8

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

TXR No.: 0052013

MEMORANDUM

DATE: February 24, 2005

SUBJECT: **FLONICAMID:** Report of the Cancer Assessment Review Committee
PC Code: 128016

FROM: Jessica Kidwell, Executive Secretary for JK 2/24/05
Cancer Assessment Review Committee
Health Effects Division (7509C)

TO: Meta Bonner, Toxicologist
Jack Arthur, Risk Assessor
Registration Branch 3
Health Effects Division (7509C)

Ann Sibold, PM 10
Insecticide Branch,
Registration Division (7505C)

The Cancer Assessment Review Committee met on January 5, 2005 to evaluate the carcinogenic potential of Flonicamid. Attached please find the Final Cancer Assessment Document.

cc: J. Pletcher
Y. Woo

MAR 1 1 2005 5

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF **FLONICAMID**

PC CODE 128016

FINAL
FEBRUARY 24, 2005

CANCER ASSESSMENT REVIEW COMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

FLONICAMID CANCER ASSESSMENT DOCUMENT FINAL

DATA PRESENTATION: _____
Meta Bonner, Ph.D.

DOCUMENT PREPARATION: _____
Jessica Kidwell, Executive Secretary

COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the assessment unless otherwise stated).

Karl Baetcke _____

Lori Brunsman _____

William Burnam _____

Marion Copley _____

Vicki Dellarco _____

Nancy McCarroll _____

Tim McMahon _____

Jess Rowland _____

Linda Taylor _____

NON-COMMITTEE MEMBERS IN ATTENDANCE (Signature indicates concurrence with the pathology report and statistical analysis of data, respectively)

John Pletcher, Consulting Pathologist _____

OTHER ATTENDEES: Jack Arthur (HED/RAB3), Lisa Austin (HED/RAB1), Steve Dapson (HED/RAB3), Whang Phang (HED/RRB1)

TABLE OF CONTENTS

EXECUTIVE SUMMARY

On January 5, 2005, the Cancer Assessment Review Committee of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of Flonicamid.

Meta Bonner of Registration Branch 3 presented the chronic toxicity/carcinogenicity studies in Wistar rats and CD-1 mice. In the rat carcinogenicity study, flonicamid was administered in the diet to groups of Wistar rats (52/sex/dose) at dose levels of 0, 50 (males only), 100 (males only), 200, 1000 or 5000 (females only) ppm (0, 1.84, 3.68, 7.32 mg/kg/day for males; 0, 8.92, 44.1, 219 mg/kg/day for females) for 24 months. In the mouse studies, flonicamid was administered in the diet to groups of CD-1 mice. In the first mouse study, mice (60/sex/dose) received dose levels of 0, 250, 750 or 2250 ppm for a period of 78 weeks. These doses corresponded to mean daily compound consumption of 0, 29, 88, and 261 mg/kg/day for males and 0, 38, 112, and 334 mg/kg/day for females. In the second mouse study mice (50/sex/dose) received dose levels of 0, 10, 25, 80, or 250 ppm for 78 weeks. This resulted in mean daily compound consumption of 0, 1.2, 3.1, 10, or 30.3 mg/kg/day for males and 0, 1.4, 3.7, 11.8, or 36.3 mg/kg/day for females.

The CARC concluded the following:

Carcinogenicity

Rat

- The incidence of nasolacrimal duct squamous cell carcinomas in male rats was 2/51 (4%), 3/50 (6%), 4/48 (8%), 2/52 (4%), 6/52 (12%) for the control, 50, 100, 200, and 1000 ppm dose groups, respectively. Although the high dose incidence was slightly outside the historical control range (0-10%), there were no significant trends or pair-wise comparisons of the dosed groups with the controls for nasolacrimal duct squamous cell carcinomas in male rats. The nasolacrimal duct tumor findings for males is confounded by the lack of a dose-response and the biological significance is questionable. However, there is an indication for a correlation between the incidence of inflammation and the fluctuating incidence of nasal tumors across dose groups. The sponsor has presented reasonable arguments regarding the origin of the tumors in relation to inflammation and malocculusion of incisor teeth. The CARC did not consider the nasolacrimal duct tumors to be treatment-related.

- The incidence of nasolacrimal duct squamous cell carcinomas in female rats was 0/52 (0%), 0/51 (0%), 0/52 (0%), 3/52 (6%) for the control, 200, 1000, and 5000 ppm dose groups, respectively. Female rats had a significant increasing trend in nasolacrimal duct squamous cell carcinomas at $p<0.05$, however, no significant differences in the pair-wise comparisons of the dosed groups with the controls were seen for this tumor type. This is considered to be a rare tumor in female rats and the incidence at the high dose is slightly

above the historical control mean (0.8%) and range (0-4%). Unlike the male rats, the incidence of nasolacrimal duct tumors in female rats could not be clearly correlated with the inflammatory response due to the low incidence of both neoplastic and non-neoplastic lesions. The CARC considered the nasolacrimal duct squamous cell carcinomas to be possibly treatment related, but that a clear association with treatment could not be made.

- The incidence of cerebellar granular cell tumors (GCT) was 3/52 (6%) in high dose females and showed a positive trend but no pair wise significance. These tumors occurred late in the study (≥94 weeks). The occurrence of these slow growing benign cerebellar granular cell tumors in females, although slightly outside of the historical controls range (0-4.35%), was not considered to be treatment related by the CARC. As stated by the Agency's reviewing pathologist "...this is a benign neoplasm that is often only discovered microscopically and, therefore, is probably underreported in the historical data. To my knowledge, granular cell tumors have never been identified as a chemically induced neoplasm. This fact, and the absence of any indication of a similar response in the males, persuades me to agree ... that the occurrence of GCT's in the Wistar study is incidental."

- The CARC considered dosing at the high dose in male (1000 ppm) and female (5000 ppm) rats to be adequate, but not excessive, for the assessment of carcinogenicity. This conclusion was based on overall decreased body weights, body weight gains (Weeks 0-104: 8%, male; 11% female), nephrotoxicity, hepatotoxicity, and ocular toxicity.

Mouse (MRID 45854615)

- In male mice, the incidences (percent) of alveolar/bronchiolar adenomas, carcinoma, and combined adenomas and/or carcinomas for the control, 250, 750, and 2250 ppm dose groups, respectively, were as follows:

  Adenomas: 7/55 (13%), 25/59 (42%), 25/58 (43%), and 32/55 (58%)
  Carcinomas: 4/55 (7%), 6/59 (10%), 12/58 (21%), and 12/55 (22%)
  Combined: 10/55 (18%), 27/59 (46%), 29/58 (50%), and 35/55 (64%)

  Male mice had a significant increasing trend, as well as significant differences in the pair-wise comparisons of all the dosed groups with the control, for alveolar/bronchiolar adenomas, all at $p<0.01$. There was a significant increasing trend, as well as significant differences in the pair-wise comparisons of the 750 and 2250 ppm dose groups with the control, for alveolar/bronchiolar carcinomas, all at $p<0.05$. There was also a significant increasing trend, as well as significant differences in the pair-wise comparison of all the dosed groups with the control for combined alveolar/bronchiolar adenomas and/or carcinomas, all at $p<0.01$. The incidences of adenomas at dose levels ≥250 ppm were greater than the historical control mean (11.6%) and range (0-26%). The incidence of carcinomas was within the historical control mean (5.2%) and range (0-23.2%). The

CARC considered the alveolar/bronchiolar tumors to be treatment related.

- In female mice, the incidences (percent) of alveolar/bronchiolar adenomas, carcinoma, and combined adenomas and/or carcinomas for the control, 250, 750, and 2250 ppm dose groups, respectively, were as follows:

  Adenomas: 9/56 (16%), 20/57 (35%), 29/57 (51%), 24/56 (43%)
  Carcinomas: 0/56 (0%), 3/57 (5%), 3/57 (5%), 7/56 (13%)
  Combined: 9/56 (16%), 22/57 (39%), 31/57 (54%), 25/56 (45%)

  Female mice had a significant increasing trend, at $p<0.05$, as well as significant differences in the pair-wise comparisons of the 250 ppm ($p<0.05$), 750 ppm ($p<0.01$), and 2250 ppm ($p<0.01$) dose groups with the control for alveolar/bronchiolar adenomas. There was a significant increasing trend, as well as a significant difference in the pair-wise comparison of the 2250 ppm dose group with the control for alveolar/bronchiolar carcinomas, both at $p<0.01$. There was also a significant increasing trend, at $p<0.05$, as well as significant differences in the pair wise comparisons of all the dosed groups with the controls, for combined adenomas and/or carcinomas, all at $p<0.01$. The incidences of adenomas at dose levels $\geq$250 ppm were greater than the historical control mean (9.4%) and range (0-16%). The incidence of carcinomas at 2250 ppm (13%) was outside the historical control range (0-12%). The CARC considered the alveolar/bronchiolar tumors to be treatment related.

- The CARC concluded that dosing in both male and female mice was adequate and not excessive. This was based on increased incidence of tissue masses/nodules in the lungs and microscopic findings in the liver, spleen, bone marrow, and lungs.

Mouse (MRID 46205801)

- In male mice, the incidences (percent) of alveolar/bronchiolar adenomas, carcinomas, and combined adenomas and/or carcinomas for the control, 10, 25, 80, or 250 ppm dose groups, respectively, were as follows:

  Adenomas: 8/46 (17%), 11/48 (23%), 12/45 (27%), 11/46 (24%), 21/48 (44%)
  Carcinomas: 3/46 (7%), 6/48 (13%), 3/45 (7%), 4/46 (9%), 9/48 (19%)
  Combined: 11/46 (24%), 16/48 (33%), 15/45 (33%), 14/46 (30%), 27/48 (56%)

  Male mice had a significant increasing trend, as well as a significant difference in the pair-wise comparison of the 250 dose group with the control for alveolar/bronchiolar adenomas, both at $p<0.01$. There was a significant trend only for alveolar/bronchiolar carcinomas, at $p<0.05$. There was also a significant increasing trend, as well as a significant difference in the pair-wise comparison of the 250 ppm dose groups with the control, for combined alveolar/bronchiolar adenomas and/or carcinomas, both at $p<0.01$. The incidence (44%) of adenomas at the high dose (250 ppm) was outside the historical mean (19.9) and range (9.6-32). The incidence of carcinomas at the high dose was within the historical control mean(12.1%) and range (2-30.8). The CARC considered the

alveolar/bronchiolar tumors to be treatment related.

- In female mice, the incidences (percent) of alveolar/bronchiolar adenomas, carcinoma, and combined adenomas and/or carcinomas for the control, 10, 25, 80, or 250 ppm dose groups, respectively, were as follows:

  Adenomas: 10/46 (22%), 8/47 (17%), 11/39 (28%), 14/38 (37%), 13/41 (32%)
  Carcinomas: 1/49 (2%), 4/48 (8%), 2/41 (5%), 3/42 (7%), 3/45 (7%)
  Combined: 10/49 (20%), 12/48 (25%), 12/41 (29%), 16/42 (38%), 16/45 (36%)

  Female mice had a significant increasing trend, as well as significant differences in the pair-wise comparisons of the 80 and 250 ppm dose groups with the control, for combined alveolar/bronchiolar adenomas and/or carcinomas, all at $p<0.05$. The incidence of adenomas (32%) at the high dose was outside the historical mean (13.7%) and range (3.8-26.9%). The incidence of carcinomas (7%) at the high dose was within the historical control mean (8%) and range (2-15.7%). The CARC considered the alveolar/bronchiolar tumors to be treatment related.

- Dosing at the high dose in male and female mice was considered to be adequate, and not excessive, based on lung masses and terminal bronchiole epithelial cell hyperplasia/hypertrophy in both sexes.

Mutagenicity

There is no mutagenicity concern for flonicamid. Flonicamid technical did not cause mutations in the bacterial reverse mutation or mouse lymphoma tests with or without metabolic activation, chromosome damage in the mouse micronucleus or *in vitro* cytogenetics tests with and without metabolic activation. Similarly, there was no increase in DNA damage in the comet assay or in an *in vivo* rat unscheduled DNA synthesis (UDS) study.

