAFF, 12 NouSan n° 8147

In the main developmental study in rats, groups of 24 naturally-mated young adult female Wistar rats (Jcl:Wistar strain; 13 w old at mating; bw = 182-236g) were administered by gavage 0; 20; 100 and 500 mg/kg bw/d of flonicamid technical suspended in 1% aqueous caroxymethylcellulose (dosing volume = 10 mL/kg bw) from d6 through d19 *post coitum*.

The stability of 1 and 200mg/mL flonicamid technical suspensions for at least 14 d was demonstrated in the preliminary study (Hojo H., 2002a) and therefore dosing suspensions were prepared twice during the study at 11-d interval. The homogeneity of 2 and 50 mg/mL formulations was determined on the first formulations prepared, and the achieved concentrations of all formulations, were confirmed by analysis during the study.

The females were observed at least once daily for morbidity, mortality and clinical signs and a detailed physical examination was performed at weighing intervals. Bw and food consumption were recorded on d0; d6; d9; d12; d15; d18 and d20 of gestation. All mated females were killed and subjected to necropsy and gross examination on d20 of gestation; liver and kidney weights were recorded and samples were preserved for histopathological examination (samples from controls and high dose groups were examined). The gravid uterus was weighed, the fetuses were removed and the placentae were weighed. Apparently non-gravid uteri were stained with ammonium sulfide to detect early resorptions. The position of fetuses in the uterine horns, the numbers of implantation sites and corpora lutea, fetal weights and sexes were recorded. Resorbed embryos or dead fetuses were classified as implantation sites, placental remnants or macerated fetuses. Fetuses were externally examined, including orifices, for malformations. Approximately one half of the fetuses from each litter were examined fresh for thoracic and abdominal visceral abnormalities. The Bouins-fixed heads were examined by Wilson's razor sectioning technique for abnormalities of the eyes, brain, nasal passages and tongue. The carcasses of the remaining fetuses were eviscerated and double-stained for the examination of cartilage and the ossified skeleton for skeletal abnormalities and variations.

Test diet analysis: The 2 and 50 mg/mL formulations were found to be homogeneous, with coefficients of variation of 0.4 and 0.5%, respectively, for 3 samples/concentration. Achieved concentrations in the first formulations prepared were in the range 96 - 101% nominal concentration.

Maternal findings:
There were no deaths or treatment-related clinical signs in maternal animals at any dose level. The food consumption and bw gain were unaffected by treatment with flonicamid technical at all dose levels, although the mean food consumption in the 500 mg/kg bw/d group was 7.3% lower than that of controls on gestation d6 - d9.

Necropsy findings
There were no treatment-related gross findings at necropsy at any dose level in maternal rats. Absolute and relative liver weights were significantly increased at 500 mg/kg bw/d and were associated with centrilobular hepatocyte hypertrophy in 13/24 females; although 1 low dose female also exhibited hepatocyte hypertrophy,

the alteration was not apparent in any animals of the control and 100 mg/kg bw/d groups. All females of the 500 mg/kg bw/d group also showed vacuolation of the kidney proximal tubular cells.

Table 92. Group mean absolute and relative liver and kidney weights - maternal animals

Group (ppm)	Adjusted[a] Bw on d-20 (g)	Group mean organ weight:			
		Liver		Kidneys	
		Absolute (g)	Relative[b] (%)	Absolute (g)	Relative[b] (%)
0	252	12.39	4.91	0.780	0.310
20	252	12.34	4.89	0.801	0.319
100	254	12.51	4.92	0.778	0.306
500	254	13.96**	5.49**	0.811	0.319
** $p < 0.01$; [a] bw less gravid uterus weight; [b] relative to bw					

Reproductive parameters

There were no treatment-related effects on reproductive parameters at any dose level: the pregnancy incidence, the gravid uterine weight, the mean number of corpora lutea and the implantation sites, pre- and post-implantation losses, the number of live fetuses, the sex ratio and the fetal weight were comparable to control values. The significantly increased mean placental weight at 500 mg/kg bw/d (by 9.7%) is of unclear significance.

Table 93. Summary of reproduction data

Parameter	Group values for animals treated at (mg/kg bw/d):			
	0	20	100	500
No. pregnant / no. mated	22 / 24	24 / 24	24 / 24	24 / 24
No. dams with live fetuses	22	24	23[a]	24
Gravid uterus weight (g)	68 ± 10	71 ± 8	67 ± 12	73 ± 8
Mean no. corpora lutea/dam	15.9 ± 1.4	15.9 ± 1.2	15.7 ± 1.4	15.7 ± 1.5
Mean no. implantation sites/dam	14.9 ± 1.3	15.0 ± 1.6	14.1 ± 2.8	14.8 ± 1.5
Pre-implantation loss (%)	5.8	5.2	10.7	5.2
Total no. dead fetuses	0	3	0	1
Total post-implantation loss (%)	9.4	6.6	6.4	4.2
Mean no. live fetuses/dam	13.5 ± 2.1	14.0 ± 1.7	13.1 ± 2.6	14.2 ± 1.6
Sex ratio (% males)	51	51	49	49
Mean male fetal weight (g)	3.25	3.21	3.33	3.22
Mean female fetal weight (g)	3.05	3.02	3.13	3.01
Placental weight (g)	0.433	0.459	0.456	0.475**
[a] one dam had implantation sites visible only by staining and was excluded from calculation of mean data				

Fetuses examination:

No treatment-related fetal abnormalities occurred at any dose level. The overall fetal incidences of spontaneous abnormalities were 0.7; 0.9; 0.3 and 0.6% in the groups treated at 0; 20; 100 and 500 mg/kg bw/d, respectively. There were no external abnormalities at any dose level and very low incidences of visceral and skeletal abnormalities in all treatment groups. Furthermore, the nature of the abnormalities did not suggest an effect of treatment. There was a treatment-related increase in the incidence of skeletal variations at 500 mg/kg bw/d, which was entirely due to a significant excess in the incidence of cervical rib. (i.e. 6.5; 2.3; 3.2 and 34.1% in the groups treated at 0; 20; 100 and 500 mg/kg bw/d, respectively). The only other statistically significant differences between control and treated groups were reduced total incidences of

skeletal variations at 20 and 100mg/kg bw/d. The nature and incidence of all other skeletal variations at all dose levels were comparable in the treated and control groups.

Table 94. Summary incidences of external, visceral and skeletal / cartilaginous tissue findings

Parameter	**No. and (%) fetuses at (mg/kg bw/d):**			
	0	**20**	**100**	**500**
No. litters evaluated (external)	22	24	23	24
No. fetuses evaluated (external)	298	337	302	341
External abnormalities	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0.0)
No. litters evaluated (visceral)	22	24	23	24
No. fetuses evaluated (visceral)	143	160	146	165
Abnormal fetuses (visceral)	0 (0.0)	2[a] (1.3)	0 (0.0)	1[b] (0.6)
No. litters evaluated (skeletal)	22	24	23	24
No. fetuses evaluated (skeletal)	155	177	156	176
Abnormal fetuses (skeletal)	2[c] (1.3)	1[d] (0.6)	1 [e] (0.6)	1 [f] (0.6)
Total abnormal fetuses	2 (0.7)	3 (0.9)	1 (0.3)	2 (0.6)
Total abnormal litters	2 (9.1)	1 (4.2)	1 (4.3)	2 (8.3)
Fetuses with visceral variations	2 (1.4)	3 (1.9)	4 (2.7)	6 (3.6)
Fetuses with skeletal variations	18 (11.6)	8* (4.5)	8* (5.1)	70*** (39.8)

* $p < 0.05$; *** $p < 0.001$
[a] one fetus with retroesophageal subclavian aortic arch, one fetus with right-sided aortic arch;
[b] one fetus with malpositioned ovary;
[c] 2 fetuses with fused and/or absent ribs, fused rib cartilage, absent and/or fused thoracic arches and centra, dumbbell-shaped cartilage and abnormalities of the thoracic and lumbar centra;
[d] one fetus with hemicentric thoracic centrum;
[e] one fetus with dumbbell-shaped cartilage of the thoracic centrum;
[f] one fetus with fused rib cartilage

In order to clarify the relevance of the increased incidence of cervical ribs (categorized as variation) a re-assessment with regards to the presence or absence of cartilage on cervical ribs was performed; this was summarized in a position paper (**"cervical ribs observed in the teratogenicity study in rats treated with Flonicamid", Hojo, July 2006**).

The incidence of cervical ribs is summarized in the table below:

Table. Incidence of skeletal variations in rats

Fetal findings	Incidence in fetuses Dose level (mg/kg/day) 0	20	100	500	Incidence in litters Dose level (mg/kg/day) 0	20	100	500
Number examined	155	177	156	176	22	24	23	24
Cervical rib without distal cartilage	10	4	5	↑58	7	4	3	↑23
Cervical rib with distal cartilage	0	0	0	2	0	0	0	1

↑, P≤0.001 (Fisher's exact probability test).

Only 2 fetuses of the same litter in the top dose group exhibited cervical ribs with distal cartilage. Other cervical ribs were revealed as completely ossified and rudimentary (or small) ribs which were adjacent to the 7th cervical vertebra unilaterally or bilaterally.

> **Supernumerary rib, a rib developing adjacent to the 7th cervical or 1st lumbar vertebrae, is often seen in laboratory developmental toxicity. Supernumerary ribs consist of two different structures: an extra rib that has a cartilaginous segment at the distal end, and an ossification site that is small or rudimentary rib without distal cartilage. Extra ribs are permanent structures in contrast to ossification sites that disappear postnatally, probably becoming part of the lateral transverse vertebral processes. When lumbar ribs are chemically induced using developmental toxicants, the incidence of extra ribs exhibits a clear dose response, but the incidence of ossification sites, in contrast, is independent of dose. The sum of these evidences supports the idea of supernumerary rib being composed of two different structures: extra ribs that are permanent dysmorphological structures that may be induced by xenobiotics and/or maternal stress, and ossification sites that may be transient variations in the formation of the lateral processes of the vertebrae.**

As a conclusion, only 2 fetuses (of the same litter) out of 60 having cervical ribs in the top dose group were found with distal cartilage, which is not significant compared to control animals; 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. Moreover these effects were observed at a dose level which caused toxicity to the dams.

An additional study was submitted to support the absence of treatment-related teratogenic effect of flonicamid in rats (Takahashi, 2006). This study is a preliminary non-GLP teratogenicity study in the SD rat that was performed in 1998 (the final report was prepared in 2006 only) in order to select the doses of the teratogenicity study. In this study, doses of 0, 10, 100, 500 mg/kg flonicamid were administered via gastric gavage daily from day 6 through day 19 of gestation. Treatment-related effects (increase of liver weight) were observed in females of the 500 mg/kg/d group. Histopathological observations revealed centrilobular hepatocellular hypertrophy of the liver and cytoplasmic vacuolation of proximal tubular cell of the kidney in all maternal animals of this dose group. No changes were noted in the maternal animals at dose levels of 10 and 100 mg/kg/d. No effects were observed on fetuses in any of the treated groups (no difference of pre-implantation loss was observed, no extra cervical rib observed).