Mode of Action

The registrant has presented plausible data showing that the lung tumors in CD-1 mice fed flonicamid are due to mitogenesis, a non-linear, non-genotoxic mode of action for which a threshold has been established. The Agency has reviewed and evaluated the data submitted and concluded that the data support this mode of action. This conclusion is based on the following:

1) *In vivo* and *in vitro* mutagenicity studies confirm that flonicamid is not mutagenic.
2) The BrdU labeling index studies demonstrated the following:
   - There was a *dose-response concordance* between the lung tumors and cell proliferation. The threshold level is between 80 and 250 ppm;
   - *A temporal relationship* was demonstrated, supporting the mode of action. The proliferative response was identified as early as 3 days and was present through 28 days of administration;

- The early mitogenic effect is *reversible;*
- The data support direct mitogenesis as a cause for the increased proliferation rather than cytotoxicity and regeneration. There was *no evidence of necrosis* by light microscopy or by transmission electron microscopy.
- A clear species difference was observed between mice and rats in the incidence of lung tumors and the BrdU Index studies. No tumors were seen in the lungs of rats. The flonicamid induced increase in the BrdU Index appears to be related to the different sensitivity of strains of mice, with the CD-1 mice being a relatively sensitive strain.

The Agency cannot, however, dismiss human relevancy. Although the sponsor has shown that Clara cells are involved in the toxicological response to treatment with the test compound, they have not ruled out if other cell types are involved in the tumorgenic response to treatment. Further, although Clara cells are more numerous in the mouse than in humans, Clara cells are present in the human lung and have been shown to be responsive to the metabolic activity of xenobiotics.

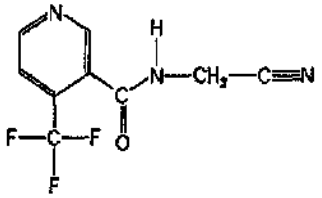
In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 1999), the CARC classified Flonicamid into the category **"Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"**. The quantification of human cancer risk is not recommended.

I. INTRODUCTION

On January 05, 2005, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs will meet to evaluate the carcinogenic potential of Flonicamid. This was the first time that this compound was assessed for carcinogenicity by the CARC.

II. BACKGROUND INFORMATION

Chemical Name:	Flonicamid
Activity:	Insecticide (nicotinoid)
Empirical Formula:	$C_9H_6F_3N_3O$
Molecular Weight:	229.16
CAS Name:	N-(cyanomethyl)-4-(trifluoromethyl)-3-pyridinecarboxamide
CAS Registry No.:	158062-67-0
PC Code:	128016
Structure:	



Flonicamid is an insecticide containing a new active ingredient not included in any previously registered compound. Flonicamid is a systemic insecticide that immediately suppresses the feeding of sucking insects. Its mode of action in insects is unknown, but it appears to be unique. USDA Release, 4/16/02 (http://www.wrpmc.ucdavis.edu/NewsAlerts/flonicamid.html): On April 9, 2002 the OPP Reduced Risk Committee granted organophosphate pesticide (OP) alternative status to ISK Biosciences and FMC's insecticide, flonicamid (F 1785 GH), for use on ornamentals grown in indoor greenhouses. Flonicamid does not work on acetylcholine esterase (OPs and carbamates), or nicotinic acetylcholine receptors (neo-nicotinoids) (Pesticide Briefs (5/10/02):http://ag.udel.edu/extension/information/pesticide/pesticide%20briefs/briefs%2002/briefsmay02.htm#op). Flonicamid is an alternative to the OPs: chlorpyrifos, acephate, dimethoate, and oxydementon methyl; the carbamate: fenoxycarb; and the pyrethroids: bifenthin, and fluvalinate, for use on indoor greenhouse ornamentals to control sucking insects (e.g. aphids, trips, and whiteflies). Its proposed use will be on potatoes, pome fruit, cotton, stone fruit, fruiting vegetables, cucurbits, and leafy vegetables.

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study with Flonicamid in F-344 Rats

Reference: Kuwahara, M. (2002) IKI-220 technical: combined chronic toxicity and carcinogenicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project Study ID: IET 98-0142, December 12, 2002. **MRID 45863801.**

Re-Read: Kuwahara, M. (2004) IKI-220: Combined chronic toxicity and carcinogenicity study in rats histopathological examination of nasal cavity. Laboratory Project ID: IET 04-0067, August 19, 2004. **MRID 46362202.**

Historical Control Data: Kuwahara, M. (2002) Response to EPA review and submission of supplemental data to report: Historical control data for IKI-220 technical: combined chronic toxicity and carcinogenicity study in rats. Document No.: IET 98-0142 SUPP.1, December 12, 2002 & May 18, 2004 - Supplement. **MRID 46275601.**

Historical Control Data: Kuwahara, M. (2002) Response to EPA review and submission of supplemental data to report: Historical control data (RCC LTD., Itingen, Switzerland) for IKI-220 technical: combined chronic toxicity and carcinogenicity study in rats. Document No.: IET 98-0142 SUPP.2, December 12, 2002 & June 25, 2004 - Supplement 2. **MRID 46323701.**

Additional Historical Control Data in Rats for Nasal Cavity: Kuwahara, M. (2004) IKI-220: Combined chronic toxicity and carcinogenicity study in rats - To provide historical control data in rats. Laboratory Project ID: IET 98-0142, August 27, 2004. **MRID 46362205.** (Data from the same and/or similar studies submitted in MRID 46275601)

A. Experimental Design

In this study (MRID 45863801), 52 Wistar rats/sex/dose were exposed to IKI-220 (98.7% a.i.; Batch No.: 9809) for up to 24 months in the diet at concentrations of 0, 50 (males only), 100 (males only), 200, 1000, or 5000 (females only) ppm (equivalent to 0/0, 1.84, 3.68, 7.32/8.92, 36.5/44.1, and 219 mg/kg/day in males/females) for up to 24 months. Additional satellite groups of 10 or 14 rats/sex/dose were similarly treated and sacrificed at 6 and 12 months, respectively.

B. Discussion of Mortality and Tumor Data

Mortality

For male rats, the statistical evaluation of mortality indicated significant differences in the pair-wise comparisons of the 100 and 1000 ppm dose groups with the control (Table 1). No significant trend was noted in males. There were no statistically significant incremental changes in mortality with increasing doses of flonicamid for female rats (Table 2). (Memo, J. Kidwell, 11/22/04, TXR No. 0052829).

Table 1. Flonicamid -Wistar Rat (MRID 46362202) (Re-read)
Male Mortality Rates[a] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	27-52	53[i]	53-78	79-105[f]	Total
0	0/62	1/62	10/61	2/51	6/49	9/52(17)
50	1/62	0/61	10/61	2/51	9/49	12/52(23)
100	0/62	3/62	10/59	2/49	22/47	27/52(52)**
200	0/62	0/62	10/62	5/52	13/47	18/52(35)
1000	0/62	0/62	10/62	4/52	16/48	20/52(38)*

[a]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim Sacrifice at week 53. [f]Final sacrifice at week 105. ( )Percent
Note:Time intervals were selected for display purposes only. Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Flonicamid -Wistar Rat (MRID 46362202) (Re-read)
Female Mortality Rates[+] and Cox or Generalized K/W Test Results
Weeks

Dose (ppm)	1-26	27-52	53[I]	53-78	79-105[F]	Total
0	0/62	0/62	10/62	7/52	14/45	21/52(40)
200	0/62	1/62	10/61	4/51	15/47	20/52(38)
1000	0/62	0/62	10/62	4/52	18/48	22/52(42)
5000	0/62	0/62	10/62	4/52	17/48	21/52(40)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[I]Interim sacrifice at week 53. [F]Final sacrifice at week 105. ( )Percent
Note: Time intervals were selected for display purposes only. Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumors

The incidence of microscopic lesions was known only for controls and high dose groups because not all animals were examined in the other dose groups. For these control vs. high dose comparisons, there were no statistically significant comparisons in the males. Females had statistically significant comparisons of the control vs. the high dose group for nasal cavity squamous cell carcinomas ($p < 0.01$) and cerebellum benign granular cell tumors ($p < 0.05$).

Consequently, the nasal and paranasal tissues from all male and female rats used for 52- and 104-week treatment were histopathologically examined by The Institute of Environmental Toxicology, Ibaraki, Japan for Ishihara Sangyo Kaisha Ltd, Osaka, Japan (Laboratory Project ID: IET 04-0067, August 19, 2004, MRID 46362202). For animals of which the nasal and paranasal tissues had not been examined in the original toxicity study, specimens of the tissues were newly prepared and examined. The rat study had a 26-week interim sacrifice group but the tissues of these animals were not re-read and, therefore, were not included in the statistical analyses.

There were no significant trends or pair-wise comparisons of the dosed groups with the controls for nasolacrimal duct squamous cell carcinomas in male rats (Table 3). Female rats had a significant increasing trend in nasolacrimal duct squamous cell carcinomas at $p<0.05$. However, no significant differences in the pair-wise comparisons of the dosed groups with the controls were seen for this tumor type (Table 4). (Memo, J. Kidwell, 11/22/04, TXR No. 0052829).

Note: The Sponsor has amended the final report (in MRID 46415501 - received 12/01/2004) total number of tumors (all tumors types) due to a calculation error. These adjustments do not alter the information for the nasolacrimal duct tumors.

Note: A discussion and table of cerebellar granular tumors (benign) in female rats are located in the Appendix 1. (Table: Email from Jessica Kidwell, 12/8/04).

Table 3. Flonicamid -Wistar Rat Study (MRID 46362202) (Re-read)
Male Nasolacrimal Duct Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)				
	0	50	100	200	1000
Squamous cell carcinoma (%)	2/51 (4)	3/50 (6)	4/48 (8)	2/52 (4)	6[a]/52 (12)
p =	0.0911	0.3375	0.5341	0.6833	0.2721

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First squamous cell carcinoma observed at week 54, dose 1000 ppm.
Note: Interim sacrifice animals are not included in this analysis. There were no tumors observed in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Flonicamid - Wistar Rat Study (MRID 46362202) (Re-read)
Female Nasolacrimal Duct Tumor Rates+ and Fisher's Exact Test and Exact Test for Trend Results

	Dose (ppm)			
	0	200	1000	5000
Squamous cell carcinoma (%)	0/52 (0)	0/51 (0)	0/52 (0)	3*/52 (6)
p =	0.0152*	1.0000	1.0000	0.1214

+Number of tumor bearing animals/Number of animals examined, excluding those that died before Week 53.

*First squamous cell carcinoma observed at week 74, dose 5000 ppm.

Note: Interim sacrifice animals are not included in this analysis. There were no tumors observed in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Historical Control Data

Historical data of the histopathological examination for the nasal cavity of control Wistar rats came from five studies from the Institute of Environmental Toxicology's (IET) combined chronic and carcinogenicity studies dating from 1982 to 1987 (MRID 46362202 & 46362205) (Table 5). The historical control incidence for squamous cell tumors in the nasolacrimal duct runs below that found for high dose treated groups, but is similar to that found for the concurrent controls. Historical control data are also presented for non-neoplastic lesions (Table 6).

Table 5. Incidence of neoplastic lesions of the nasal cavity in historical control Wistar rats from IET's combined chronic and carcinogenicity studies.

Combined Historical Control Incidence of Squamous Cell Tumors in the Nasolacrimal Duct	Male	Female
Total number of rats examined	255	254
Number of tumors observed	9	2
Incidence (Range)	0% - 10.0%	0% - 4.1%
Incidence (Mean)	3.5%	0.8%

Data from page 17 from MRID 46362202.

Table 6. Incidence of non-neoplastic lesions of the nasal cavity in control Wistar rats from IET combined chronic and carcinogenicity studies.

		Study ID		A	B	C	E
Male rats		Total	%				
	No. of rats examined	205		56	49	50	50
Nasal cavity	Squamatization	1	0.49	1	0	0	0
	Mucosal epithelial hyperplasia	1	0.49	0	1	0	0
	Nasolacrimal duct hyperplasia	8	3.90	0	0	0	8
	Goblet cell proliferation	2	0.98	0	0	0	2
	Rhinitis	14	6.83	0	1	10	3
	Nasolacrimal duct inflammation	25	12.2	0	4	6	15
Female rats		Total	%				
	No. of rats examined	204		56	49	49	50
Nasal cavity	Nasolacrimal duct hyperplasia	4	1.96	0	0	0	4
	Goblet cell proliferation	1	0.49	0	0	0	1
	Rhinitis	16	7.84	5	2	8	1
	Nasolacrimal duct inflammation	16	7.84	0	2	1	13
	Abscess	1	0.49	0	1	0	0

Study A - Nov.1982; Study B - Mar. 1984; Study C - May 1984; Study E - Jul. 1987.
Data from pages 6, 30-31 from MRID 46362205.