Conclusion
The NOAEL for maternal rats was 100 mg/kg bw/d and the NOAEL for fetuses was 100 mg/kg bw/d in the main study. Flonicamid is not teratogenic in rats under the conditions of both preliminary and main studies.

Rabbit studies

Reference: Takahashi (2002c)
GLP: Yes
Guidelines: not applicable (dose range finding study)

In the preliminary developmental study in rabbits, groups of 6 artificially inseminated female Japanese White rabbits (Kbl:JW strain; 18 w old at mating; bw = 3.43 - 4.19 kg) were administered by gavage 0; 3; 10 and 30 mg/kg bw/d flonicamid technical suspended in 1% aqueous carboxymethylcellulose from d6 to d27 of gestation[43] (dosing volume = 5 mL/kg bw).

The stability at ca. 5°C for 14 days of suspensions containing 1 or 200 mg/mL flonicamid technical had been confirmed by analysis in a previous study (Hojo, 2002a) and therefore dosing solutions were prepared weekly. The homogeneity of suspensions corresponding to the 3 and 30 mg/kg bw dose level, the stability of the 3 mg/kg bw dosing suspension and the achieved concentrations of all formulations were confirmed by analysis.

All rabbits were observed at least once daily for mortality and clinical signs and a detailed physical examination was performed at weighing intervals. Females found dead were immediately necropsied on discovery; females showing signs of abortion were killed and subjected to necropsy. Bw were recorded on gestation d0; d6; d9; d12; d15; d18; d21; d24; d27 and d28 and indicidual food consumption was determined on d0-3; d3-6; d6-9; d9-12; d12-15; d15-18; d18-21; d21-24; d24-27 and d27-28 of gestation. All surviving rabbits were killed on d28 of gestation and subjected to necropsy and *post mortem* examination of major organs and tissues. Gross lesions were recorded but no organs were retained. The ovaries and uterine contents of pregnant animals were examined. The gravid uterus weight, the numbers of corpora lutea and implantation sites, the numbers of live and dead fetuses was recorded. Resorbed embryos or dead fetuses were classified as implantation site, placental remnants or macerated fetuses. Live fetuses were sexed by examination of the internal reproductive organs and the weights of individual placentae and live fetuses were recorded. The fetuses were examined to detect visible abnormalities including those in the body orifices. All fetuses and placentae were discarded.

Test diet analysis: The formulations were found to be homogeneous, with coefficients of variation of 0.0-0.4%, for 3 samples/concentration. The formulations were stable at ca. 5°C for 14 days at which time 101 and 100% nominal concentrations remained, respectively (therefore, dosing suspensions were prepared twice during the study at an interval of 12 days). Achieved concentrations in the first formulations prepared were in the range 87 -105 % nominal concentrations.

Maternal findings:

Two females at 30 mg/kg bw/d aborted and were killed and necropsied on d22 or d25 of gestation (one showed posterior paralysis and soiled fur on the abdomen prior to aborting and the other showed reddish coloured urine). There were no deaths, abortions or treatment-related clinical signs at dose levels of 3 or 10 mg/kg bw/d. Bw loss or slightly reduced bw gain occurred throughout the treatment period in the group treated at 30 mg/kg bw/d, and group mean weight gain was significantly lower than the controls during the period d6 to d24. No effect on bw gain occurred at 3 or 10 mg/kg bw/d. The food consumption of the

[43] Initially, the study was started using 0; 30; 100; 300 and 1000mg/kg bw/d of flonicamid technical. Since 2 animals/group died after a single dose of 100; 300 and 1000mg/kg bw/d, the study was terminated, the protocol was amended and the lower dose levels were selected.

30mg/kg bw/d group was slightly reduced from d12 of gestation but not statistically different from that of controls. Food consumption at 3 or 10mg/kg bw/d was unaffected by treatment.

Table 95. Summary of group mean bw and food consumption

Parameter	**Group mean value at (mg/kg bw/d):**			
	0	**3**	**10**	**30**
Bw (kg)				
d- 0	3.77	3.84	3.85	3.77
d- 6	3.88	4.01	4.00	3.88
d- 12	3.91	4.02	3.96	3.86
d- 18	4.01	4.05	4.05	3.82
d- 24	4.12	4.07	4.13	3.79
d- 28	4.17	4.04	4.16	3.93
Bw gain (g)				
d- 6-9	14	-13	-27	-25
d- 6-12	30	9	-39	-24
d- 6-18	129	39	51	-58
d- 6-24	236	60	126	-157*
d- 6-28	287	37	159	-23
Gravid uterus weight (g)	482	465	474	260*
Adjusted bw (kg) on d-28	3.69	3.58	3.69	3.67
Food consumption (g/day)				
	206	198	182	181
d0 - 3	190	200	187	190
d3 - 6	186	192	180	151
d6 - 9	164	183	157	144
d9 - 12	156	159	148	109
d12 - 15	164	141	150	90
d15 - 18	171	128	148	74
d18 - 21	145	107	126	83
d21 - 24	126	71	103	89
d24 - 27	105	66	104	83
d27 - 28				
* $p < 0.05$				

Necropsy findings:
There were no treatment-related gross necropsy findings in maternal rabbits at any dose level, although equivocal findings were found in the 2 females of the 30 mg/kg bw/d group that aborted and were killed prematurely (one female exhibited haemorrhage in the pelvic cavity and the other one had a yellow-coloured liver and black contents in the stomach).

Reproductive parameters:
All females surviving at termination of the study were proved to be pregnant and had live fetuses, but there were only 4 full-term litters at 30 mg/kg bw/d due to abortion in 2 females In the 30 mg/kg bw/d group, the mean gravid uterine weight was significantly reduced, the mean numbers of implantations and live fetuses were markedly reduced as a consequence of markedly elevated pre-implantation loss and the high pre-implantation loss was due, in part, to a single animal with 15 corpora lutea and only one implantation. These

parameters were not affected by treatment at lower dose levels. Post-implantation loss was not affected by treatment at any dose level.

Fetuses examination:

Male and female fetal weights at 30 mg/kg bw/d were 11.1 and 5.9% lower than control values respectively, but neither was significantly different from the controls. Fetal weights at lower dose levels were comparable to control values. Sex ratios and the incidences of externally abnormal fetuses were unaffected by treatment at all dose levels. No external abnormalities occurred in live fetuses from females treated at 0, 3 or 30 mg/kg bw/d. One fetus at 10mg/kg bw/d showed omphalocele.

Table 96. Group mean reproductive and fetal data

Parameter	**Group mean value at (mg/kg bw/d):**			
	0	**3**	**10**	**30**
No. pregnant / no. mated	6 / 6	6 / 6	6 / 6	6 / 6
No. with live fetuses on day 28	6	6	6	4[a]
Gravid uterus weight (g)	482 ± 72	465 ± 84	474 ± 94	260 ± 163*
Number corpora lutea (mean/dam)	13.3 ± 3.3	12.0 ± 1.3	11.7 ± 2.8	11.3 ± 3.9
Number implantations (mean/dam)	9.7 ± 3.3	10.5 ± 1.4	9.3 ± 2.5	5.0 ± 3.7
Pre-implantation loss (%)	25.8	12.2	18.6	52.6
Number live fetuses (mean/dam)	8.8 ± 2.3	9.0 ± 2.6	7.8 ± 1.7	4.8 ± 1.3
Post-implantation loss (%)	6.5	14.8	14.2	2.8
Male fetal weight (g)	38.8 ± 6.7	37.1 ± 7.3	41.4 ± 2.2	34.5 ± 9.3
Female fetal weight (g)	37.0 ± 3.5	34.2 ± 8.6	38.0 ± 4.1	34.8 ± 7.2
Placental weight (g)	5.07	5.17	6.12	6.09
Sex ratio (% males)	57	67	62	37

[a] 2 females aborted and were killed before day 28; * $p < 0.05$

Conclusion
Dose levels ≥ 100 mg/kg bw/d (in the initial study) clearly exceeded the maternal maximum tolerated dose level. The NOAEL for dams and fetuses was 10 mg/kg bw/d, based on maternal bw loss and reduced food consumption, and reduced live fetus number at 30 mg/kg bw/d.

Reference: Takahashi (2002d)
GLP: Yes
Guidelines: OECD 414; US-EPA 870.3700; JMAFF 12 NouSan n° 8147

In the main develomental study in rabbits, groups of 25 artificially inseminated female Japanese White rabbits (Kbl:JW strain; 18 w old at mating; bw = 3.26 - 4.19 kg) were administered, by gavage, 0; 2.5; 7.5 and 25 mg/kg bw/d of flonicamid technical suspended in 1% aqueous carboxymethylcellulose from d6 trhough d27 of gestation (dose volume = 5mL/kg bw).

The stability at ca. 5°C for 14 days of suspensions containing 1 or 200mg/mL flonicamid technical was confirmed by analysis in previous studies (Hojo, 2002a; Takahashi, 2002b) and therefore dosing suspension were prepared weekly. The homogeneity of suspensions containing 0.5 or 5 mg/mL, the stability of the 0.5 mg/mL suspension and the achieved concentrations of all formulations were confirmed by analysis

The females were observed at least once daily for mortality and clinical signs and a detailed physical examination was performed at weighing intervals. Females found dead were immediately necropsied on discovery; females showing signs of abortion were killed and subjected to necropsy. Bw were recorded on d0; d6; d9; d12; d15; d18; d21; d24; d27 and d28 of gestation and food consumption consumption was

determined on d0-3; d3-6; d6-9; d9-12; d12-15; d15-18; d18-21; d21-24; d24-27 and d27-28 of gestation. All surviving rabbits were killed on d28 of gestation and subjected to necropsy and *post mortem* examination of major organs and tissues. Gross lesions were recorded but no organs were retained. The ovaries and uterine contents of pregnant animals were examined and the apparently non-gravid uteri were stained with ammonium sulfide solution to detect early resorptions. The gravid uterus weight, the numbers of corpora lutea and implantation sites, the numbers of live and dead fetuses were recorded. Resorbed embryos or dead fetuses were classified as early resoprtions (implantation sites or placental remnants) or late resorptions (macerated fetuses including dead fetuses at term). Live fetuses were sexed by examination of the internal reproductive organs and the weights of individual placentae and live fetuses were recorded. The thoracic and abdominal viscera of all fetuses were examined fresh by dissection. The eyes of 50% of the fetuses/litter were also examined after removal of the palpebral skin and the brain was examined by making a transverse razor section through the coronal suture of the skull. The heads of the remaining fetuses were preserved in Bouin's fluid and the eyes, brain, nasal passages and tongue examined by Wilson's razor sectioning technique. All carcasses were stained with alizarin red S for examination of the ossified skeleton for abnormalities and variations.

Test diet analysis: The 0.5 mg/mL formulation was shown to be stable at 5°C for 14 days, at which time 101% of starting concentration remained. The 0.5 and 5 mg/mL formulations were found to be homogeneous, with coefficients of variation of 0.5 and 0.3%, respectively, for 3 samples/concentration. Achieved concentrations of all formulations were in the range 97 - 101% nominal concentration.