In addition, a set of historical control data (MRID 46323701) from 2-year bioassays performed at RCC LTD Itingen in Switzerland was submitted in response to EPA request on 07/02/2004 for historical controls in Wistar rats. The current study (MRID 458638010) was conducted by the Institute if Environmental Toxicology, Ibaraki, Japan. Historical control data from the same lab conducting the study were submitted later, and therefore, the control data from Switzerland were not used in this case.

D. Non-Neoplastic Lesions and Body Weight Data

The Sponsor argues that the incidence of lesions of the nasolacrimal duct seen in rats treated with Flonicamid are in part related to inflammatory changes related to malocclusion of incisor teeth. As evidence of the non-neoplastic lesions in nasolacrimal duct, MRID 46362202 supplies incidence tables for 52 weeks and 104 weeks, Table 7 and Table 8 respectively, of inflammation, squamous cell metaplasia, and squamous cell hyperplasia. There does not appear to be any treatment relation for inflammation, squamous cell metaplasia or hyperplasia in the nasolacrimal duct.

Table 7. Non-neoplastic lesions in nasolacrimal duct incidence in rats treated with IKI-220 in the diet for up to 52 weeks.[a]

Nasolacrimal duct non-neoplastic lesion	Dose (ppm)					
	0	50	100	200	1000	5000
Males						
Inflammation	3	4	2	0	4	NA
Squamous cell metaplasia	3	5	2	1	4	NA
Squamous cell hyperplasia	0	1	0	0	0	NA
Females						
Inflammation	0	NA	NA	1	1	0
Squamous cell metaplasia	0	NA	NA	1	0	0
Squamous cell hyperplasia	0	NA	NA	0	0	0

a Data (n=10) obtained from pages 14 of MRID 46362202.
NA Not tested in this sex

The incidence table of the non-neoplastic lesions in nasolacrimal duct at 104-weeks is in the following table. The incidence of the nasolacrimal duct non-neoplastic lesions are greater in male dose groups than those in female dose groups. The incidence, severity grade, and laterality of inflammation, squamous cell metaplasia and squamous cell hyperplasia were similar in controls and treated groups within each sex.

Table 8. Non-neoplastic lesions in nasolacrimal duct incidence in rats treated with IKI-220 in the diet for up to 104 weeks.[a]

	Dose (ppm)					
Nasolacrimal duct non-neoplastic lesion	**0**	**50**	**100**	**200**	**1000**	**5000**
Males						
Number of animals examined.	52	51	52	52	52	NA
Inflammation	11	11	12	9	12	NA
Squamous cell metaplasia	9	11	10	7	11	NA
Squamous cell hyperplasia	4	3	2	3	6	NA
Females						
Number of animals examined.	52	NA	NA	52	52	52
Inflammation	2	NA	NA	5	5	4
Squamous cell metaplasia	2	NA	NA	2	2	3
Squamous cell hyperplasia	0	NA	NA	0	1	0

a Data obtained from pages 15 of MRID 46362202.

NA Not tested in this sex

Other selected non-neoplastic histological findings are in Tables 9a-9d. Most significant incidences were between the control and high dose groups, but in some cases not all the mid-dose animals were examined. With regard to hyaline droplet deposition in renal proximal tubular cells, the Sponsor makes the case that the hyaline droplets contained alpha-2-u-globulin. Hepatic alpha-2-u-globulin deposition in the kidney can result in nephrotoxicity; humans do not synthesize alpha-2-u-globulin. The effect is minor however, treatment related changes in the kidney were also seen in other studies for rats, mice and dogs.

Table 9a. Selected other non-neoplastic histological findings (%) in rats treated with IKI-220 in the diet for up to 26 weeks.[a]

Non-neoplastic lesion	Dose (ppm)					
	0	50	100	200	1000	5000
Males						
Kidney						
Hyaline droplet deposition in proximal tubular cells (Slight-Moderate Total)	0	0	20	80**	100**	NA
Tubular basophilic change (Slight-Moderate Total)	30	50	40	50	80*	NA
Granular casts in dilated tubules (Slight/Total)	0	0	0	0	50*	NA
Females						
Kidney						
Cytoplasmic vacuolation in the proximal tubular cells (Slight-Moderate Total)	0	NA	NA	0	0	100**
Liver						
Centrilobular hepatocyte hypertrophy (Slight/Total)	0	NA	NA	0	0	100**

a Data (n=10) obtained from pages 390, 409, 1250-1299, and 1610-1649 of MRID 45863801.
NA Not tested in this sex
* Statistically different ($p \leq 0.05$) from the controls
** Statistically different ($p \leq 0.01$) from the controls

Table 9b. Selected other non-neoplastic histological findings (%) in rats treated with IKI-220 in the diet for up to 52 weeks.[a]

Non-neoplastic lesion	Dose (ppm)					
	0	50	100	200	1000	5000
Males						
Kidney						
Hyaline droplet deposition in proximal tubular cells (Slight/Total)	0	0	0	0	100**	NA
Tubular basophilic change (Slight-Moderate-SevereTotal)	40	60	60	80	100**	NA
Females						
Kidney						
Cytoplasmic vacuolation in the proximal tubular cells (Slight/Total)	0	NA	NA	0	0	100**
Liver						
Centrilobular hepatocyte hypertrophy (Slight/Total)	0	NA	NA	0	0	90**

a Data (n=10) obtained from pages 391, 410, 1300-1349, and 1650-1689 of MRID 45863801.
NA Not tested in this sex
** Statistically different ($p \leq 0.01$) from the controls

Table 9c. Selected non-neoplastic histological findings (%) in male rats treated with IKI-220 in the diet for up to 104 weeks.[a]

Non-neoplastic lesion	Dose (ppm)				
	0	50	100	200	1000
Bone marrow, increased hematopoiesis					
Vertebra (Slight-Moderate-Severe Total)	25 (13/52)	36 (4/11)[b]	38 (10/26)[b]	50 (9/18)[b]	38 (20/52)
Sternum (Slight-Moderate-Severe Total)	25 (13/52)	29 (15/51)	24 (12/50)	29 (15/52)	40 (21/52)
Femur (Slight-Moderate-Severe Total)	25 (13/52)	29 (15/51)	26 (13/51)	29 (15/52)	40 (21/52)
Forestomach, erosion/ulcer (Slight-Moderate-Severe Total)	8 (4/52)	18 (2/11)[b]	35 (9/26)[b]	39 (7/18)[b]	29** (15/52)
Kidney, pelvic dilatation (Slight-Moderate Total)	33	31	24	27	46
Hyaline droplet deposition in proximal tubular cell (Slight-Moderate-Severe Total)	21	16	8*	15	37
Eye, keratitis (Slight-Moderate-Severe Total)	12	(5/29)[b]	(2/37)[b]	(3/30)[b]	23

a Data (main group only) obtained from pages 403-408 and 1350-1609 of MRID 45863801. Due to rounding approximations, the reported total may differ by 1% from the sum of the grades.

b Reported as # affected/# examined. The percentage affected may not be reflective of the entire group, because only animals that were sacrificed in extremis or found dead or had macroscopic lesions in the organ were examined histologically. These groups were not subjected to statistical analyses.

c A unilateral effect was observed and the worst severity is reported in this table.

** Statistically different ($p \leq 0.01$) from the controls

Table 9d. Selected non-neoplastic histological findings (%) in female rats treated with IKI-220 in the diet for up to 104 weeks.[a]

Non-neoplastic lesion	Dose (ppm)			
	0	200	1000	5000
Muscle, striated muscle fiber atrophy (Slight-ModerateTotal)	35 (18/52)	40[c] (21/52)	48 (25/52)	100** (52/52)
Kidney, chronic nephropathy (Slight-Moderate-Severe Total)	21	33	21	40*
Cytoplasmic vacuolation in proximal tubular cell (Slight/Total)	0	0	0	71**
Brown pigment deposition in proximal tubular cell (Slight-Moderate-Severe Total)	12	13	10	27*[c]
Liver				
Centrilobular hepatocyte hypertrophy (Total/Slight)	0	0	0	38**
Foci of cellular alteration (eosinophilic-type) (Slight-Moderate-Severe Total)	12	13	19	29*
Eyes, cataract (Slight-Moderate-Severe Total)	46[c]	31	42	65*
Retinal atrophy (Slight-Moderate-Severe Total)	17	10	21	58**
Forestomach, erosion/ulcer (Slight-Moderate-Severe Total)	6 (3/52)	25 (5/20)[b]	27 (6/22)[b]	23*[c] (12/52)
Nasal cavity, rhinitis (Slight-Moderate Total)	10 (5/52)	10 (2/20)[b]	9 (2/22)[b]	23 (12/52)
Pituitary, anterior cell hyperplasia (Slight-Moderate-Severe Total)	15 (8/52)	9 (4/43)[b]	22 (9/41)[b]	31 (16/52)

a Data (main group only) obtained from pages 347-349 and 359-361 of MRID 45863801. Due to rounding approximations, the reported total may differ by 1% from the sum of the grades.

b Reported as # affected/# examined. The percentage affected may not be reflective of the entire group, because only animals that were sacrificed in extremis or found dead or had macroscopic lesions in the organ were examined histologically. These groups were not subjected to statistical analyses.

c The value reported in the summary table does not agree with the reviewer's tabulated result from the individual data.

d A unilateral effect was observed and the worst severity is reported in this table.

* Statistically different ($p \leq 0.05$) from the controls

** Statistically different ($p \leq 0.01$) from the controls

Body Weight and Body Weight Gains

Decreased body weights were observed in the 1000 ppm males during the last year of the study and were significant ($p \leq 0.05$) at Weeks 100 and 104 (↓5-6%; Table 10), which contributed to body weight gain decrease of 61% during this time. Decreased ($p \leq 0.05$) body weights were observed in the 5000 ppm females at Weeks 1-64 (↓3-8%). A minor, incidental decrease ($p \leq 0.05$) in body weight was observed in the 100 ppm males at Week 100. Decreased ($p \leq 0.05$) body weights were observed in the 5000 ppm females that were sacrificed at 52 weeks on Weeks 9-28 (↓4-6%); however these were considered as minor, transient decreases and the weight of the treated group at 52 weeks was similar to the controls. Body weight in the other treated groups were similar to controls.

The decreased body weight gain in the 1000 ppm males during the last year of the study (↓61%) resulted in a decreased overall (Weeks 0-104) body weight gain (↓8%). Decreased body weight gain was generally observed in the 5000 ppm females throughout the study resulting in a decreased overall (Weeks 0-104) body weight gain (↓11%). Body weight gain in the other treated groups were similar to controls.

Table 10. Mean (±SD) body weights (g) at selected intervals in rats treated with IKI-220 in the diet for up to 104 weeks. [a]

Weeks on study	Dose (ppm)					
	0	50	100	200	1000	5000
Males						
0	135±5	135±5	135±5	135±5	135±5	NA
13	425±28	428±23	423±27	422±26	421±20	NA
52	561±38	562±29	552±34	553±35	549±34	NA
104	605±71	604±55	591±129	593±46	566±56* (↓6)	NA
BWG 0-13	290	293	288	287	286	NA
BWG 13-52	136	134	129	131	128	NA
BWG 52-104	44	42	39	40	17 (↓61)	NA
BWG 0-104	470	469	456	458	431 (↓8)	NA
Females						
0	127±6	NA	NA	127±6	127±6	127±6
1	153±8	NA	NA	153±7	153±8	148±7** (↓3)
13	261±17	NA	NA	260±14	258±15	241±15** (↓8)
52	331±33	NA	NA	339±34	333±29	308±28** (↓7)
104	436±63	NA	NA	440±70	436±56	401±59
BWG 0-13	134	NA	NA	133	131	114 (↓15)
BWG 13-52	70	NA	NA	79	75	67 (↓4)
BWG 50-104	105	NA	NA	101	103	93 (↓11)
BWG 0-104	309	NA	NA	313	309	274 (↓11)

a: Data (n=27-52, main group only) obtained from pages 240-242 and 245-247 of MRID 45863801.
Percent difference from controls, calculated by reviewers, is included in parentheses. Body weight gain (BWG) was also calculated by reviewers. NA = Not tested in this sex . * =Statistically different ($p \leq 0.05$) from the controls.
** =Statistically different ($p \leq 0.01$) from the controls.

D. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was considered adequate, based on decreased body weights, body weight gains, nephrotoxicity, hepatotoxicity, and ocular toxicity. Hyaline droplet deposition in renal proximal tubular cells, tubular basophilic changes, and kidney pelvic dilatation were observed starting at 1000 ppm. Also, cytoplasmic vacuolation and brown pigment (lipofuscin) deposition in the renal proximal tubular cells were observed at higher doses. Indications of hepatotoxicity in the high dose group was indicated by decreased triglycerides, increased total cholesterol, and increased relative liver weights, along with increased incidences of dark-colored livers with accentuated lobular pattern and slight centrilobular hepatocyte hypertrophy. Cataracts and retinal atrophy were seen in high dose females.

E. Literature

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 organ. J Environ Pathol Toxicol Oncol 1994; 13(1):49-57.

The Sponsor presented this reference in a presentation on July 27, 2004 to support the idea that malocculusion of incisor teeth may be a contributing factor for the incidence of rats with squamous cell carcinoma of the nasolacrimal duct. First the article noted that the spontaneous occurrence of nasal tumors in ageing rats is very low. Referencing some other studies the article points out incidences of nasal tumors in untreated control Cpb:WU (Wistar random) rats of 7/662 males and 0/442 females, where all seven of the tumors were squamous cell carcinomas associated with malocclusion. Further, another study showed that chronic irritation and disruption of tissue in the mouth, pharynx, and nose of untreated outbred NOL:Wistar (SPF) rats caused by chaff from oat and barley in the feed gave rise to oral and nasal squamous cell carcinomas with an incidence of 8%. From the authors own study of eight long-term toxicity/carcinogenicity studies, they concluded that nasal squamous cell carcinomas in Cpb:WU (Wistar random) rats occurs more in males and is primarily associated with chronic inflammation of the nasolachrymal duct rather than maloccusion, however, they did suggest that maloccusion was probably an indirectly contributing factor probably by aggravation of the nasolachrymal duct damage.

2. Carcinogenicity Studies in Mice

I. The 78 Week CD-1 Mouse Carcinogenicity Study (MRID 45854615)

Reference: Ridder, W.E. and M. Watson. (2003) An oncogenicity study in mice with IKI-220 technical. Toxicology and Pharmacology, Ricerca Biosciences, LLC, Painesville, OH. Laboratory Document #: 011885-1, January 3, 2003. **MRID 45854615.**

A. Experimental Design

The study design allocated groups of 60 CD-1 mice per sex to dietary dose levels of 0, 250, 750, or 2250 ppm of Flonicamid for 78 weeks. This resulted in a mean daily compound consumption of 0, 29, 88, and 261 mg/kg/day for males and 0, 38, 112, and 334 mg/kg/day for females. An additional 10 mice/sex/dose from the control and 2250 ppm dose groups were sacrificed at Week 26 (Interim Sacrifice 1) and Week 52 (Interim Sacrifice 2).

B. Discussion of Mortality and Tumor Data

Mortality

There were no statistically significant incremental changes in mortality with increasing doses of flonicamid in male and female mice (Memo, J. Kidwell, 11/22/04, TXR No. 0052829).

Table 11. Flonicamid - CD-1 Mouse Study (MRID 45854615), Male Mortality Rates[+] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	26 Week Interim Sacrifice	27-52	52 Week Interim. Sacrifice	53-78[f]	Total
0	1/80	10/79	4/69	10/65	8/55	13/60(22)
250	0/60	--	1/60	--	9/59	10/60(17)
750	0/60	--	2/60	--	4/58	6/60(10)
2250	0/80	10/80	5/70	10/65	9/55	14/60(23)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 78. ( )Percent.
Note: Time intervals were selected for display purposes only. Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 12. Flonicamid - CD-1 Mouse Study (MRID 45854615)
Female Mortality Rates[†] and Cox or Generalized K/W Test Results

Dose (ppm)	Weeks 1-26	26 Week Interim Sacrifice	27-52	52 Week Interim Sacrifice	53-79[f]	Total
0	1/80	10/79	3/69	10/66	10/56	14/60(23)
250	0/60	--	3/60	--	8/57	11/60(18)
750	1/60	--	1/58[a]	--	11/57	13/59(22)
2250	2/80	10/78	2/68	10/66	10/56	14/60(23)

[†]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[a]One accidental death at week 47, dose 750 ppm.
[f]Final sacrifice at week 78/79. ( )Percent
Note: Time intervals were selected for display purposes only. Significance of trend denoted at control. Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumors

Gross Pathology: Incidence (%) of selected gross lesions in mice fed IKI-220 in the diet for up to 78 weeks.[a] (MRID 45854615)

Gross lesion	Dose (ppm) 0	250	750	2250
Males				
Lung, Masses	3	12	25	25
Females				
Lung, Masses	5	7	12	12

a Data (n=60) were obtained from pages 32 and 74-77 of MRID 45854615.

Microscopic Pathology: The alveolar/bronchiolar tumors from this mouse carcinogenicity study (MRID 45854615) have been evaluated by a Pathology Work Group (PWG) as requested by Ishibara Sangyo Kaisha Ltd through FMC Corporation, Agricultural Products Group. The PWG review was completed on November 12-13, 2002. The results of the PWG are presented in this document.

Male mice had a significant increasing trend, as well as significant differences in the pair-wise comparisons of all the dosed groups with the control, for alveolar/bronchiolar adenomas, all at $p<0.01$ (Table 13). There was a significant increasing trend, as well as

significant differences in the pair-wise comparisons of the 750 and 2250 ppm dose groups with the control, for alveolar/bronchiolar carcinomas, all at $p<0.05$. There was also a significant increasing trend, as well as significant differences in the pair-wise comparison of all the dosed groups with the control for combined alveolar/bronchiolar adenomas and/or carcinomas, all at $p<0.01$.

Female mice had a significant increasing trend, at $p<0.05$, as well as significant differences in the pair-wise comparisons of the 250 ppm ($p<0.05$), 750 ppm ($p<0.01$), and 2250 ppm ($p<0.01$) dose groups with the control for alveolar/bronchiolar adenomas (Table 14). There was a significant increasing trend, as well as a significant difference in the pair-wise comparison of the 2250 ppm dose group with the control for alveolar/bronchiolar carcinomas, both at $p<0.01$. There was also a significant increasing trend, at $p<0.05$, as well as significant differences in the pair wise comparisons of all the dosed groups with the controls, for combined adenomas and/or carcinomas, all at $p<0.01$.

Table 13. Flonicamid - CD-1 Mouse Study (MRID 45854615)
Male Alveolar/Bronchiolar Tumor Rates+ and Fisher's Exact and Trend Tests Results

	Dose (ppm)			
	0	250	750	2250
Adenomas (%)	7/55 (13)	25[a]/59 (42)	25/58 (43)	32/55 (58)
p =	0.00003**	0.0004**	0.0003**	0.0000**
Carcinomas (%)	4/55 (7)	6[b]/59 (10)	12/58 (21)	12/55 (22)
p =	0.0136*	0.4168	0.0364*	0.0279*
Combined (%)	10[c]/55 (18)	27[d]/59 (46)	29[e]/58 (50)	35[f]/55 (64)
p =	0.0000**	0.0015**	0.0003**	0.0000**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First adenoma observed at week 55, dose 250 ppm.
[b]First carcinoma observed at week 62, dose 250 ppm
[c]One animal in the control group had both an adenoma and a carcinoma.
[d]Four animals in the 250 ppm dose group had both an adenoma and a carcinoma.
[e]Eight animals in the 750 ppm dose group had both an adenoma and a carcinoma.
[f]Nine animals in the 2250 ppm dose group had both an adenoma and a carcinoma.
Note: Interim sacrifice animals are not included in this analyses. There were no tumors observed in any interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 14. Flonicamid - CD-1 Mouse Study (MRID 45854615)
Female Alveolar/Bronchiolar Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Results

	Dose (ppm)			
	0	250	750	2250
Adenomas (%)	9/56 (16)	20/57 (35)	29[a]/57 (51)	24/56 (43)
p =	0.0125*	0.0174*	0.0001**	0.0017**
Carcinomas (%)	0/56 (0)	3[b]/57 (5)	3/57 (5)	7/56 (13)
p =	0.0059**	0.1250	0.1250	0.0064**
Combined (%)	9/56 (16)	22[c]/57 (39)	31[c]/57 (54)	25[d]/56 (45)
p =	0.0123*	0.0063**	0.0000**	0.0009**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First adenoma observed at week 56, dose 750 ppm.
[b]First carcinoma observed at week 78, dose 250 ppm
[c]One animal in each of the 250 and 750 ppm dose groups had both an adenoma and a carcinoma.
[d]Six animals in the 2250 ppm dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals and accidental kill animal are not included in this analyses. An adenoma was observed in the accidental kill animal at week 47, dose 750 ppm.

Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Historical Control Data

Historical control ranges for lung tumors reported from Charles River Laboratories (Giknis and Clifford, 2000) are shown below. Data were obtained from MRID 46362201.

Historical control incidence (%) ranges for lung tumors.[a] (MRID 46362201)

Neoplastic lesion		Historical Controls[c]
Males		
Lung,	Adenoma	11.6% (0-26%)
	Carcinoma	5.2% (0-23.2%)
Females		
Lung,	Adenoma	9.4% (0-16%)
	Carcinoma	6.1% (0-12%)

aData were obtained from page 20.
cData from Giknis and Clifford (2000).

D. Non-Neoplastic Lesions and Body Weight Data

The non-neoplastic lesions observed in both sexes of mice are presented in Tables 15.

Table 15. Incidence (%) of selected non-neoplastic microscopic lesions in mice treated with IKI-220 for up to 78 weeks[a] MRID 45854615.

Microscopic lesion	Dose (ppm)			
	0	250	750	2250
Males				
Liver				
centrilobular hepatocellular hypertrophy	7	23	60	67
Spleen				
extramedullary hematopoiesis	28	50	47	75
pigment deposition	2	0	3	13
Bone marrow, femur				
decreased cellularity	0	2[b]	12[b]	40
pigment deposition	0	7[b]	20[b]	53
Bone marrow, sternum				
decreased cellularity	0	2	8	37
pigment deposition	0	12	25	52
Lung				
hyperplasia/hypertrophy, epithelial cells terminal bronchioles	33	37	77	77

Microscopic lesion	Dose (ppm)			
	0	250	750	2250
	(2/60)	(22/60)	(46/60)	(46/60)
Females				
Liver				
centrilobular hepatocellular hypertrophy	0	0	2	18
Spleen				
extramedullary hematopoiesis	55	57	72	77
pigment deposition	25	15	25	47
Bone marrow, femur				
decreased cellularity	2	12	18	37
pigment deposition	7	3	12	42
Bone marrow, sternum				
decreased cellularity	2	5	10	20
pigment deposition	8[b]	10	17	40
Lung				
hyperplasia/hypertrophy, epithelial cells terminal bronchioles	7 (4/60)	33 (20/60)	68 (41/60)	70 (42/60)

a Data obtained from pages 794-799 of the study report. n=60

b n=59

( ) = incidence/number of animals examined

Body Weight and Body Weight Gains

No treatment-related effects were observed on body weight or body weight gains (Table 16). Differences ($p \leq 0.05$) in body weight were observed, but were minor and unrelated to dose. Transient increases ($p \leq 0.05$) in body weight gains were noted in males. Increases ($p \leq 0.05$) in body weight gain were generally observed in all treated female groups throughout the study, but the effect was not clearly related to dose.

Table 16. Mean (±SD) body weights and body weight gains (g) at selected intervals in mice treated with IKI-220 in the diet for up to 78 weeks[a]

Weeks on Study	Dose (ppm)			
	0	250	750	2250
Males (n=47-80)				
1	29.7±2.30	29.5±2.22	30.7±2.50	30.1±2.22
13	37.4±2.95	37.8±2.52	37.6±2.83	37.7±2.93
25	39.7±3.40	39.2±3.38	39.9±3.57	40.4±3.68
53	41.4±4.40	41.7±3.69	41.8±3.75	42.2±4.56
77	42.2±4.32	42.4±4.92	42.1±3.91	42.6±5.28
Weeks 1-13[b]	7.7	8.3	6.9	7.6
Weeks 13-25[b]	2.3	1.4	2.3	2.7
Weeks 25-53[b]	1.7	2.5	1.9	1.8
Weeks 53-77[b]	0.8	0.7	0.3	0.4
Weeks 1-77[b]	12.5	12.9	11.4	12.5
Females (n=46-80)				
1	23.5±1.94	24.5±2.05* (14)	24.6±1.76** (15)	24.1±2.12
13	30.8±2.98	30.8±2.80	31.4±3.10	31.3±2.59
25	32.9±3.62	32.8±3.40	33.5±3.53	32.5±2.88
53	34.0±3.52	35.3±4.35	35.7±3.68	35.2±3.42
77	36.9±3.87	36.9±4.26	38.6±4.45	36.8±4.08
Weeks 1-13[b]	7.3	6.3	6.8	7.2
Weeks 13-25[b]	2.1	2.0	2.1	1.2
Weeks 25-53	1.1	2.5	2.2	2.7
Weeks 53-77[b]	2.9	1.6	2.9	1.6
Weeks 1-77[b]	13.4	12.4	14.0	12.7

a Data obtained from pages 42-47 of the study report (MRID 45854615). Percent difference from controls, calculated by the reviewers, is included in parentheses.
b Calculated by reviewers
* Significantly different from controls; p≤0.05
** Significantly different from controls; p≤0.01

E. Adequacy of the Dosing for Assessment of Carcinogenicity

Dosing was considered adequate based on increased incidence of tissue masses/nodules in the lungs and microscopic findings in the liver, spleen, bone marrow, and lungs. In the liver at study termination, the high dose group demonstrated increased absolute and relative weights. Centrilobular hepatocellular hypertrophy was observed in all treated groups for males and high dose females, and incidence and severity increased with dose.

In the spleen at terminal sacrifice, extramedullary hematopoiesis was noted in all treated doses for males and for doses 750 ppm and above for females. Increased pigment deposition in the spleen was also noted at the high dose. In the bone marrow decreased cellularity was observed in the femur of the 750 ppm and above treated males and in all treatment groups for females, and in the sternum of the high dose males and in females at doses 750 ppm and above. Pigment deposition was observed in the femur and sternum.

II. 78-Week CD-1 Mouse Carcinogenicity Study (MRID No. 46205801)

Reference: Nagaoka, T. (2004) Dietary carcinogenicity study of IKI-220 technical in mice. Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan. Laboratory Study No.: SBL40-50, January 22, 2004. **MRID 46205801.**

Historical Control Data: Nagaoka, T. (2004) Response to EPA review and submission of supplemental data addendum to report: Historical data for Dietary carcinogenicity study of IKI-220 technical in mice. Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan. Laboratory Study No.: SBL40-50 - ADDENDUM, January 22, 2004 & April 2, 2004 - ADDENDUM. **MRID 46242202.**

A. Experimental Design

The study design allocated groups of 50 CD-1 mice per sex to dietary dose levels of 0, 10, 25, 80, or 250 ppm of Flonicamid for 78 weeks. This resulted in a mean daily compound consumption of 0, 1.2, 3.1, 10, or 30.3 mg/kg/day for males and 0, 1.4, 3.7, 11.8, or 36.3 mg/kg/day for females.

B. Discussion of Mortality and Tumor Data

Mortality

There were no statistically significant incremental changes in mortality with increasing doses of flonicamid in male mice (Table 17). The statistical evaluation of mortality indicated a statistically significant increasing trend with increasing doses of flonicamid in female mice, as well as significant differences in the pair-wise comparisons of the 25 and 80 ppm dose groups with the controls (Table 18). (Memo, J. Kidwell, 11/22/04, TXR No. 0052829).

Table 17. Flonicamid - CD-1 Mouse Study (MRID 46205801)
Male Mortality Rates[+] and Cox or Generalized K/W Test Results
Weeks

Dose (ppm)	1-26	27-52	53-79[f]	Total
0	0/50	4/50	12/46	16/50(32)
10	0/50	2/50	11/48	13/50(26)
25	0/50	5/50	12/45	17/50(34)
80	0/50	4/50	17/46	21/50(42)
250	0/50	2/50	13/48	15/50(30)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 79.
( )Percent
Note: Time intervals were selected for display purposes only. Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 18. Flonicamid - CD-1 Mouse Study (MRID 46205801)
Female Mortality Rates[+] and Cox or Generalized K/W Test Results
Weeks

Dose (ppm)	1-26	27-52	53-79[f]	Total
0	0/50	0/50	6/50	6/50(12)*
10	0/50	0/50	5/50	5/50(10)
25	0/50	7/50	6/43	13/50(26)*
80	0/50	5/50	9/45	14/50(28)*
250	0/50	3/50	10/47	13/50(26)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[f]Final sacrifice at week 79.
( )Percent
Note: Time intervals were selected for display purposes only. Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Tumors

Gross pathology: At 250 ppm, lung masses were increased in males (44% treated vs 20% controls; p≤0.01) and females (34% treated vs 24% controls; not statistically significant [NS]; Table 19). An increased incidence of lung spots was observed in the males (24% treated vs 12% controls; NS). The incidence of thickened uterine wall was increased (42% treated vs 28% controls; NS). Other findings in the treated groups occurred at a similar incidence as controls.

Table 19. Incidence (%) of selected gross lesions in mice fed IKI-220 in the diet for up to 78 weeks.[a] (MRID 46205801)

	Dose (ppm)				
Gross lesion	**0**	**10**	**25**	**80**	**250**
Males					
Lung					
Masses	20	28	26	20	44**
Spot(s)	12	8	8	10	24
Females					
Lung, Masses	24	26	22	28	34
Uterus, Thickened wall	28	34	32	36	42

a Data (n=50) were obtained from pages 583-586 and 593-596 of MRID 46205801.
** Significantly different from controls; p≤0.01

Microscopic pathology: Male mice had a significant increasing trend, as well as a significant difference in the pair-wise comparison of the 250 dose group with the control for alveolar/bronchiolar adenomas, both at p<0.01 (Table 20). There was a significant trend only for alveolar/bronchiolar carcinomas, at p<0.05. There was also a significant increasing trend, as well as a significant difference in the pair-wise comparison of the 250 ppm dose groups with the control, for combined alveolar/bronchiolar adenomas and/or carcinomas, both at p<0.01.

Female mice had a significant increasing trend, as well as significant differences in the pair-wise comparisons of the 80 and 250 ppm dose groups with the control, for combined alveolar/bronchiolar adenomas and/or carcinomas, all at p<0.05 (Table 21).

Table 20. Flonicamid - CD-1 Mouse Study (MRID 46205801)
Male Alveolar/Bronchiolar Tumor Rates[+] and Fisher's Exact Test and Exact Test for Trend Results

	Dose (ppm)				
	0	10	25	80	250
Adenomas (%)	8/46 (17)	11/48 (23)	12/45 (27)	11[a]/46 (24)	21/48 (44)
p =	0.0022**	0.3417	0.2077	0.3037	0.0051**
Carcinomas (%)	3/46 (7)	6/48 (13)	3/45 (7)	4[b]/46 (9)	9/48 (19)
p =	0.0330*	0.2647	0.6510	0.5000	0.0698
Combined (%)	11/46 (24)	16[c]/48 (33)	15/45 (33)	14[c]/46 (30)	27[d]/48 (56)
p =	0.0006**	0.2177	0.2230	0.3199	0.0013**

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 53.
[a]First adenoma observed at week 53, dose 80 ppm.
[b]First carcinoma observed at week 62, dose 80 ppm
[c]One animal in each of the 10 and 80 ppm dose groups had both an adenoma and a carcinoma.
[d]Three animals in the 250 ppm dose group had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 21. Flonicamid - CD-1 Mouse Study (MRID 46205801)
Female Alveolar/Bronchiolar Tumor Rates[+] and Peto's Prevalence Test Results

	Dose (ppm)				
	0	10	25	80	250
Adenomas (%)	10/46 (22)	8/47 (17)	11/39 (28)	14/38 (37)	13[a]/41 (32)
p =	0.0646	0.7183	0.2383	0.0594	0.1339
Carcinomas (%)	1/49 (2)	4/48 (8)	2/41 (5)	3/42 (7)	3[b]/45 (7)
p =	0.3428	0.0718	0.2299	0.1094	0.1506
Combined (%)	10[c]/49 (20)	12/48 (25)	12[c]/41 (29)	16[c]/42 (38)	16/45 (36)
p =	0.0354*	0.3530	0.1655	0.0202*	0.0400*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before observation of the first tumor.
[a]First adenoma observed at week 72, dose 250 ppm.
[b]First carcinoma observed at week 61, dose 250 ppm
[c]One animal in each of the control, 25 and 80 ppm dose groups had both an adenoma and a carcinoma.

Note: Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

C. Historical Control Data

Historical control ranges for lung tumors reported from IET (Nakashima, 2004) are shown in Table 22. Data were obtained from MRID 46362201.

Table 22. Historical control incidence (%) ranges for lung tumors.[a] (MRID 46362201)

Neoplastic lesion	Historical Controls[c]
Males	
Lung, Adenoma	19.9% (9.6-32%)
Carcinoma	12.1% (2.0-30.8%)
Females	
Lung, Adenoma	13.7% (3.8-26.9%)
Carcinoma	8.0% (2.0-15.7%)

aData were obtained from page 21.
cData from Nakashima (2004).

D. Non-Neoplastic Lesions and Body Weight Data

The non-neoplastic lesions observed in both sexes of mice are presented in Table 23.

Table 23. Incidence (%) of selected non-neoplastic microscopic lesions in mice treated with IKI-220 for up to 78 weeks.[a] (MRID 46205801)

Microscopic lesion	Dose (ppm)				
	0	10	25	80	250
Males					
Lung, Hyperplasia/hypertrophy, terminal bronchiole epithelial cell	10 (5/50)	10 (5/50)	10 (5/50)	6 (3/50)	28* (14/50)
Females					
Lung, Hyperplasia/hypertrophy, terminal bronchiole epithelial cell	6 (3/50)	10 (5/50)	4 (2/50)	8 (4/50)	22* (11/50)
Lung, Hyperplasia, alveolar epithelial cell	4 (2/50)	8 (4/50)	4 (2/50)	2 (1/50)	14 (7/50)
Liver, Fatty change, centrilobular hepatocyte	0 (0/50)	0 (0/50)	0 (0/50)	6 (3/50)	10* (5/50)
Uterus, Proliferation, endometrium [b]	54 (12/22)	62 (15/24)	77 (23/30)	77 (24/31)	76 (26/34)
Adrenal, Deposition of brown pigment at the cortico-medullary junction [b]	83 (5/6)	83 (5/6)	54 (7/13)	73 (11/15)	100 (14/14)

a Data were obtained from pages 612-616, 623-627, and 629-1129 of MRID 46205801.

b Reported as # affected/# examined. The percentage affected may not be reflective of the entire group, because only animals that were sacrificed *in extremis* or found dead or had macroscopic lesions in the organ were examined histologically. Data were not tested statistically.

* Significantly different from controls; $p \leq 0.05$

Body Weight and Body Weight Gains

No treatment-related effects were observed on body weight or body weight gains (Table 24). Differences ($p \leq 0.05$) in body weight were observed, but were minor and unrelated to dose.

Table 24. Mean (±SD) body weights and body weight gains (g) at selected intervals in mice treated with IKI-220 in the diet for up to 78 weeks [a]

Weeks on Study	Dose (ppm)				
	0	10	25	80	250
Males (n=30-50)					
1	31.12±1.79	31.14±1.43	31.24±1.67	31.57±1.56	31.10±1.69
13	41.53±3.18	42.06±2.87	41.24±3.01	42.21±2.82	42.05±4.23
53	46.97±4.77	49.98±4.96* (16)	47.17±4.93	48.90±4.79	48.19±5.38
77	46.64±5.59	49.45±5.27	47.88±5.92	49.61±5.64	48.03±5.01
Weeks 1-13 [b]	10.41	10.92	10.00	10.64	10.95
Weeks 13-77 [b]	5.11	7.39	6.64	7.40	5.98
Weeks 1-77 [b]	15.52	18.31	16.64	18.04	16.93
Females (n=36-50)					
1	24.39±1.43	24.17±1.37	24.23±1.35	24.26±1.26	24.34±1.21
13	35.74±3.74	35.66±4.27	34.12±3.05	35.04±3.08	35.44±3.38
53	46.37±6.44	48.45±8.42	45.45±5.96	45.90±7.23	47.07±6.50
77	47.57±7.17	48.66±8.16	47.51±6.58	46.25±6.57	48.20±6.88
Weeks 1-13 [b]	11.35	11.49	9.89	10.78	11.10
Weeks 13-77 [b]	11.83	13.00	13.39	11.21	12.76
Weeks 1-77 [b]	23.18	24.49	23.28	21.99	23.86

a Data obtained from pages 271-274 of MRID 46205801 Percent difference from controls, calculated by the reviewers, is included in parentheses.

b Calculated by reviewers

* Significantly different from controls; $p \leq 0.05$

E. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing was considered adequate based on lung masses and terminal bronchiole epithelial cell hyperplasia/hypertrophy in both sexes.