Maternal findings:

There were no treatment related deaths or treatment-related clinical signs in maternal animals at any dose level, but 1 female from the 2.5 mg/kg bw/d group was found dead on gestation d9 (death was attributed to a mechanical intubation damage beause of findings such as rhinorrhagia, subcutaneous hemorrhage in the brachial and axillary regions, hydrothorax, atelectasis, bone fracture in the humerus…) and 1 female in each of the 2.5; 7.5 and 25 mg/kg bw/d groups were killed on d23; d24 and d26 of gestation, respectively because of abortion without other clinical abnormalities (these females stopped eating before abortion; no gross changes were found in these females at necropsy and abortion were not likely to be related to treatment because of the absence of a dose response relationship). Group mean bw were not significantly different from control values, but reduced bw gain occurred throughout the treatment period in the 25mg/kg bw/d group (achieving statistically significance on d12-28 of gestation); bw gains at 2.5 or 7.5mg/kg bw/d were lower than control group bw gains but did not achieve statistical significance. The food consumption was significantly reduced from d9 through d21 of gestation in the 25 mg/kg bw/d group only.

Table 97. Summary of cumulative group mean body weight gain and food consumption

Parameter	Group mean value at (mg/kg bw/d):			
	0	**2.5**	**7.5**	**25**
Bw gain (kg) on days:				
6 - 9	-10 ± 55	-32 ± 68	-10 ± 38	-48 ± 55
6 - 12	21 ± 70	-8 ± 86	3 ± 59	-64 ± 89**
6 - 15	72 ± 95	46 ± 114	37 ± 105	-50 ± 131**
6 - 18	104 ± 113	48 ± 145	23 ± 154	-90 ± 189***
6 - 21	130 ± 124	58 ± 142	14 ± 204	-85 ± 221**
6 - 24	174 ± 134	82 ± 149	81 ± 201	-50 ± 238**
6 - 28	225 ± 126	126 ± 202	124 ± 258	39 ± 243*
Gravid uterus weight (g)	418 ± 114	398 ± 172	405 ± 88	384 ± 114
Adjusted bw (kg) on day 28	3.63 ± 0.28	3.56 ± 0.25	3.54 ± 0.33	3.49 ± 0.25
Food cons.(g/day) on days:				
0 - 3	179 ± 36	181 ± 33	181 ± 28	182 ± 25
3 - 6	187 ± 34	181 ± 29	185 ± 29	184 ± 21
6 - 9	174 ± 34	164 ± 37	175 ± 26	148 ± 29
9 - 12	159 ± 37	159 ± 33	156 ± 25	129 ± 38*
12 - 15	145 ± 46	140 ± 43	126 ± 49	93 ± 34**
15 - 18	161 ± 43	134 ± 58	121 ± 55	90 ± 62***
18 - 21	153 ± 46	136 ± 51	120 ± 55	97 ± 61*
21 - 24	137 ± 47	121 ± 55	116 ± 51	102 ± 51
24 - 27	110 ± 39	106 ± 51	99 ± 42	106 ± 45
27 - 28	109 ± 38	102 ± 47	101 ± 44	103 ± 47
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$				

Necropsy findings

There were no treatment-related gross findings at necropsy in maternal animals killed on gestation d 28.

The pregnancy incidence in all groups was uniformly high and 23; 22; 21 and 23 females treated at 0; 2.5; 7.5 and 25mg/kg bw/d, respectively, had viable young on d28. There were no treatment-related effects at any dose level on gravid uterus weight, the numbers of corpora lutea and implantations, pre-implantation loss, number of live fetuses, and post-implantation loss from resorption and fetal death.

Table 98. Group mean reproductive and fetal data

Parameter	Group mean value at (mg/kg bw/d):			
	0	**2.5**	**7.5**	**25**
No. pregnant / no. mated	24 / 25	25 / 25	25 / 25	24 / 25
No. females with resorptions only	1	1	3	0
No. with live fetuses on day 28	23	22[a]	21[b]	23[b]
Gravid uterus weight (g)	418 ± 114	398 ± 172	405 ± 88	384 ± 114
Number corpora lutea (mean/doe)	10.1 ± 1.9	10.5 ± 2.1	10.0 ± 1.5	10.3 ± 1.9
Number implantations (mean/doe)	8.1 ± 2.7	8.3 ± 4.0	8.0 ± 2.2	8.2 ± 2.8
Pre-implantation loss (%)	20.4	25.4	19.1	20.7

Number live fetuses (mean/doe)	7.5 ± 2.6	7.6 ± 3.9	7.4 ± 2.0	7.4 ± 2.5
Total no. dead fetuses	6	7	6	5
Post-implantation loss (%)	6.9	8.1	7.0	8.7
Male fetal weight (g)	39.2 ± 5.7	36.7 ± 7.7	38.0 ± 6.2	35.4 ± 5.5
Female fetal weight (g)	38.6 ± 5.6	37.3 ± 7.6	36.7 ± 6.7	34.9 ± 5.3
Placental weight (g)	5.30 ±0.92	5.37 ± 1.09	5.14 ± 0.83	5.27 ± 0.86
Sex ratio (% males)	48.0	51.5	55.8	49.4
[a] one female died and one female aborted; [b] one female aborted; * p < 0.05				

Fetus examinations:

The fetal weights in treated groups were not statistically reduced, although they were 9.7 and 9.6% lower than control in both sexes. There were no treatment-related effects on mean placental weight and sex ratio at any dose level.

Higher incidences of abnormal fetuses occurred in all treated groups, but the total fetal and litter incidences of abnormal fetuses in the groups treated at 2.5 or 25 mg/kg bw/d were not significantly different from control values. The incidence of fetuses with visceral abnormalities and the overall litter incidence of abnormalities at 7.5mg/kg bw/d were significantly higher than the control values: the nature of the observed abnormalities (malpositioned innominate; malpositioned subclavian branch; thymic remnant in the neck…) in all treatment groups was diverse and all individual abnormalities occurred at very low frequencies of 1 or 2 fetuses only; therefore, no statistically significant differences at any dose level in the incidence of individual abnormalities was found and these findings should be considered as incidental. There were no treatment-related effects at any dose level on the incidences of visceral and skeletal variations. There were no statistically significant differences between control and treated groups in the incidences of skeletal variations, but the incidence of visceral variations at 2.5mg/kg bw/d was significantly lower than the controls, due to a lower incidence of thymic remnant.

Table 99. Summary incidences of external, visceral and skeletal findings

Parameter	**No. and (%) fetuses at (mg/kg bw/d):**			
	0	**2.5**	**7.5**	**25**
No. litters evaluated (external)	23	22	21	23
No. fetuses evaluated (external)	173	167	156	170
External abnormalities	0 (0.0)	2 (1.2)	2 (1.3)	1 (0.6)
No. litters evaluated (visceral)	23	22	21	23
No. fetuses evaluated (visceral)	173	167	156	170
Abnormal fetuses (visceral)	1 (0.6)	2 (1.2)	6* (3.8)	5 (2.9)
No. litters evaluated (skeletal)	23	22	21	23
No. fetuses evaluated (skeletal)	173	167	156	170
Abnormal fetuses (skeletal)	0 (0.0)	3 (1.8)	3 (1.9)	3 (1.8)
Total abnormal fetuses	1[a] (0.6)	7[b] (4.2)	11[c] (7.1)	9[d] (5.3)
Total abnormal litters	1 (4.3)	4 (18.2)	6* (28.6)	3 (13.0)
Fetuses with visceral variations	7 (4.0)	1* (0.6)	10 (6.4)	7 (4.1)
Fetuses with skeletal variations	55 (31.8)	59 (35.3)	43 (27.6)	65 (38.2)

* p < 0.05

[a] one fetus with malpositioned testis

[b] 2 fetuses with malpositioned testis, one fetus with anal atresia, one fetus with omphalocele, 2 fetuses with fused sternebrae, one fetus with absent cervical vertebral arch

[c] one fetus with local edema, one fetus with omphalocele, one fetus with multiple malformations (retroesophageal subclavian aortic arch, absent kidney and ureter, fused rib and supernumerary thoracic vertebral arch and centrum), 2 fetuses with abnormal lung lobation, one fetus with narrowed pulmonary trunk, one fetus with small lung, one fetus with malpositioned testis, one fetus with fused sternebrae, one fetus with absent rib and hemicentric thoracic vertebral centrum, one fetus with supernumerary thoracic vertebral arch

[d] one fetus with amelia, short tail and gastroschisis, one fetus with ventricular septal defect and interrupted aortic arch, one fetus with fused sternebrae, one fetus with absent lung, 2 fetuses with abnormal lung lobation, one fetus with absent kidney and ureter with small bladder, one fetus with fused caudal vertebral centrum, one fetus with multiple vertebral and long-bone abnormalities

The NOEL in maternal rabbits was 7.5mg/kg bw/d, based on the occurrence of reduced bw gain and food consumption at 25mg/kg bw/d.

Relevance of external, visceral and skeletal malformations

The relevance of external, visceral and skeletal malformation was further discussed in the light of historical control data and additional information submitted by the performing laboratory IET. Historical control data were obtained from 15 studies performed at IET during the 1992-2001 period (the rabbit teratogenicity study was performed in 2001).

External abnormalities

Table 100. Type and incidence of external abnormalities

Fetal findings	**Incidence (%) in this study at (mg/kg bw/d)**				**Literature historical control incidence[44] (%)**	**IET historical control data (%) 1992-2001**
	0	2.5	7.5	25		
Short tail	0	0	0	0.59	0 - 2.22	0
Anal atresia	0	0.60	0	0	0 - 1.48	0
Gastroschisis	0	0	0	0.59	0 - 0.92	0
Local oedema	0	0	0.64	0	Not reported	0.05
Amelia	0	0	0	0.59	Not reported	0
Omphalocele	0	0.6	0.64	0	0-1.13	0.05

Although the incidence of external abnormalities (short tail, anal atresia, gastroschisis and omphalocele) is above the HCD of the facility, they fall well within the historical control data reported in the literature. In addition they are scattered throughout all dose level groups without any dose-response. Thus they can be considered as not relevant.