1. Metabolism

Rat metabolism studies were conducted using ^{14}C-pyridyl-flonicamid. Absorption was rapid as radioactivity was detected in the plasma within 10 minutes of dosing, with maximum plasma concentrations being achieved within 24-54 minutes. The majority of the dose was rapidly excreted. Overall recovery of the radioactive dose from all group was 94-99% by 168 hours post-dose. Flonicamid was a major component of rat urine 48 hours after dosing. TFNA-AM (4-trifluoromethylnicotinamide) was the major metabolite found in rats (urine). TFNG was found between 8%-24% of the total radioactive residue (TRR) in the livers of rats sacrificed at intervals between 0.5-6 hours after dosing. The liver samples at these time intervals had ^{14}C-residues of 2.3%-4.6% of the dose. TFNA was not a major component in animal tissues. The metabolism of flonicamid in animals shows the main pathway of metabolism involves hydrolysis of -CN and - $CONH_2$ functional groups in the molecule, identical to plant metabolism. The main metabolic reactions were hydrolysis of cyano to the amide function and ring hydroxylation. In rats flonicamid was further metabolized by several routes, including nitrile hydrolysis, amide hydrolysis, N-oxidation, and hydroxylation of the pyridine ring, leading to multiple metabolites. The metabolism of flonicamid was also examined in livestock: goat and hen. Metabolite toxicology: The main metabolites of flonicamid were examined in acute oral toxicity studies in rats and bacterial reverse mutation tests. All the metabolites were less toxic than flonicamid and not mutagenic. **(MRID Nos: 45863802-45863805 and 45854703)**

2. Mutagenicity

Flonicamid technical did not cause mutations in the bacterial reverse mutation or mouse lymphoma tests with or without metabolic activation, chromosome damage in the mouse micronucleus or *in vitro* cytogenetics tests with and without metabolic activation. Similarly, there was no increase in DNA damage in the comet assay or in an *in vivo* rat unscheduled DNA synthesis (UDS) study. Based on the weight of evidence, it is concluded, that flonicamid technical is not genotoxic. With the exception of the comet assay, all studies were acceptable according to Subdivision F Guideline requirements for mutagenicity testing. Therefore, there is no mutagenic concern for flinicamid.

(i) In repeat reverse gene mutation assays in bacteria employing the preincubation procedure (**MRID 45656725**), four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, TA100) and the tryptophan deficient (*try*$^-$) WP2 μvrA strain of *Escherichia coli* were exposed at five doses (61.7 to 5000 μg/plate +/- S9 – EXPERIMENT I; 313 to 5000 μg/plate +/- S9 -- EXPERIMENT II) to the test compound (IKI 220 technical, lot 9809, 98.7% a.i., dissolved in dimethylsulfoxide, DMSO) in the presence and absence of metabolic activation (± S9). IKI-220 (flonicamid) is concluded to be nonmutagenic to *S. typhimurium* and *E. coli* tested up to limiting dosing.

(ii) In repeat *in vitro* cytogenetic (chromosome aberrations) assays (**MRID 45656727**), cultures of Chinese hamster lung (CHL) cells were exposed to the test compound (IKI 220 technical, lot no. 9809, 98.7% a.i., in dimethylsulfoxide, DMSO) at 3 concentrations of 573, 1145 and 2290 μg/mL, in the presence and absence of metabolic activation: (i) for 6 hours ("short-term treatment or (ii) for 24 or 48 hours ("continuous treatment", nonactivated conditions only). Minimal cytotoxicity was shown at the HDT, 2290 μg/mL (10 mM, the limit concentration) with no precipitation at any dose or time exposure. Also, no increases in mutant frequency ($TK^{+/-}$) were reported at any dose up to the HDT.

(iii) In an *in vivo* cytogenetic (micronucleus) test (**MRID 45656728**) groups of male and female (5M:5F) ICR mice were administered the test substance (IKI-220 Technical, in 0.5% carboxymethylcellulose, sodium salt, lot 9809, 98.7% a.i.) twice orally by intragastric gavage at doses of 250, 500 and 1000 mg/kg/day for males and 125, 250 and 500 mg/kg/day for females. IKI 220 technical (flonicamid) does not induce micronuclei in male mice up to the limit dose or in females up to a level approaching a lethal dose.

(iv) In an independent repeat (initially by plate incorporation, repeated by pre-incubation) assays (**MRID 45854617**), cultures of four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, and TA100) and the tryptophan-deficient (*try*$^-$) strain of *Eschericha coli* (WP2 *uvr*A) were exposed to TFNA (Batch No. 0006, 99.4% a.i., dissolved in dimethyl sulfoxide, DMSO), in the presence and absence of rat liver microsomal activation (± S9), at six concentrations ranging from 33 to 5000 *ug*/plate. TFNA (flonicamid) is considered non-mutagenic in this standard battery of bacterial strains.

(v) In independent repeat (initial plate incorporation, repeated following preincubation) assays (**MRID 45854618**), cultures of four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* and the tryptophan-deficient (*try*$^-$) WP2 *uvra* strain of *Escherichia coli* were exposed to TFNA-AM (Batch No. 0006, 100% a.i., dissolved in dimethyl sulfoxide, DMSO) at six concentrations ranging from 33 to 5000 *ug*/plate, in the

presence and absence of exogenous metabolic activation (± S9). TFNA-AM is considered non-mutagenic in this battery of bacterial strains.

(vi) In independent repeat (initial plate incorporation, repeated following pre-incubation) assays (**MRID 45854619**), four histidine-deficient (*his*$^-$) strain of *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and the tryptophan-deficient (*try*$^-$) WP2 *uvr*A strain of *Escherichia coli* were exposed to TFNG-AM (Batch No. 0006, 99.5% a.i., dissolved in dimethyl sulfoxide, DMSO), at six concentrations ranging from 33 to 5000 *u*g/plate, in the presence and absence of an exogenous metabolic preparation (± S9). TFNG-AM is considered to be non-mutagenic in this bacterial reverse mutation assay.

(vii) In independent, (initial plate incorporation, repeated following a pre-incubation step) assays (**MRID 45854620**) four histidine-deficient (*his* $^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, and TA100) and the tryptophan deficient (*try* $^-$) WP2 *uvr*A strain of *Escherichia coli* were exposed to TFNA-OH (Batch No. 0010, 100% a.i., dissolved in dimethyl sulfoxide, DMSO), in the presence and absence of exogenous metabolic activation, at six concentrations ranging from 33 to 5000 ug/plate. TFNA-OH is considered non-mutagenic in these bacterial strains.

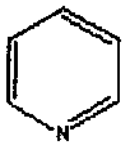
(viii) In independent repeat (initially by plate incorporation, repeated following preincubation) assays (**MRID 45854621**), cultures of four histidine-deficient (*his*$^-$) strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, and TA100) and the tryptophan-deficient (*try*$^-$) WP2 *uvr*A strain of *Escherichia coli* were exposed to TFNG (Batch No. 0006, 99.4% a.i., dissolved in dimethyl sulfoxide, DMSO), in the presence and absence of an exogenous metabolic activation preparation (± S9), at seven concentrations ranging from 5 to 5000 ug/plate (Range-Finding and Trial I, by standard plate incorporation); or five concentrations ranging from 50 to 5000 ug/plate (Trial II, including a preincubation step). TFNG is considered to be nonmutagenic in these bacterial strains.

(ix) In an *in vivo* comet assay, designed to detect DNA lesions in mammalian tissues (**MRID 45854622**), groups of four male mice were administered Flonicamid Technical (Lot No. 9809, suspended in 0.5% aqueous sodium carboxymethylcellulose, CMC-Na) by oral gavage at single doses of 375, 750 and 1500 mg/kg. **While the EPA agrees that Flonicamid Technical was not positive for nuclear migration up to 1500 mg/kg in the three tissues sampled, certain major deficiencies (*e.g.*, no females were tested; no adverse effects were observed with the highest dose tested, despite being 75% of the acute lethal dose; and no rationale was given for the selection of the sampled tissues) classifies this non-guideline assay as performed to be <u>not</u> acceptable for regulatory purposes.**

(x) In an *in vivo* unscheduled DNA synthesis (UDS) repair assay (**MRID 45854623**), groups of four male rats were administered IKI-220 (Batch No. 9809, 98.7% a.i., suspended in 1% methylcellulose, MC) once orally at 600 and 2000 mg/kg, and sacrificed either 2 or 14 hours later. IKI-220 technical (flonicamid) is not genotoxic in hepatocytes from treated rats, *i.e.*, does not cause DNA repair by UDS.

3. Structure-Activity Relationship

Pyridine



Chemical Formula: C_5H_5N
CAS Number: 110-86-1

The CARC noted that pyridine is a simple heterocyclic aromatic compound and is structurally related to benzene, with one CH group in the six-membered ring replaced by a nitrogen atom. [http://en.wikipedia.org/wiki/Pyridine]

The National Toxicology Program (report NTP -TR No. 470) conducted drinking water toxicity studies on pyridine. Male and female F344/N rats, male Wistar rats, and male and female B6C3F1 mice were exposed to pyridine (approximately 99% pure) in drinking water for 13 weeks or 2 years. [http://ntp.niehs.nih.gov/INDEX.CFM?OBJECTID=070A864E-C4F9-A4C1-0565F4A56E271B99] Under the conditions of these 2-year drinking water studies, there was some evidence of carcinogenic activity of pyridine in male F344/N rats based on increased incidences of renal tubule neoplasms. There was equivocal evidence of carcinogenic activity of pyridine in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was equivocal evidence of carcinogenic activity in male Wistar rats based on an increased incidence of interstitial cell adenoma of the testis. There was clear evidence of carcinogenic activity of pyridine in male and female B6C3F1 mice based on increased incidences of malignant hepatocellular neoplasms.

Pyridine was not mutagenic in Salmonella typhimurium strains TA98, TA100, TA1535, or TA1537 or in L5178Y mouse lymphoma cells, with or without S9 metabolic activation, and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells, with or without S9. Pyridine was tested for induction of sex-linked recessive lethal mutations in adult male Drosophila melanogaster, and mixed results were obtained.

4. Subchronic Toxicity

1) M. Kuwahara (2002), IKI-220 Technical: 90 Day oral toxicity study in rats. Institute of Environmental Toxicology, Uchimoriya-machi 4321, Misukaido-shi. Ibaraki 303-0043, Japan. 19 February 2002. Report No. IET 98-0141 MRID 45656721.

In a subchronic rat feeding study (MRID 456567-21), Wistar (Jcl:Wistar) rats (12/sex/dose) were administered technical IKI-220 (98.7% a.i.) in diets at levels of 0, 50 (males only), 200, 1000, 2000 (males only) and 5000 (females only) ppm for a period of 13 weeks. These diets resulted in average dose levels of 3.08, 12.11, 60.0, and 119.4 mg/kg/day for the male rats treated with 50, 200, 1000 and 2000 ppm diets, respectively. The females received 14.52, 72.3 and 340.1 mg/kg/day for the 200, 1000 and 5000 ppm diets, respectively.

The animals were observed daily for clinical signs of toxicity; weekly for body weight changes, food consumption; and routine hematology, clinical chemistry, and urinalyses at the end of the study. Ophthamological observations were made prior to treatment and during week 13. The animals were also evaluated weekly for behavioral changes (functional observation battery) and for additional motor activities during week 12. At the end of the study each animal was necropsied for gross pathological changes, selected organ weights and for routine organ histopathological assessment.