Skeletal abnormalities

[44] 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
Morita et al., Spontaneous malformations in laboratory animals: frequency of external, internal and skeletal malformations in rats, rabbits and mice. Cong Anom, 27: 147-206, 1987

Table 101. Type and incidence of skeletal abnormalities

Fetal findings	**Fetuses affected : number and (%)** **Dose level (mg/kg bw/d)**				**Literature historical control incidence[1] (%)**	**IET historical control data** **1992-2001 (%)**
	0	**2.5**	**7.5**	**25**		
No. of fetuses examined	173	167	156	170		2177
Fused sternebra	0	2 (1.20)	1 (0.64)	1 (0.59)	(0 - 5.05)	(0.64)
Absent rib	0	0	1 (0.64)	0	(0 – 1.54)	(0.05)
Fused rib	0	0	1 (0.64)	0	(0 – 1.96)	0
Absent cervical arch	0	1 (0.60)	0	0	0 – 1.75)	(0.05)
Supernumerary thoracic arch	0	0	1 (0.64)	0	Not reported	0
Hemicentric thoracic centrum	0	0	1 (0.64)	0	Not reported	0
Supernumerary thoracic centrum	0	0	1 (0.64)	0	Not reported	0
Absent lumbar arch	0	0	0	1 (0.59)	(0 – 1.16)	0
Fused lumbar centrum	0	0	0	1 (0.59)	(0 – 1.59)	0
Absent sacral arch	0	0	0	1 (0.59)	Not reported	0
Fused sacral centrum	0	0	0	1 (0.59)	(0 – 1.59)	0

Skeletal malformations were observed simultaneously in the same fetus at 25 mg/kg bw/d: absent lumbar arch, fused lumbar centrum, absent sacral arch, fused sacral centrum, absent caudal arch, fused caudal centrum, and multiple other skeletal anomalies; they fall in the range of the historical control data reported in the literature. Other skeletal abnormalities were scattered throughout all dose level groups without any dose-response.

Visceral abnormalities

Table 102. Type and incidence of visceral abnormalities

Fetal findings	Fetuses affected : number and (%) **Dose level (mg/kg bw/d)**				IET historical control data **1992-2001 (%)**
	0	**2.5**	**7.5**	**25**	
No. of fetuses examined	173	167	156	170	2177
No. of fetuses with malformations	1	2	6*	5	
Membranous ventricular septum defect	0	0	0	1 (0.59)	(0.09)
Interrupted aortic arch	0	0	0	1 (0.59)	(0.09)
Narrowed pulmonary trunk	0	0	1 (0.64)	0	(0.09)
Retroesophageal subclavian	0	0	1 (0.64)	0	(0.09)
Abnormal lung lobation	0	0	2 (1.28)	2 (1.18)	(0.09)
Absent lung	0	0	0	1 (0.59)	0
Small lung	0	0	1 (0.64)	0	(0.09)
Absent kidney	0	0	1 (0.64)	1 (0.59)	0
Small bladder	0	0	0	1 (0.59)	0
Absent ureter	0	0	1 (0.64)	1 (0.59)	0
Malpositioned testis	1 (0.57)	2 (1.19)	1 (0.64)	0	Not known

* Significantly different from control at P <0.05

The number of fetuses having one or more visceral malformations regardless the type of malformation was increased in the 7.5 and 25 mg/kg bw/d groups, with a statistically significant difference between the control group at 7.5 mg/kg bw/d.

A Cochran Armitage test trend was performed for incidence of fetuses having visceral malformations, abnormal lung lobation, absent kidney and absent ureter, individually : no significant trend was detected in any of the parameters analyzed. Moreover, the type of malformations varied widely among fetuses and though exceeding the incidence in the historical control data 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)[45] .

[45] 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

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) [4] 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 %.

Conclusion
Flonicamid is not teratogenic in the rabbit. The NOEL in maternal rabbits was 7.5mg/kg bw/d, based on the occurrence of reduced bw gain and food consumption at 25mg/kg bw/d. The NOEL in the fetus was > 25mg/kg bw/d, based on the absence of developmental toxicity at the highest dose level.

4.11.3.2 *Human information*

No data available.

4.11.4 Other relevant information

None

4.11.5 Summary and discussion of reproductive toxicity

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

- In **the rat - 2 generation** reproductive toxicity study, flonicamid does not affect fertility and fecundity in both sexes; 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 F1 & F2 generation and slightly delayed vaginal opening in F1 progeny), but all other aspects of F1 development and, specifically the reproductive capacity, are unaffected by treatment with flonicamid; reduced ovary weights are 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 w at up to 5000 ppm from 6 w of age. Sperm analysis did not reveal any treatment effect on sperm count and morphology.
- Investigation of serum gonadotrophin and sex hormone concentrations in male F1 progeny reveals no effects at any dose level up to 125 mg/kg bw/d on LH, FSH and testosterone concentrations, but in females, raised serum LH and slightly reduced serum 17β-estradiol concentrations occur at dose levels ≥ 30.5 and 177mg/kg bw/d, respectively. These variations were 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. Serum progesterone concentration is unaffected by flonicamid. Further investigation of estrogen receptor binding affinity reveals 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 relate to the kidney: morphologically distinct histopathological alterations occur in the kidneys of males and females in both P and F1 generations; both renal lesions were already seen in previous short-term toxicity studies: in males, the kidney changes are considered to be mediated by the male rat-specific protein, α2-microglobulin; in females, the kidney changes consist of vacuolation of proximal tubular cells. F1 males and females are no more susceptible than the P generation to these renal lesions.

- The parental NOAEL was 18 mg/kg bw/day based on degenerative renal tubular lesions in males, reduced ovary/adrenal weight and renal tubular vacuolation in females. The NOAEL for the reproductive parameters was 109.1 mg/kg bw/day based on the absence of effects on mating, fertility or gestation. The NOAEL for the offspring was 30 mg/kg bw/day based on delayed vaginal opening and reduced uterus weights in F1 progeny.

- Flonicamid is **not teratogenic in either the rat or the rabbit**. However in rats, flonicamid elicits a marked increase in the incidence of cervical ribs at a dose level of 500 mg/kg bw/d which causes overt maternal effects, 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/day, based on effects observed in the kidneys and liver. The developmental NOAEL was also 100 mg/kg bw/day, related to an increased incidence of skeletal variations, namely extra cervical ribs. In rabbits, the maternal NOAEL was 7.5 mg/kg bw/day, 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 was observed; they were never dose-related, they fall within the historical control data and thus can be considered as incidental.

Note:
The EFSA conclusion of the peer review of flonicamid has come to 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”, was proposed.

4.11.6 Comparison with criteria

Reprotoxic substances can be toxic to the development of the unborn child or can cause impairment of fertility in male and female subjects.

Reprotoxic substances are divided into 2 groups;
- Effects on male or female fertility, including adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response.
- Developmental toxicity, including any effect interfering with normal development before and after birth.

1) Criteria for classification under Directive 67/548/EEC:

- Fertility:

The placing in category 1 is done on the basis of epidemiological data. Placing in categories 2 and 3 is done primarily on the basis of animal data. Data from *in vitro* studies are regarded as supportive evidence. When effects have been demonstrated only in high doses, classification in category 3 or even no classification will be warranted. Also when studies are performed in only 1 species, without other relevant supporting evidence, classification in category 3 may be appropriate.

- Developmental toxicity

For classification in category 2 there should be clear evidence of adverse effects in more species. The route of exposure is also important. Classification in category 3 or no category would be assigned when the only effects recorded are small changes in the incidences of spontaneous defects or small differences in postnatal developmental assessments.

2) Criteria in the CLP classification :

- Fertility and developmental toxicity

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.

3) Comparison with criteria:

Flonicamid was not found toxic for the reproduction nor the development in animal experiments and thus does not fulfill the criteria for classification.

4.11.7 Conclusions on classification and labelling

No classification is required for flonicamid under either Directive 67/548/EEC or the CLP Regulation.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 *Neurotoxicity*

Table 103.

Method	Results	Remarks	Reference
OECD guideline no. 424; US-EPA OPPTS 870.6200 **Acute oral neurotoxicity**; **Rat SD** 0, 100, 300, 600 (M), 1000 (F)mg/kg	No neurotoxicity Minor behavioural effects reflecting acute toxicity only	-	Ridder & Watson (2002)
OECD 424; US-EPA OPPTS 870.6200 **28-d oral range-finding neurotoxicity**; **Rat SD** 0, 200, 500, 1000, 5000, 10000, 20000ppm	↓ bw gain & food consumption	-	Schaefer (2003a)
OECD 424; US-EPA OPPTS 870.6200 **90-d oral neurotoxicity** **Rat SD** 0, 200, 1000, 10000ppm	↓ weight gain and food consumption	-	Schaefer (2003b)

A single oral dose of flonicamid technical of up to 600 mg/kg bw to males and 1000 mg/kg bw to females does not elicit neurohistopathological changes in central and peripheral nervous tissues, and does not elicit signs of neurobehavioral toxicity. Therefore, the NOAEL for neurotoxicity were 600 and 1000 mg/kg bw in males and females, respectively. The NOAEL values for all effects were 600 and 300 mg/kg bw in males

and females, respectively, based on the occurrence of transient behavioural effects at 1000 mg/kg bw which were considered to reflect general toxicity associated with dose levels that exceed or approach the acute oral LD_{50} values (Ridder & Watson, 2002).

In the 28-d oral range-finding study peformed by Schaeffer (2003 a), a diet concentration of 20000 ppm clearly exceeded the maximum tolerated concentration. The NOAEL were 1000 and 5000 ppm, equivalent to dose levels of 84 and 429 mg/kg bw/d, based on slightly and/or transiently reduced weight gain and food consumption at 5000 ppm in males and 10000 ppm in females. More marked effects were evident at 10000 ppm in males and 20000 ppm in females.The highest diet concentration suitable for use in a subsequent 90-day dietary study was considered to be 10000 ppm.

In the Schaeffer (2003 b) study, the NOAEL for neurotoxicity was > 10000 ppm, equivalent to dose levels of > 625 and > 722 mg/kg bw/d in males and females, respectively, based on the absence of neurobehavioural and neurohistopathological effects at the highest dose level employed. The NOAEL for all effects was established as 1000 ppm, equivalent to dose levels of 67 and 81 mg/kg bw/d in males and females, respectively, based on reduced bw gain and food consumption at 10000 ppm.

Under the conditions of the studies, flonicamid has no neurotoxic potential. Moreover as flonicamid has no chemical similarities to structures known or implicated in producing delayed neurotoxicity, no studies designed to identify delayed neurotoxicity were conducted.

4.12.1.2 *Immunotoxicity*

No data available

4.12.1.3 *Specific investigations: other studies*

No data available

4.12.1.4 *Human information*

No data available

4.12.2 Summary and discussion

The acute toxicity of flonicamid was evaluated following 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; no deaths occur in response to a limit dose of 5000 mg/kg by semi-occluded topical application for 24h (dermal LD_{50} > 5000 mg/kg); the 4-hour nose-only LC_{50} is >4.9 mg/L. Flonicamid is not a skin or eye irritant; it is not sensitizing to the guinea pig. Flonicamid has no genotoxic properties. A number of adequate studies have demonstrated that flonicamid has no potential for carcinogenicity or reproductive toxicity. Flonicamid is not a neurotoxicant.

4.12.3 Comparison with criteria

Flonicamid exhibited an acute oral toxicity 200 < LD_{50} < 2000 mg/kg, according to Dir 67/548 EC this substance meets the criterion for classification with Xn R22.

Flonicamid exhibited an LD_{50} of 884 mg/kg bw, therefore this substance meets the CLP criterion for classification “oral (mg/kg bw) 300 < ATE ≤ 2000”.

4.12.4 Conclusions on classification and labelling

Accordingly, flonicamid requires classification in respect with oral acute toxicity with:

Xn R22, harmful if swallowed

Warning; H302; hazard category 4; harmful if swallowed; P264; P270; P301+P312; P330; P501

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate properties assessment for flonicamid is based on the Draft Assessment Report, the Addenda to the Draft Assessment Report and the EFSA Scientific Report on the peer review of flonicamid.

All the studies on the fate and behaviour of flonicamid in the environment were performed on GLP and according to SETAC or OECD guidelines. Then their reliability factor was stated to 1.

5.1 Degradation

5.1.1 Stability

5.1.1.1 *Hydrolysis*

A single hydrolysis study of flonicamid is available.

Walsh K.J. and Murray M.D. (2000).
This study is performed according to OECD 111 guidelines and is GLP.

Hydrolysis of pyridyl ^{14}C-flonicamid (> 97.7 % purity) was studied at 1 mg/L in sterile buffers at pH 4.0, 5.0, 7.0 and 9.0 for 120 d at 50° C, in sterile buffers at pH 5.0, 7.0 and 9.0 for up to 120 d at 25° C, and in sterile buffer at pH 9.0 for up to 59 d at 40° C with 2 replicates. Acetonitrile was < 1 % in buffers. Solutions were analysed by HPLC and DT_{50} were calculated (single 1st order kinetic with linear regression adjustment). The chemical structures were confirmed by LC-MS.

Because volatiles were not trapped, mass balance could not be determined but the recovered RA was always close to 100 % (Table 104). Whatever the temperature (25 - 50° C), flonicamid was stable at pH 4 and 5. At pH 7, it was stable at 25° C and slowly hydrolysed at 50° C (DT_{50} 578 d, small amounts of TFNG-AM max 5.8 % and TFNG max. 6.8 % could be detected in addition to the parent). 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 main degradation products were TFNG-AM (max. 30.5 % after 120 d, 63.3 % after 43 d and 65.1 % after 20 d at 25, 40 and 50° C, respectively) and TFNG (max. 2.0 % after 120 d, 25.9 % after 59 d and 85.7 % after 120 d at 25, 40 and 50° C, respectively).

Table 104. Hydrolysis of flonicamid at selected sampling time (% of AR, mean of 2 repl.)

Temp.	pH	DAT	Flonicamid	TFNG-AM	TFNA-AM	TFNG	Balance
25° C	5	0	97.9	-	2.1	-	98.6
		15	97.6	-	2.4	-	97.6
		30	97.5	-	2.6	-	101.5
	7	0	97.4	-	2.6	-	100.4
		15	97.3	-	2.7	-	100.5
		30	97.1	-	2.9	-	103.9
	9	0	97.5	-	2.5	-	100.2
		15	93.0	4.2	2.8	-	97.6
		30	88.0	9.3	2.7	-	102.2
		77	75.2	21.5	2.3	1.0	97.9
		120	64.8	30.5	2.8	2.0	106.2
50° C	4	0	98.3	-	1.7	-	101.0
		10	97.0	-	3.0	-	101.6
		120	97.1	-	2.9	-	106.6
	5	0	97.9	-	2.1	-	101.5
		10	97.5	-	2.5	-	99.2
		120	97.2	-	2.9	-	104.8
	7	0	97.5	-	2.5	-	100.4
		10	96.4	0.7	2.8	0.2	105.2
		120	84.6	5.8	2.8	6.8	106.0
	9	0	97.3	-	2.7	-	103.3
		10	35.7	54.7	2.8	6.8	98.1
		20	14.0	65.1	2.4	18.5	96.9
		120		11.4	3.0	85.7	101.9
40° C	9	0	97.5	-	2.5	-	103.0
		5	79.8	17.5	2.7	-	105.2
		10	66.0	30.0	2.4	1.6	100.9
		20	43.8	48.0	3.2	5.1	106.6
		43	17.3	63.3	2.9	16.5	104.7
		59	8.9	62.3	2.9	25.9	108.8

5.1.1.2 *Photolysis*

In water

A single study of the photolysis of flonicamid in water is available.

Walsh K.J. (2002b)
This study is performed according to SETAC guideline and is GLP.

Photolysis of pyridyl ^{14}C-flonicamid (97.6 % purity) was studied at 1.1 mg/L in sterile buffer at pH 7, with 2 replicates. Solutions were kept in darkness or exposed to Xenon lamp (Suntest, 2200 Watt, > 290 nm) for up to 15 d at 23° C. Solutions were analysed by HPLC. The chemical structures were confirmed by LC-MS. The wavelength average quantum yield was calculated by means of the equation proposed by Swanson and co-workers using the measured first order rate constant, wavelength, spectral irradiance and molar absorptivity.
Because volatiles were not trapped, mass balance could not be determined but the recovered RA was always close to 100 % (98.7-102.8 %), data not shown. At pH 7, flonicamid was stable in the dark (> 97.3 %) and limited degradation was observed in the light (flonicamid was 93.6 %, TFNA-AM was 2.9 % and polars were 3.5 % after 15 d). In the light, DT_{50} for flonicamid was calculated to be 267 d at 23° C and the quantum yield to be 0.000319.
At pH 7 and at 23° C, flonicamid is stable in the dark and slowly degraded in the light (DT_{50} 267 d continous artifical light). Thus photodegradation of flonicamid is not expected to occur in surface water.

On soil

A single study of the photolysis of flonicamid on soil is available.

Walsh K.J. (2002a).
This study is performed according to SETAC guideline and is GLP.

Pyridyl ^{14}C-flonicamid (97.9 % purity, TFNA-AM was present in traces) in acetonitrile was applied at 0.1 mg/kg to layers (10 g samples, 3 mm thick) of the dry US loamy sand soil No 072 with 2 replicates. Samples were kept in the dark or continuously exposed to Xenon lamp (Suntest, > 290 nm) for 15 d at 20° C. Soil was extracted with acetonitrile : water (0.1 % phosphoric acid at 85 %) and concentrated extracts were analysed by HPLC. The chemical structures were confirmed by MS. Volatiles were not trapped because a preliminary study showed no volatilisation. Unextractable RA was determined by combustion.

Volatiles were not trapped but extractable + unextractable RA was always close to 100 % of AR (Table 105). In addition metabolites were the same as observed for aerobic degradation where no volatiles other than CO_2 were detected. Accordingly it is reasonable to assume that volatiles were negligible. In the dark, flonicamid was slowly degraded on soil (DT_{50} = 53.3 d, linear 1st order, R^2 0.95) 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, linear 1st order, R^2 0.97) and TFNG-AM was the main degradation product (max. 29.5 % after 15 d).

Table 105. Photodegradation of flonicamid on soil (US loamy sand, % of AR, mean of 2 replicates)

	DAT	Extractable	Unextractable	Balance	Flonicamid	TFNG-AM	TFNG	TFNA-AM
Dark	0	101.3	0.3	101.6	99.0			2.3
	1	99.6	0.7	100.3	95.8	1.2		2.5
	3	99.4	1.1	100.4	93.2	3.8		2.3
	7	99.5	1.2	100.7	88.3	7.8	1.0	2.5
	9	100.3	1.2	101.6	86.1	10.1	1.4	2.8
	11	99.4	0.9	100.3	85.7	11.1		2.6
	15	98.9	1.8	100.7	80.4	13.8	2.0	2.8
Light	0	101.3	0.3	101.6	99.0			2.3
	1	97.8	0.8	98.5	92.0	2.9		2.9
	3	96.5	1.2	97.7	88.0	5.9		2.6
	7	99.8	2.0	101.8	79.8	15.0	0.4	4.6
	9	99.1	1.7	100.7	77.2	17.0	1.0	3.9
	11	98.5	1.7	100.2	69.9	22.0	1.6	5.0
	15	96.0	1.7	97.7	59.5	29.5	2.0	5.0

On dry soil, flonicamid is slowly degraded in the dark (DT_{50} 53 d). Under continous artifical light, degradation is faster (DT_{50} 22 d) but for a 12 h photoperiod difference would be small. Thus photodegradation on soil is not expected to play a significant role as compared to aerobic degradation. TFNG-AM is the only significant degradation product (13.8 % in the dark and 29.5 % in the light after 15 d).

5.1.2 Biodegradation

5.1.2.1 *Biodegradation estimation*

No study on the readily biodegration of flonicamid is available.

5.1.3 Summary and discussion of degradation

5.1.3.1 *Fate and behaviour in water*

In laboratory sterile aqueous hydrolysis experiments flonicamid was stable at pH 4, 5 and 7 at environmentally relevant temperatures. At pH 9 flonicamid degraded with a first order DT_{50} estimated at 204 days at 25°C. The major breakdown product formed was TFNG-AM accounting for a maximum of 30.5% AR at study end (120 days). In laboratory sterile aqueous hydrolysis experiments where TFNA was dosed it was stable at pH 4, 5, 7 and 9 even at 50°C. In a laboratory sterile aqueous photolysis experiment at pH 7 flonicamid degradation was minimal over the 15 days of the experiment.
The water-sediment study (2 systems studied at 20°C in the laboratory) demonstrated flonicamid dissipated exhibiting moderate persistence in water (single first order DT_{50} 30-37 days). In the total system flonicamid also exhibited moderate persistence, (single first order DT_{50} were 36-44 days). In 1 sediment water system (0.74% oc river system) levels of metabolites remained low (max. 1.5% AR in water and 0.9% AR in sediment). In the second (10.2%oc pond system) TFNA accounted for a maximum of 9.6% AR in water (at 30 days) and 9.2% AR in sediment (at 42 days). TFNA-OH accounted for a maximum of 12.5% AR in water (at 42 days) and 2.2% AR in sediment (after 30 days). Levels of both metabolites declined at subsequent sampling times following these maxima. The terminal metabolite, CO_2, was the most significant degradation product accounting for 16-59% AR at 136-145 days (study end). Residues not extracted from sediment by acidified water / acetonitrile were also a significant sink for radioactivity representing 38-75 % AR at study end.