There were no mortalities during the study. Assessments of toxicity for males in the 50 and 200 ppm dose groups and for females in the 50, 200 and 1000 ppm dose groups were comparable to corresponding control animals.

Red periocular staining was seen in all groups of animals (including controls) ranging in incidences from 1/12 or 6/12 without relevance to dose level.

Measurements for motor activity at week 12 indicated that activity rates decreased with each succeeding 10 min bin periods for males with two periods statistically affected for 1000 ppm male groups - on decreased (1st bin) and one increased (4th bin) compared to controls. The overall effects for the 60 min. were comparable to controls. Motor measurements for females were also decreased over each succeeding 10 min. period with measurements in the 2nd , 3rd, and 4th bin periods statistically different from control but not in relationship to dose.

In females in the 5000 ppm group food consumption was significantly decreased at several weeks during the treatment period and average food consumption was 11% lower than that by the controls. Females in the 5000 ppm group, showed a significant decrease in hematocrit (37.3% vs 58.6% for controls) while mean corpuscular hemoglobin

concentration (38.5% vs 37.6% for controls) was significantly increased. Females in the 5000 ppm group also saw significant decreases over controls in glutamic pyruvic transaminase [88% (28/32 (U/L)] and triglyceride [59% (52/88 (mg/dL)]. In males of the 2000 ppm group, significant decreases over controls were noted in glutamic pyruvic transaminase [87% (34/39 U/L)] , creatine phosphokinase [83% (111/134 U/L)] in controls) and triglycerides ([80% (140/175 mg/dL)]. In the 1000 ppm group, females showed a significant decrease in inorganic phosphorous [86% (4.2/4.9 (mg/dL)]. There were no corresponding significant changes in the 1000 ppm males.

At necropsy, males in the 2000 ppm group had an increased incidence of pale kidneys (11/12) which was significantly increased over control animals (0/12). A pale kidney was also seen in one male (1/12) in the 1000 ppm group. There were no significant macroscopic changes in females or of males in other treatment groups. In females treated at 5000 ppm, absolute and relative weights of the liver and kidneys were significantly increased. In males treated at 2000 ppm, absolute and relative weights of the kidneys were significantly increased. In the 1000 ppm group, absolute and relative weights of the kidneys were significantly increased in males. There were no significant changes in organ weight in 1000 ppm females. In males of the 50 ppm group, relative weight of the epididymides was significantly increased. There were no significant changes in organ weight in males and females treated at 200 ppm.

The incidence centrilobular hepatocellular hypertrophy of the liver was significantly increased in high dose males and females: 12/12 for males in the 2000 ppm dose group and 12/12 for females in the 5000 ppm dose group.

Kidneys showed several pathological changes at the upper dose levels, more prominent in males than females. Cytoplasmic vacuolization for the proximal tubular cells of the kidneys of females (12/12) in the HD group (5000 ppm) occurred but not in any of the lower dose females or in any treatment males. Hyaline droplets in proximal tubules occurred with increasing frequency in the 200 ppm males (8/12), and in the 1000 ppm and 2000 ppm males (12/12 in each). These lesions were not seen in low dose males or in any of the corresponding female groups. Granular casts in dilated kidney tubules were significantly increased in the upper dose group males: 5/12 (1000 ppm) and 12/12 (2000 ppm) but not seen in any female groups. Similarly basophilic change of tubular cells occurred in upper dose males: 11/12 (1000 ppm) and 12/12 (2000 ppm) but not in any female test animals.

Inflamation of the nasolacrimal ducts were seen also in all male groups (incidences of 1 to 5 per test group) and females (1 to 4 per test group) but these effects were not treatment (dose) related (see following table).

90-Day Subchronic Oral Toxicity Study in Rats, MRID45656721 Severity of Nasal Cavity, Inflammation, Nasolacrimal Duct						
Sex	**Dose: 0 ppm**	**50 ppm**	**200 ppm**	**1000 ppm**	**2000 ppm**	**5000 ppm**
Male						NA
slight	1	2	4	1	0	
moderate	0	0	1	1	2	
severe	0	0	0	0	0	
Female		NA			NA	
slight	1		1	4		1
moderate	0		0	0		2
severe	0		0	0		0

Nasal Cavity, Inflammation, Nasolacrimal Duct - Totals						
Sex	**Dose: 0 ppm**	**50 ppm**	**200 ppm**	**1000 ppm**	**2000 ppm**	**5000 ppm**
Male	1/12 (8%)	2/12 (17%)	5/12 (42%)	2/12 (17%)	2/12 (17%)	NA
Female	1/12 (8%)	NA	1/12 (8%)	4/12 (33%)	NA	3/12 (25%)

Data from pages 88-89, 293-401 of MRID 45656721. NA = not tested in this sex.
Percent follows observed number over number of animals examined.

The NOAEL of IKI-220 technical to Wistar (Jcl:Wistar) rats under the conditions of the present study was 200 ppm (12.11 mg/kg/day) for males and 1000 ppm (72.3 mg/kg/day) for females. The LOAELs were 1000 ppm (60.0 mg/kg/day) for males based on changes in the kidney (hyaline deposition) and 5000 ppm (340 mg/kg/day) for females based on kidney (hyaline deposition) and liver changes (centrilobular hypertrophy).

The study is considered ACCEPTABLE for meeting the requirements for a 90 day subchronic toxicity study in rats.

2. Ridder, W. E., Yoshida, M. and M. Watson (2001) A 13-week feeding study in mice with IKI-220 technical. Toxicology& Metabolism, Ricerca, LLC, Painesville, Ohio. Document No. 8090-1, December 11, 2001. MRID 45656719.

In a 13-week oral toxicity study (MRID 45656719) flonicamid, 98.7% a.i. lot # 9809)] was administered to 10 Charles River Crl:CD-1 (ICR) BR mice/sex/dose in the diet at dose levels of 0, 100, 1000 or 7,000 ppm (equivalent to 0, 15.25, 153.9 or 1069 mg/kg bw/day in males, and 0, 20.10, 191.5, or 1248 mg/kg bw/day in females).

Body weight gain was decreased in males and females in the 7000 ppm groups from 0-30 days (males: 0.3 g wt. loss; females: 23.7% of controls). Food consumption was significantly decreased (83-90% of controls) in females in the 7000 ppm group from weeks 3-7. Erythrocyte counts were significantly decreased in males and females in the 7000 ppm group (76.9 and 81.5% of controls, respectively, as well as hemoglobin (89.2 and 90.4% of controls, respectively) and hematocrit (88.1 and 90.0% of controls, respectively). MCV was significantly increased in males and females in the 7000 ppm group (115 and 112% of controls, respectively), as well as MCH (116 and 111% of controls, respectively) and reticulocyte counts (511 and 367% of controls, respectively). Males in the 7000 ppm group had a significant increase in total bilirubin (150% of controls). Absolute liver with gall bladder weights were significantly increased in males (121% of controls) and spleen weights were significantly increased in males and females (146 and 163% of controls, respectively) in the 7000 ppm group. Relative liver with gall bladder weights and spleen weights were significantly increased in males and females in the 7000 ppm group (129 and 121% of controls, respectively; and 159 and 174% of controls, respectively). Minimal to moderate centrilobular hepatocellular hypertrophy was observed in all males and females in the 7000 ppm group and in males (minimal grade) in the 1000 ppm group. Minimal to moderately severe extramedullary hematopoiesis of the spleen was observed in all males and females in the 7000 ppm group and in several males and females in the 1000 ppm group. Minimal to moderate increased pigment deposition in the spleen was observed in all animals in the 7000 ppm group. Minimal to mild hypocellularity of the bone marrow was observed only in 8 of 10 males in the 7000 ppm group and increased pigment deposition in the bone marrow was only observed in 7 males and 5 females in the 7000 ppm group. The severity of these lesions of the liver, spleen and bone marrow increased with increasing dose.

The LOAEL is 1000 ppm in (males: 153.9 mg/kg bw/day; females: 191.5 mg/kg bw/day) based on extramedullary hematopoiesis of the spleen. The NOAEL is 100 ppm (males: 15.25 mg/kg bw/day, females: 20.10 mg/kg bw/day).

This 13-week oral toxicity study in the mouse is acceptable (non-guideline) and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100;

OECD 408) in mice. The majority of the tissues/organs recommended by Guideline 870.3100 were not histologically examined in any dose group, including the high dose group. It was determined that this is not a required study and was used as a range-finding study for the mouse carcinogenicity study.

5. Mode of Action Studies

Lung tumors in CD-1 mice following 18-months of dietary administration with flonicamid technical were identified in study reports as alveolar/bronchiolar tumors. The Sponsor has presented the idea that since flonicamid is non-genotoxic that the increased cell proliferation is likely related to Clara cell proliferation by mitogenic activity. The Sponsor provided five studies of BrdU labeling index in the lungs that showed the following:

- treatment with flonicamid in mice increased labeling as early as day 3;
- the level of labeling was less at day 7 compared to day 3;
- day 7 and day 28 had similar labeling levels;
- animals on withdrawal from treatment with flonicamid recovered in one week;
- labeling occurs in the mouse but not in the rat;
- labeling in mouse lungs shows strain sensitivity;
- labeling does not occur with the investigated flonicamid metabolites.

In addition the Sponsor has provided evidence from electron microscopic examination of lung tissue from flonicamid treated mice after 28 days of feeding administration have increased Clara cells numbers, length and size (MRID 4585416, page 104). The increases in length and size of Clara cells returned to normal after one week of recovery (MRID 4585416, page 115-117). Following 28 days of treatment with flonicamid, histopathological study of the mouse lung revealed no evidence of cell necrosis, damage or inflammatory response around the examined activated Clara cells.

Information on focal alveolar/bronchiolar hyperplasia in mouse lung presented below (Table 25).

Table 25. Hyperplasia/ hypertrophy of the pulmonary terminal bronchiolar epithelial cells and tumors seen in mouse lung.

	Dose (ppm)								
Males	0[a]	0	10	25	80	250[a]	250	750	2250
No. animals examined	55[b]	50	50	50	50	59[b]	50	60	60
Hyperplasia/hypertrophy Epithelial cells, Terminal Bronchioles	2	5	5	5	3	22**	14*	46**	46**
Focal Alveolar/Bronchiolar Hyperplasia [c]	2	-	-	-	-	5	-	11**	13**
Adenomas	7	8	11	12	11	25**	21**	25**	33**
Carcinomas	4	3	6	3	4	6	9	12*	12*
Females									
No. animals examined	57[b]	50	50	50	50	58[b]	50	60	60
Hyperplasia/hypertrophy Epithelial cells, Terminal Bronchioles	4	3	5	2	4	20**	11*	41**	42**
Focal Alveolar/Bronchiolar Hyperplasia [c]	5	-	-	-	-	3	-	6	11
Adenomas	9	10	8	11	14	20*	13	30**	24**
Carcinomas	0	1	4	2	3	3	3	3	7**

Data from pages 9- 10 of MRID 45854616, from page 21 MRID 46362201, & from the presentation on July 27, 2004.

*p<0.05, **p<0.01

a, first mouse study; b, animal number = 60 in MRID 45854616 for hyperplasia data;

c, second mouse study, MRID 46205801 did not provide Focal Alveolar/Bronchiolar Hyperplasia data.

The Sponsor conducted a series of studies (MRID 45854616) on Clara cells in an effort to determine the mechanism of action of this compound. A summary of these findings is provided following the discussion on Clara cells.

Clara cells are commonly thought to be the precursor cell for airway epithelium. (Pathology of the Mouse, Maronpot et al., 1999). Clara cells serve as stem cells for airway cell renewal. Thus, tumors derived from Clara cells may differentiate into various bronchiolar cell types, or undergo squamous cell metaplasia (Devor, 1993). Although the sponsor has shown that Clara cells are involved in the toxicological response to treatment with the test compound, they have not ruled out if other cell types are involved in the tumorgenic response to treatment. Further, although Clara cells are more numerous in the mouse than in humans, Clara cells are present in the human

lung and have been show to responsive to the metabolic activity of xenobiotics (Hukkanen et al., 2002).

In the literature there has been some question regarding the differentiation of murine pulmonary adenomas (Mason et al., 2000). According to Kauffman (1981) mouse lung adenomas have two characteristic histologic patterns, alveolar and bronchiolar or papillary. He noted that papillary tumors are said to grow faster and become larger and possibly malignant and that the progression from alveolar to papillary tumors possibly involves a step-wise transformation from benign to malignant tumors. There is evidence from his ultrastructural studies that the brochiolar tumors consist of Clara cells and alveolar tumors are made up of Type II alveolar epithelium, and further that papillary tumors are exclusively from Clara cells.