5.1.3.2 *Fate and behaviour on soil*

In soil experiments on four different soils with texture varying from sand to loamy sand (pH 6.2-7.2 organic carbon (oc) 0.6-2.7%) carried out under aerobic conditions in the laboratory (20°C, 45% maximum water holding capacity (MWHC)) in the dark, the predominant pathway of flonicamid degradation was microbially intermediated mineralisation (pyridyl ring radiolabel) to carbon dioxide (47-57 % of applied radioactivity (AR) after 30 days, study end). The formation of residues not extracted by acidified water / acetonitrile was also a significant sink for this applied radiolabel (30-43 % AR after 30 days). Three major (>10% AR) metabolites TFNA (formed by hydrolysis of the amide bond), TFNA-OH (hydroxylation of the piridyl ring of TFNA) and TFNG-AM (hydrolysis of the cyanogroup of flonicamid) were measured at maximum levels of 12-36% AR (at 1-3 days); 12-21% AR (at 2-7 days) and 7.8-10.2%AR (at 0.3-2 days) respectively. Two minor (<10% AR) metabolites TFNG (<3.9% AR) and TFNA-AM (7.6% AR) were also identified and their fate and behaviour was investigated further (soil half lives and adsorption determined).
Degradation under anaerobic conditions was not investigated as it is not pertinent to the season of application of the applied for intended uses and this active substance degrades quickly so would not remain in soil over the autumn / winter period.
In a laboratory soil photolysis study, the rate of degradation on light exposed air dried soil was faster (single first order DT_{50} 22 days under continuous irradiation) than in dark controls (single first order DT_{50} 53 days). Assuming a 12 hour photoperiod the rate of degradation (single first order DT_{50} 45 days) is significantly longer than that measured in the dark higher moisture content aerobic soil incubation experiments where the microbially intermediated hydrolysis reactions would appear to have occurred more readily. No novel metabolites were identified compared to the higher moisture content dark laboratory incubations. The only degradation product identified was TFNG-AM which accounted for 29% AR at study end (15 days).
The rate of degradation of flonicamid determined in the 4 soils gave single first order DT_{50} in the range of 0.7-1.8 days (arithmetic mean 1.1 days, geometric mean 1 day). Flonicamid is considered to exhibit very low to low persistence.
The rate of degradation of the 5 identified flonicamid soil metabolites when applied as test substances on 3 of the 4 soils used for flonicamid (sand to loamy sand pH 5.7-6.9 organic carbon (oc) 0.6-3.2%) gave low calculated single first order DT_{50} with TFNA and TFNG-AM exhibiting very low persistence ($DT_{50} \leq 1$ day) and TFNA-OH, TFNG and TFNA-AM exhibiting very low to low persistence ($DT_{50} \leq 2.6$ days). Arithmetic mean DT_{50} values were TFNA 0.4 days, TFNA-0H 1.6 days, TFNG-AM and TFNG 0.5 days and TFNA-AM 1.6 days.

The adsorption / desorption of flonicamid was investigated in four soils. Calculated adsorption $K_d$oc values varied from 2.5 to 8.7 mL/g, indicating that flonicamid exhibits very high mobility in soil (arithmetic mean 5.9 mL/g). The adsorption / desorption properties of the 5 identified flonicamid soil metabolites was studied in 4 soils or 9 soils for TFNA-AM. Adsorption $K_d$oc values were in the range of <3 to 13.2 mL/g indicating that these 5 metabolites exhibit very high mobility in soil. Arithmetic mean values were TFNA *ca*. 2mL/g, TFNA-0H *ca*. 3 mL/g, TFNG-AM 9.2 mL/g, TFNG *ca*. 1.6 mL/g and TFNA-AM 6.2 mL/g. There was no indication that adsorption of any of these compounds was pH dependant, though this would be very difficult to identify when the measured adsorption was always so minimal.
The meeting of experts discussed if the adsorption study designs were acceptable particularly with respect to the issue of estimating adsorption equilibrium times (that might have been too short) and the use of mercuric chloride to inhibit microbial activity (additional details clarifying the experimental design and deviations from OECD 106 year 2000 guidelines was included in B.8 Addendum 2 of October 2006, that is included in France (2009)). The experts agreed with the assessment of the RMS that as in these cases adsorption is very low, the deviations from the OECD 106 year 2000 study guideline would not be expected to change the Koc values significantly and the expected effect of there being no deviation from this guideline would have been that higher Koc values would be determined.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

From the available studies, flonicamid may be considered to exhibit high to very high mobility in soil ($K_d$oc = 2.5 – 8.7 mL/g; $K_d$oc arithmetic mean = 5.9 mL/g)

5.2.2 Volatilisation

Based on the low vapour pressure (9.43×10^{-7} Pa at 20° C) and the low Henry law constant (4.2×10^{-8} Pa m^3 $mole^{-1}$) flonicamid is not considered as a volatile substance.

5.2.3 Distribution modelling

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5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

Based on its log Kow value of -0.24 (pH not measured at 20°C), no concern over any potential for bioaccumulation could be concluded for flonicamid.

5.3.2 Summary and discussion of aquatic bioaccumulation

See 5.3.1.

5.4 Aquatic toxicity

Only validated ecotoxicity tests accepted for risk assessment from Draft Assessment Reports were used. All the aquatic toxicity studies of flonicamid were performed on GLP and according to EPA or OECD guidelines. Then, the reliability factor would be indicated in the summary only when different of 1.

The reliability factors of the aquatic toxicity studies are reported in the Table 106, which summarised the available data on the toxicity for aquatic organisms.

5.4.1 Fish

Two short-term toxicity studies to fish are available for flonicamid.

5.4.1.1 *Short-term toxicity to fish*

B.9.2.1.1. Acute toxicity of flonicamid to rainbow trout (*Oncorhynchus mykiss*; static test)

Peither, A. (2001a)
This test was GLP and performed according to OECD guideline no 203 (1992). The tested species was *Oncorhynchus mykiss* (rainbow trout).

After one week of acclimatization, seven fish (average length of 5.4 cm and 1.4 g body weight at study start) were tested at the nominal test concentration of 100 mg a.s./L and in a control. Each test vessel (glass aquarium) contained 15 L test medium. The fish were not fed during the test. Temperature in water was 15°C, the pH-value ranged from 7.8 to 7.9 and the dissolved oxygen concentration ranged from 8.7 to 9.2 mg/L (> 60% oxygen saturation). A 16-hour light to 8-hour darkness photoperiod occurred during the test and the light intensity ranged from 50 to 500 Lux.
The concentration of IKI-220 (flonicamid) in the test water was measured at initiation and termination of the test. The test fish were observed after approximately 3, 24, 48, 72 and 96 hours for mortality and symptoms of intoxication.
The measured concentration of IKI-220 in the analysed test medium was 98% of nominal at the start and the end of the test period.
Mortality and other effects: In the control and at the test concentration of 100 mg a.s./L no mortality or other signs of intoxication were determined during the test period of 96 hours.

LC_{50} (96 h) > 100 mg a.s./L
NOEC (96 h) = 100 mg a.s./L

Peither, A. (2001b)
This test was GLP and performed according to OECD guideline no 203 (1992). The tested species was *Lepomis macrochirus* (bluegill sunfish).

After one week of acclimatization, seven fish (average length of 4.5 cm and 1.5 g body weight at study start) were tested at the nominal test concentration of 100 mg a.s./L and in a control. Each test vessel (glass aquarium) contained 15 L test medium. The fish were not fed during the test.
Temperature in water ranged from 22 to 23°C, the pH-value ranged from 7.8 to 8.0 and the dissolved oxygen concentration ranged from 7.4 to 8.2 mg a.s./L (higher than 60% oxygen saturation). A 16-hour light to 8-hour darkness photoperiod occurred during the test and the light intensity ranged from 50 to 500 Lux.
The concentration of IKI-220 (flonicamid) in the test water was measured at initiation and termination of the test. The test fish were observed after approximately 2, 24, 48, 72 and 96 hours for mortality and symptoms of intoxication.
The measured concentration of IKI-220 in the analyzed test medium was 99% of nominal at the start and the end of the test period.
Mortality and other effects: In the control and at the test concentration of 100 mg as/L no mortality or other signs of intoxication were determined during the test period of 96 hours.

LC_{50} (96 h) > 100 mg a.s./L
NOEC (96 h) = 100 mg a.s./L

5.4.1.2 *Long-term toxicity to fish*

A single long-term toxicity study to fish is available for flonicamid.

Palmer, S. J. et al.(2002), Kendall, T. Z. and Nixon, W. B. (2003)

This test (Early life stage toxicity) was GLP and performed according to OECD guideline no 210 (1992). The tested species was *Pimephales promelas* (fathead minnow).

Four replicate test chambers (9-L glass aquarium filled with 7 L of test solution) were maintained in each treatment and control group, with one incubation cup (glass cylinders, ∅ 50 mm with 425 µm nylon mesh screen) in each test chamber. Each incubation cup contained 20 embryos (< 24 hours old), resulting in a total of 80 embryos per treatment. They were exposed to the nominal concentrations of 1.3. 2.5, 5.0, 10 and 20 mg a.s./L and a water control. A continuous-flow diluter was used to deliver approximately 8 volume additions of test water per 24 hours. After a 5-day embryo hatching period, the larvae were released into the test chambers, where exposure continued during a 28-day post hatch juvenile growth period. Newly hatched larvae were fed live brine shrimp nauplii (*Artemia* sp.) 2-3 times per day until 48 hours prior to the test termination.

The test chambers were placed in a temperature-controlled environmental chamber. Temperature of water ranged from 24.2 to 26.0°C, the pH-value ranged from 8.1 to 8.4 and the dissolved oxygen concentration ranged from 7.5 to 8.2 mg/L (92% of saturation) throughout the test. A 16-hour light to 8-hour darkness photoperiod, with a 30-minute transition period of low light intensity, occurred during the test (light intensity at test initiation was 296 Lux).

The concentrations of IKI-220 (flonicamid) were determined from each treatment and control group at the beginning of the test, on Day 5 and at weekly intervals during the test and at test termination on Day 33.

Mortality of the embryos was observed twice during the first 24 hours of exposure and daily until hatching was complete. Then, mortalities and other clinical signs of toxicity of the newly hatched larvae were observed daily during the 28-day post-hatch exposure period. From these observations, hatching success, time to hatch and post-hatch growth and survival were evaluated. Growth was evaluated at the end of the test by measuring the total length and wet and dry weights of each surviving fish.

Mortalities were analyzed using 2 X 2 contingency tables to identify treatment groups that showed a statistically significant difference ($p \leq 0.05$) from the control. The growth parameters (e.g. body length and weight) were evaluated for normality using Shapiro-Wilk's test and for homogeneity of variance using Bartlett's test ($p=0.01$). Dunnett's test was used to evaluate differences between treatment and control means ($p=0.05$). The results of the statistical analysis were used to calculate the NOEC, the LOEC and the maximum acceptable toxicant concentration (MATC) as the geometric mean of the NOEC and LOEC.

The performance of the analytical method to determine IKI-220 in freshwater was verified with fortified samples over a concentration range of 0.25 mg to 25.0 mg a.s./L (see Kendall and Nixon, 2003). The recoveries were 99.9%, 99.8%, 100.0% and 100.0%, respectively (overall mean recovery 99.9%).

The mean measured concentrations of IKI-220 were 1.2, 2.6, 4.9, 9.5 and 20 mg a.s./L, which represented 92%, 104%, 98%, 95% and 100% of nominal, respectively.

Time to hatch: All embryos hatched between Days 4 and 5 in the control and treatment groups. There were no apparent differences among the experimental groups in the time required to hatch.

Hatching success in the control was 99% and ranged from 95 to 100% in the 1.3. 2.5, 5.0, 10 and 20 mg a.s./L treatment groups. There were no statistically significant differences in hatching success between the control and the treatment groups. Therefore, the NOEC for hatching success was 20 mg a.s./L, i.e. the highest concentration tested.

Larval survival in the control at test termination (Day 33) was 91% and ranged from 92 to 96% in the 1.3. 2.5, 5.0, 10 and 20 mg a.s./L treatment groups. There were no statistically significant differences in larval survival between the control and the treatment groups. Consequently, the NOEC for larval survival was 20 mg a.s./L.

Clinical observations during the 28-day post-hatch period showed no treatment-related effects.

Growth: The mean total length was 22.2 mm in the control and wet and dry weights were 76.2 mg and 14.2 mg, respectively. Fish in the 20 mg a.s./L treatment group showed a slight statistically significant reduction in total length (21.3 mm) and dry weight (13.1 mg) measurements in comparison to the control ($p \leq 0.05$). Dunnett's test showed no significant reductions in wet weight among fish in the treatment groups (ranging from 71.9 to 83 mg) in comparison to the control ($p > 0.05$).

NOEC (33 d) = 10 mg/L

5.4.2 Aquatic invertebrates

5.4.2.1 *Short-term toxicity to aquatic invertebrates*

A single short-term toxicity study to aquatic invertebrates is available for flonicamid.

Peither, A. (2001c)
This test was GLP and performed according to OECD guideline no 202 (1984). The tested species was *Daphnia magna*.

Two replicates of 10 daphnids (< 24 hours old) were exposed to the test substance at the nominal concentration of 100 mg a.s./L and to a control. Each test vessel (100 mL glass beakers) contained 50 mL of the test medium.
All test beakers were covered and placed in an environmental chamber. Temperature in water ranged from 20 to 21°C, the pH value from 7.8 to 7.9 and the dissolved oxygen concentration ranged from 8.1 to 8.3 mg/L. A 16-hour light to 8-hour darkness photoperiod occurred during the test and the light intensity ranged from 200 to 1200 Lux.
The concentration of IKI-220 (flonicamid) in the test water was measured at initiation and termination of the test. The daphnids were observed for mortality and immobility after 24 and 48 hours of exposure.
The measured concentration of IKI-220 in the test medium was 98% of nominal at the start and 99% of nominal at the end of the test period.
Immobility and mortality: In the control and at the test concentration of 100 mg a.s./L no dead or immobile daphnids were observed during the test period.

EC_{50} (48 h) > 100 mg a.s./L
NOEC (48 h) = 100 mg a.s./L

5.4.2.2 *Long-term toxicity to aquatic invertebrates*

Two long-term toxicity studies to aquatic invertebrates are available for flonicamid.

Peither, A. (2002i)
This test was GLP and performed according to OECD guideline no 211 (1998). The tested species was *Daphnia magna*.

Ten young daphnids individually held (< 24 hours old,) were used per test concentration and the control. Each test vessel (100 mL glass beaker) contained 80 mL of the test medium (M7). The test substance was tested at the nominal concentrations of 3.1, 6.3, 12.5, 25, 50 and 100 mg a.s./L and renewed 3 times per week. The daphnids were fed every working day with a food mixture containing green algae.
All test beakers were covered and placed in an environmental chamber. Temperature in water ranged from 20 to 21°C, the pH value from 7.6 to 8.2 and the dissolved oxygen concentration ranged from 8.0 to 8.7 mg/L. A 16-hour light to 8-hour darkness photoperiod occurred during the test and the light intensity ranged from 360 to 660 Lux.
The concentration of IKI-220 (flonicamid) in the test media was measured on Days 0, 12, 14, 16 and 19 at nominal concentrations of 3.1 and 6.3 mg a.s./L.

Mortality of adults and the number of young Daphnia were recorded on Days 0, 1, 2 and thereafter three times per week before renewal of the test media. At the end of the test, the body length of each Daphnia was measured.
The NOEC and the LOEC for the reproduction rate and the body length of the adults were determined statistically in comparison with the control group using the multiple Williams-test and a one-way analysis of variance (ANOVA). The EC_{50} for the reproduction rate and the body length were calculated by Probit analysis.
The measured concentrations of IKI-220 in the test media ranged from 93 to 101% of nominal.
Mortality: In the control group and at the test concentrations up to and including 25 mg/L, a maximum mortality rate of 10% for the parental animals, was recorded after 21 days. However, at the concentration of 50 mg/L, a significant mortality rate of 60% was observed at the end of the test, when compared to the control. At the highest concentration tested of 100 mg/L, all test animals died until Day 19. No particular visible abnormalities were observed in any of the concentrations and the control during the test.
Body length: The mean body length of the daphnids in the control was 4.0 ± 0.06 mm. Significant differences in body length of the surviving adults were first observed at the concentration 12.5 mg/L compared to the control group (Williams-test, α=0.05). Therefore, the 21-day EC_{50} for the body length of the adults was calculated to be 87 mg/L (95% confidence limits: 65 – 133 mg/L).
Reproduction rate: In the control and at the test concentrations up to and including 25 mg/L, the appearance of juveniles started on Day 9, but was delayed at the concentration of 50 mg/L (first offspring observed on Day 12). At the highest concentration tested (100 mg/L), no offspring were produced during the test.
The mean number of juveniles per parent in the control group was 78.7 after 21 days. No significant toxic effect on the reproduction rate was determined at the test concentration of 3.1 mg/L. There was a statistically significant difference in the reproduction rate at the test concentrations of 6.3 to 50 mg/L, when compared to the control. Accordingly, the 21-day EC_{50} for the reproduction rate of Daphnia magna was calculated to be 16 mg a.s./L.

NOEC (21 d) = 3.1 mg a.s./L

Peither, A. (2008)
This test was GLP and performed according to OECD guideline no 211 (1998). The tested species was *Daphnia magna*.

The effect of the test item IKI-220 Technical (flonicamid) on the survival and reproduction of *Daphnia magna* was investigated in a semi-static test.

In this semi-static test, the test media of all test concentrations and of the control were renewed on Days 2, 5, 7, 9, 12, 14, 16, and 19 of the test period (every Monday, Wednesday, and Friday). At these dates, the surviving test animals were carefully transferred by means of glass tubes from the old test vessels into the freshly prepared test medium. The study was started with 10 daphnids per treatment. The test duration was 21 days. The nominal concentrations tested were 1.6, 3.1, 6.3, 12.5, 25 and 50 mg/L. Additionally, a control was tested in parallel. The test replicates were observed for mortality of adults on Day 0-2 and thereafter three times per week before renewal of the test media. On the same dates, the test replicates were observed for live and dead offspring and for the presence of aborted eggs. The reproduction rate was calculated as the total number of living offspring produced per parent female surviving until the end of the test. To determine the maintenance of the test item concentrations in the test media, stability samples were taken at the end of test medium renewal periods of 48 hours (Days 2 and 9) and at the end of one renewal period of 72 hours (Day 19).

The concentrations of IKI-220 Technical measured in the test media with nominal test concentrations of 6.3 and 12.5 mg/L were between 97 and 105% of the nominal values. This shows the correct dosage of the test media and the stability of IKI-220 Technical during the renewal periods of two and three days. Therefore, all reported biological results are related to the nominal concentrations of the test item. In the control and at all test concentrations up to and including 25 mg/L, the survival of the test animals at the end of the test was 100%. At the highest test concentration of 50 mg/L, three test animals were dead at test end (30% mortality). Thus, the survival rate of the parent test animals (female daphnids) in the control was at least 80% at the end

of the test as requested by the guidelines. The mean reproduction rate of the daphnids in the control was 122.4 ± 50.3 living offspring per adult (mean ± standard deviation). Thus, the mean number of living offspring in the control was at least 60 per surviving adult *Daphnia* after 21 days, as requested by the guidelines. No significant inhibitory effect of the test item on the mean reproduction rate was determined up to and including the test concentration of 6.3 mg/L (Williams' test, one-sided, $\alpha = 0.05$), where the mean reproduction rate corresponded to 98.7% of the control. At the next higher test concentration of 12.5 mg/L, the mean reproduction rate was statistically significantly reduced with 76.6% compared to the control value. At the next higher test concentrations of nominal 25 and 50 mg/L the inhibitory effect of the test item on the mean reproduction rate successively increased, being in line with a clear concentration effect relationship from the 6.3 mg/L (NOEC) to the highest test concentration of 50 mg/L. At the test concentration of 3.1 mg/L, which was below the NOEC determined in this test, a lower mean reproduction rate of 63.8% (not statistically significant according to the Williams test) was observed. This was considered to be an accidental occurrence and not attributed to a toxic effect of the test item since in this test a clear concentration effect relationship was observed in the range from 6.3 mg/L (NOEC) to 50 mg/L and the test concentration of 3.1 mg/L was not a relevant part of this range.

Taking into account the effects on survival and reproduction of the test animals, the highest concentration of IKI-220 Technical tested without toxic effects after the exposure period of 21 days (21-day NOEC) was 6.3 mg/L. The 21-day EC50 for the reproduction rate of the daphnids was calculated to be 22.8 mg/L (95% confidence limits: 17.6 – 29.9 mg/L).

NOEC (21 d) = 6.3 mg a.s./L

5.4.3 Algae and aquatic plants

A single long-term toxicity study to algae is available for flonicamid.

Peither, A. (2001d)
This test was GLP and performed according to OECD guideline no 201 (1984). The tested species was *Pseudokirchneriella subcapitata*.

Cell density was 1×10^4 cells/mL at initiation of the test. Each test vessel (50 mL Erlenmeyer flasks) contained 15 mL algal suspension. Three replicates per test concentrations of nominal 4.6, 10, 22, 46 and 100 mg a.s./L and six replicates of the control were tested.
All flasks were covered and incubated in a temperature controlled water bath at a temperature of 22°C. They were continuously illuminated with a light intensity ranging from 8300 to 9300 Lux. The pH value in all test media was 7.9 at the start of the test and 9.0 at the end of the test.
The algal cell densities were determined by counting after 24, 48 and 72 hours of exposure using an electronic particle counter. The EC values for biomass (b) and growth rate (μ) were calculated after 72 hours test duration. For the determination of the LOEC and NOEC, the calculated mean biomass and the mean growth rate at the test concentrations were tested for significant differences compared to the control values by a Dunnett's-test (one-sided, $\alpha = 0.05$).
The concentrations of IKI-220 (flonicamid) in the test concentrations of nominal 46 and 100 mg a.s./L were measured at the beginning and at the end of the test.
The measured concentrations in the test media varied between 94% and 97% of nominal.

E_bC_{50} (72 h) > 100 mg a.s./L
E_rC_{50} (72 h) > 100 mg a.s./L
NOEC (72 h) = 46 mg a.s./L

A single long-term toxicity study to aquatic plants is available for flonicamid.

Desjardins, D. et al. (2002)

This test was GLP and performed according to OECD guideline no 221 (Proposal, 2000). The tested species was *Lemna gibba*.

Three replicates, each with 5 plants totalising 15 fronds were used per test concentrations of nominal 7.5, 15, 30, 60 and 120 mg a.s./L and in a control (20X AAP medium, pH 7.5, sterilized by 0.22-µm filtration). Test media were renewed on Days 3 and 5. All test vessels (250 mL sterile glass beakers filled with 100 mL test medium) were covered with petri dishes and incubated in an environmental chamber at a temperature of 25±2°C. They were continuously illuminated with warm-white fluorescent lighting at an intensity of varying between 4480 and 5340 Lux.
Observations: The concentrations of IKI-220 (flonicamid) were measured in the freshly prepared test media of all test concentrations on Days 0, 3 and 5 and in the aged test media on Days 3, 5 and 7 using HPLC with UV detection.
Frond numbers and toxic effects (i.e. chlorosis, necrosis, break-up of colonies, death and other abnormalities) were recorded on Days 3, 5, and 7 to determine the growth inhibition (increase in frond number (ΔFN) and the growth rate (k)). At test termination the dry weight of all Lemna-colonies in each test flask was determined.
The 7-day EC_{50} values were calculated for each growth parameter (increase of frond number, growth rate and dry weight of the colonies) using linear interpolation. Treatment groups were compared to the negative control group (p=0.05) using ANOVA and Dunnett's t-test
The measured concentrations of IKI-220 in the freshly prepared test media ranged from 95.4 to 98.8 of nominal. During the 48- to 72-hour exposure periods no decrease in concentrations was found as demonstrated by the measured concentrations varying between 97.2 and 101% of nominal. The mean measured concentrations were 7.3, 15, 30, 59 and 119 mg a.s./L equivalent to 97.3, 100, 100, 98.3 and 99.2% of nominal. The biological test results are based on mean measured concentrations.
Temperatures ranged from 25.1 to 26.1°C and pH was between 8.0 and 8.2 at test initiation and between 9.3 and 9.4 at test termination.
No mortality occurred at any of the test concentrations and in the control. Therefore, the 7-day EC_{50} for the mortality was higher than the highest concentration tested, i.e. 119 mg a.s./L.
The growth parameters (ΔFN and k) were not statistically significantly reduced in any treatment group in the course of the test when compared to the control group, except for the 15 mg a.s/L treatment group on day 7 showing a 21 % decrease in frond number. For all other test groups percent inhibition of frond growth varied between 1.2 and –4.4%. The situation was identical with regard to the growth rate, which was inhibited by 0.35, 7.7, -1.4, -0.99 and –0.90% at test termination in the 7.3, 15, 30, 59 and 119 mg a.s./L treatment groups, respectively. Inhibition of frond growth was significantly different from the control group only in the 15 mg a.s./L treatment group. Due to the lack of concentration dependent response the effects on both frond growth and growth rate were not considered to be treatment related.
No abnormalities or signs of a toxic effect were observed in any treatment group except in one replicate of the 15 mg a.s/L treatment group, where slightly curled fronds were observed.
The mean dry weight of the plants after 7 days was not statistically significantly reduced in any treatment group. The percent inhibition ranged from 4.1 to –3.5%.

EC50 > 119 mg a.s./L
NOEL = 119 mg a.s./L

5.4.4 Other aquatic organisms (including sediment)

Two toxicity studies to sediment dwelling organisms are available for flonicamid.

5.4.4.1 *Short-term toxicity to sediment dwelling organisms*

A single short-term toxicity study to sediment dwelling organisms is available for flonicamid.

Memmert, U. (2002a)
This test was GLP and performed according to OECD guideline no 202 (1984). The tested species was *Chironomus riparius*.

The acute toxicity of IKI-220 (flonicamid) technical to first-instar larvae of the midge *Chironomus riparius* was determined in a static test over 48 hours. Four replicates of 5 animals (2-3 days old) were exposed to the test substance at the nominal concentrations of 2.0, 6.3, 20, 63 and 200 mg a.s./L and a control. Each test vessel (100 mL glass beakers) contained 50 mL of the test medium (reconstituted water, M7-medium). The chironomids were fed at the start of the study to ensure a sufficient survival of test animals during the test period.
All test beakers were covered with glass plates and placed in an environmental chamber. Temperature in water was 22°C, the pH value ranged from 7.6 to 8.0 and the dissolved oxygen concentration ranged from 7.8 to 8.4 mg/L during the test. A 16-hour light to 8-hour darkness photoperiod occurred during the test and the light intensity ranged from 400 to 700 Lux.
Observations: The concentration of IKI-220 in the test medium of the highest concentration was measured at initiation and termination of the test. The larvae were observed for mortality or symptoms of intoxication after 24 and 48 hours of exposure. The highest test concentration causing no immobilization (NOEC) was determined directly from the raw data without any data transformation.
The measured concentrations of IKI-220 in the test medium from the concentration of nominal 200 mg a.s./L, were 102% and 104% of nominal at the start and the end of the test period, respectively.
Mortality and other effects: In the control and at all test concentrations up to and including 200 mg a.s./L no mortality of the larvae or other signs of intoxication were determined after 24 hours and 48 hours of exposure.

LC_{50} (48 h) > 200 mg a.s./L
NOEC (48 h) = 200 mg a.s./L

5.4.4.2 *Long-term toxicity to sediment dwelling organisms*

A single long-term toxicity study to sediment dwelling organisms is available for flonicamid.

Memmert, U. (2002b)
This test was GLP and performed according to OECD guideline no 219 (Draft, 2001). The tested species was *Chironomus riparius*.

Four replicates of 20 larvae (first-instar larvae; 2-3 days old) were exposed to the test substance at the nominal concentrations of 6.3, 12.5, 25, 50 and 100 mg a.s./L and a control. The animals were acclimatized to the test water for five days. The bottom of each test vessel (3 L glass beakers) was covered with a 3-cm layer of artificial wet sediment (76% sand, 4% sphagnum peat, 20% kaolin clay and 0.31% calcium carbonate to adjust the pH to 7.1, equivalent to 680 g at 46% water content) and filled with 1600 mL of test water (M7 medium, 12 cm depth). One day after adding the larvae, the test substance was applied to the water column. The chironomid larvae were fed fish food three times per week until Day 26.
The test beakers were covered with watch-glasses and additionally with a mosquito net from Day 7 until the end of the test, and placed in a temperature-controlled room. Temperature in water ranged from 19.8 to 21.6°C, the pH ranged from 7.8 to 8.5 and the dissolved oxygen concentration ranged from 7.9 to 9.0 mg/L during the test. A 16-hour light to 8-hour darkness photoperiod with a 30-minute transition period between light and darkness occurred during the test. The light intensity ranged from 690 to 820 Lux. The water was gently aerated during the whole study.
Observations: The concentrations of IKI-220 (flonicamid) were determined in the overlying water, the pore water (obtained by centrifugation of the wet sediment at about 10000 g for 30 minutes) and the sediment samples, in the 25 and 100 mg/L test concentrations on Day 0, 7 and at the test termination on Day 27.
Signs of intoxication of larvae, pupae and emerged midges were recorded. The number and the sex of emerged midges were recorded daily during the period of emergence (from Day 10 to Day 27). The mean emergence ratios (ER), defined as the sum of fully emerged midges divided by the number of inserted larvae, and development rates were statistically evaluated on significant differences to the control. The ER-values were transformed to arcsin-values (ERarc) to obtain an approximate normal distribution and to equalize variances. The multivariate Williams-test was used after a one-way analysis of variance (ANOVA).

Statistical evaluations were done separately for emerged males and females (development rate) and with pooled sexes (emergence ratio). The NOEC and LOEC-values were then determined
The mean measured concentrations of IKI-220 in the overlying water on Day 0 was 93% of nominal and decreased slightly to 86% of nominal on Day 7 and 77-79% of nominal at study termination (Day 27), in both nominal concentrations of 25 and 100 mg/L. In the pore water and sediment the concentrations of IKI-220 continuously increased during the study period. At the concentration of nominal 25 mg a.s./L, the concentration in pore water was in maximum 14.2 mg a.s./L and 6.6 mg a.s./kg in the sediment at study termination on Day 27. At nominal 100 mg a.s./L, in maximum 57.5 mg a.s./L were found in the pore water, and 26.2 mg a.s./kg in the sediment.
No symptoms of toxicity were observed at the larvae, pupae and emerged midges during the study.
The emergence ratios per vessel in the controls ranged from 80 to 100%. Not completely emerged or dead midges were observed at all test concentrations, but also in one of the control beakers. First emergence occurred between Days 11 and 14 in the controls and at all test concentrations. Up to and including 25 mg a.s./L, the mean emergence ratios of male and female midges pooled were not significantly lower than in the control (α=0.05). At the two highest test concentrations of 50 and 100 mg a.s./L the mean emergence ratios (arcsin-transformed) of 1.181 and 1.052, respectively, were statistically significantly lower than in the control (i.e. 1.455).
The mean development rates of fully emerged midges in the control amounted to 0.062 and 0.070 day–1 for the females and the males, respectively. Up to and including 50 mg a.s./L, the mean development rates of the males were not statistically significantly lower than in the control. The mean development rate of the females was significantly reduced at 50 mg a.s./L and higher (α=0.05).

NOEC (27 d) = 25 mg a.s./L

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Data are summarised in Table 106 below.

Table 106. Summary of acute and long term toxicity of flonicamid to the most sensitive species within different groups of aquatic organisms

Organism	Species	Test conditions	LC_{50}/ EC_{50} (mg/L)	NOEC (mg/L)	GLP (Y/N)	Reliability
Fish	*Oncorhynchus mykiss* (Rainbow trout)	96 h static	>100 (nominal)	>100 (nominal)	Y	1
	Pimephales promelas (Fathead minnow)	Early life stage, flow-through test , 33 days	-	10 (nominal)	Y	1
Invertebrates	*Daphnia magna* (waterflea)	48 h, static	> 100 (nominal)	100 (nominal)	Y	1
	Daphnia magna (waterflea)	Growth and reproduction, semi-static, 21 days	-	3.1 (nominal)	Y	1
Algae	*Pseudokirchneriella subcapitata*	Static, 72 h Biomass: Growth rate:	 >100 (nominal) >100 (nominal)	46 (nominal) - -	Y	1
Aquatic plants	*Lemna gibba*	Semi-Static, 7 days	> 119 (measured)	119 (measured)	Y	1
Sediment dwelling organisms	*Chironomus riparius*	48 h, static	> 200 (nominal)	200 (nominal)	Y	1
	Chironomus riparius	28 d, static	-	25 (nominal)	Y	1

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

In toxicity studies for all aquatic organisms EC50s at concentrations above > 100 mg/L were obtained. In addition, flonicamid is not readily biodegradable. However, based on flonicamid physico-chemical properties (log Kow < 3), it is unlikely for the substance to bioaccumulate. Based on these findings, and according to the CLP Regulation, flonicamid should be not classified **NC** (Not Classified).

Here is the classification proposal for chronic toxicity according to the 2nd ATP to the regulation (EC) 1272/2008. The lowest chronic toxicity value was the NOEC = 3.1 mg/L (Peither, A. (2002i), determined with *Daphnia magna*. As a consequence and according to the CLP Regulation, flonicamid should be not classified NC (Not Classified).

Proposed classification based on Directive 67/548/EEC criteria:

NC (Not Classified)

Proposed classification based on CLP criteria:

NC (Not Classified)

6 OTHER INFORMATION

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8 ANNEXES

See appendix 1 for confidential data.