The Agency's consulting pathologists have given us further information and their information supports a weight of the evidence approach. The followings are e-mails from Drs. Wolf and Pletcher.

> **Doug Wolf**, email 12/03/04: The current thinking is that Clara cells are pluripotential cells that primarily differentiate into the epithelium lining the tertiary bronchioles and can develop into the bronchiolo tumors whereas the cells adjacent to the Clara cells, type II pneumocytes, develop into type I pneumocytes and are the origin of the alveolo tumors
>
> What follows is from Pathology of the Mouse (Maronpot et al., 1999): "The predominant cell type in the smaller airways (in the mouse) is the dome-shaped Clara cell which is interspersed with fewer numbers of ciliated cells. Clara cells are located at all levels of the mouse airways...and comprises up to 50-60% of the cells in the murine airway. In the primary bronchi the population of Clara cells is 47% and ciliated is 46%; in the lobar bronchi ciliated 36% and Clara cells 61%. Ciliated cells are terminally differentiated and do not divide, they are derived from Clara cells. In the unexposed 3-month-old mouse the proliferative index for bronchiolar Clara cells is 0.3%. Toxicants are metabolically activated within Clara cells which can result in selective toxicity of the Clara cells. Toxicity of the adjacent cells and structures may also occur. It is commonly thought that the Clara cell is the precursor cell for airway epithelium."
>
> "Primary hyperplasia of the alveolar epithelium is thought to be a precursor to alveolar/bronchiolar adenomas and carcinomas and derived from Type II cells. Primary hyperplasia of the larger airways is rare in mice and usually due to inhalant toxicants."
>
> The section on histogenesis of lung tumors in the mouse reports that they can originate from either Clara or Type II cells or in some cases both cell characteristics (immunohistochemistry, EM) are present in the tumor cells. However they do state that the majority of mouse lung tumors arise from Type II cells. "It may be that both cell

types are important in mouse lung tumorigenesis, with one cell type being preferentially targeted over the other based on genetics, chemical, and age of mouse."

> **J.Pletcher**, email 12/7/2004: Clara cells are found throughout the pulmonary airways of mammals but are most numerous in the smaller bronchioles. They are recognized by a lack of cilia and prominent electron-dense granules in their apical cytoplasm. Ciliated bronchiolar epithelial cells are thought to be derived through the division and differentiation of Clara cells.....just as Type 1 pneumocytes are derived from Type 2's in the alveoli deeper in the lung parenchyma. Alveolar/bronchiolar (AB) tumors are thought to arise from alveolar epithelial cells (Type 2 pneumocytes) rather than bronchiolar epithelial cells; however, cells resembling Clara cells (dense granules in apical cytoplasm) have been found in some AB neoplasms. So, the jury is still out on the exact! origin o f AB tumors. I believe that what we recognize and diagnosis as AB adenomas or carcinomas may have arisen from either the Type 2 pneumocyte or Clara cells; I don't think you will find anyone at the FDA who will argue that assumption with any conviction. As I mentioned last week, Clara cells (like Type 2 pneumocytes) have a lot more metabolic machinery (P450 enzymes, etc.) than do the ciliated epithelial cells and Type 1 pneumocytes that are considered terminally differentiated cells. The relevance of rodent AB adenomas to human cancer is, as usual, a matter of speculation. The "weight of evidence" approach is the only way to go.

BrdU Immunohistochemical Staining

> *Reference:* Nomura, M. (2003) IKI-220: Discussion on lung finding observed in mouse oncogenicity study. Safety Science Research Laboratory, Central Research Institute, Ishihara Sangyo Kaisha, Ltd., Japan. Laboratory Document #: AN-2203, January 21, 2003. **MRID 45854616**.
>
> Rationale: Cohen, SM, Hardisty, JF, and McCarty, JD (2004) Flonicamid (IKI-220): Rationale for Regulation by Reference Dose. FMC Corporation, Agricultural Products Group, Princeton, NJ. Document #: Flonicamid 04-04, August 31, 2004. **MRID 46362201**.

The Sponsor has presented the view that the findings suggested that the increased incidence of lung tumors in mice was species-specific, unique to the CD-1 mouse, and indicated mitogenic activity (see Appendix 3 for Sponsor's Mode of Action discussion, MRID 46362201). Therefore, the Sponsor conducted a series of mechanistic studies employing bromodeoxyuridine (BrdU) uptake and incorporation into newly synthesized DNA in the lungs to examine cell division. The rationale for these studies was that hyperplasia/hypertrophy of the epithelial cells of the terminal bronchioles was noted, and the terminal bronchioles have been historically observed to be the site of primary tumors in the lung of various strains of mice. Additionally, the cellular

composition of the terminal bronchioles are known to vary among species. Mice have the highest percentage of Clara cells (80-85%), while rats are intermediate (approximately 35%), and humans have an even lower number (approximately 11%). Therefore, studies were designed to examine species and strain differences in response to test substance exposure.

In all of the supplementary studies, clinical signs, mortality, body weight, body weight gains, food consumption and compound intake were measured and recorded. No treatment-related effects were observed on any of these parameters. Compound intakes were similar to those calculated for the mouse carcinogenicity study. Animals were necropsied at sacrifice, and no treatment-related effects were observed at necropsy except as noted in Study 3. In all studies, animals were injected once with BrdU 2 hours prior to scheduled sacrifice except in the first study, when animals were injected 2 times at 2 and 14 hours prior to sacrifice.

1. Cell cycle analysis using BrdU for the threshold of the effect: In the first study (ISK Study # AN-2110), groups of 5 CD-1 male mice were exposed to IKI-220 in the diet at concentrations of 0, 80, 250, 750, or 2250 ppm nominally for 3 days. The mice were injected with BrdU, killed, and the lungs removed and fixed. Lung sections were immunohistochemically stained, and the BrdU labeling index (the number of BrdU-positive nuclei per 1000 nuclei total) in the terminal bronchioles was determined. The labeling index was increased (Table 26) at ≥250 ppm ($p \leq 0.01$ for ≥750 ppm), while the 80 ppm group was comparable to controls. This indicated the test compound caused epithelial cell division in the terminal bronchioles of the treated mice, and that the effect (termed "mitogenic effect" by the Sponsor) had an apparent threshold dose between 80 and 250 ppm.

Table 26. BrdU Index in the Terminal Bronchiole of the Mouse Lung After 3-Days Exposure.

Group Number: Dose	**BrdU: Mean ± SD of positive cells/ 1000 counted cells**
G1: 0 ppm	7.8 ± 2.4
G2: 80 ppm	7.8 ± 2.2
G3: 250 ppm	16.0 ± 8.9
G4: 750 ppm	25.2 ± 6.9**
G5: 2250 ppm	28.4 ± 5.3**

Table from page 27 of MRID46362201.
Dunnett's multiple comparison test *p<0.05, **p<0.01

2. Comparative study on the cell cycle between mice and rats: In the second study (ISK Study # AN-2130), groups of 5 CD-1 female mice and 5 Wistar female rats were exposed to IKI-220 in the diet for 3 or 7 days. For each time interval, mice were given concentrations of 0 or 2250 ppm nominally, while rats were given concentrations of 0 or 5000 ppm. These concentrations yielded comparable compound consumption between the 2 species. The animals were injected with BrdU, killed, and the lungs removed and fixed. Lung sections were immunohistochemically stained, and the BrdU labeling index was determined. The labeling index (Table 27) was increased ($p \leq 0.01$) in the 2250 ppm mice at Days 3 and 7, while the 5000 ppm rats were comparable to controls at both time points. This indicated there was a clear species difference between rats and mice in the potential for IKI-220 to induce epithelial cell division in the terminal bronchioles.

Table 27. BrdU Index of 3 and 7 Day Treatment Groups in Mouse and Rats.

Group	Dose (ppm)	Species	Feeding duration	BrdU: Mean/1000 ± SD
1	0	mouse	3 days	3.6 ± 1.1
2	2250	mouse	3 days	13.6 ± 3.6**
3	0	rat	3 days	3.8 ± 0.8
4	5000	rat	3 days	3.6 ± 1.1
5	0	mouse	7 days	3.8 ± 1.3
6	2250	mouse	7 days	10.4 ± 2.1**
7	0	rat	7 days	3.2 ± 1.5
8	5000	rat	7 days	3.8 ± 1.3

Table from page 28 of MRID46362201.
Bionomial test *p<0.05, **p<0.01

3. Toxicological Effect on the Mouse Lung and Its Reversibility: In the third study (ISK Study # AN-2140), groups of 5 CD-1 male mice were exposed to IKI-220 in the diet at concentrations of 0 or 2250 ppm nominally for 28 days. Mice were sacrificed at Day 28 and following recovery periods of 7, 14, or 28 days without treatment with the test substance. The mice were injected with BrdU, killed, necropsied, and the lungs removed and fixed. Lung sections were immunohistochemically stained either for BrdU and the labeling index determined, or were stained with a Clara cell-specific antibody. Electron microscopy was also performed on lung samples. Necropsy revealed a slight enlargement of the liver at Day 28, but this enlargement resolved in all recovery groups. Reaction to the anti-Clara cell antibody was increased in the terminal bronchiole at Day 28, and electron microscopy of this area revealed trace to minimal longitudinal elongation and hyperplasia/hypertrophy of the Clara cells. These reactions returned to control levels by the 7 day recovery period. The BrdU labeling index (Table 28) was increased ($p<0.01$) on Day 28, but was comparable to controls by the 7 day recovery period. This indicated the test compound caused increased cell division and altered morphology of Clara cells in the terminal bronchioles. These alterations were readily reversible, with cells returning to normal appearance within one week of cessation of treatment.

Table 28. BrdU Index in the Terminal Bronchiole of the Mouse Lung.

Group	Dose (ppm)	Recovery period (Weeks)	BrdU: Mean/1000 ± SD
1	0	0	3.4 ± 1.1
2	2250	0	9.8 ± 2.8**
3	0	1	3.8 ± 1.3
4	2250	1	3.6 ± 1.3
5	0	2	3.2 ± 1.9
6	2250	2	3.6 ± 0.9
7	0	4	3.6 ± 1.5
8	2250	4	3.4 ± 1.1

Table from page 29 of MRID46362201.
Bionomial test *p<0.05, **p<0.01

4. Cell Cycle Analyses of Flonicamid and its Metabolites: In the fourth study (ISK Study # AN-2163), groups of 5 CD-1 male mice were exposed to IKI-220 or its metabolites *N*-(4-trifluoromethylnicotinoyl)glycine (TFNG), 4-trifluoromethylnicotinic acid (TFNA), or 4-trifluoromethylnicotinamide (TFNA-AM) at concentrations of 0 or 2250 ppm nominally in the diet for 3 or 7 days. The animals were injected with BrdU, killed, and the lungs removed and fixed. Lung sections were immunohistochemically stained, and the BrdU labeling index was determined. The labeling index (Table 29) was increased ($p \leq 0.01$) in the IKI-220 mice at Days 3 and 7, while the TFNG, TFNA, and TFNA-AM mice were comparable to controls at both time points. This indicated only the test compound and none of the metabolites had mitogenic activity in the terminal bronchioles of mice.

Table 29. BrdU Index in the Terminal Bronchiole of the Mouse Lung of 3 and 7 Day Groups.

Group	Dose (ppm)	Test material	Feeding duration	BrdU: Mean/1000 ± SD
1	0	Control	3 days	3.6 ± 1.1
2	2250	Flonicamid	3 days	12.6 ± 3.6**
3	2250	TFNG	3 days	3.0 ± 0.7
4	2250	TFNA	3 days	3.4 ± 1.5
5	2250	TFNA-AM	3 days	3.8 ± 0.8
6	0	Control	7 days	3.2 ± 1.1
7	2250	Flonicamid	7 days	9.4 ± 4.5**
8	2250	TFNG	7 days	3.4 ± 1.5
9	2250	TFNA	7 days	3.4 ± 1.1
10	2250	TFNA-AM	7 days	3.6 ± 1.5

Table from page 32 of MRID46362201.
Bionomial test *p<0.05, **p<0.01

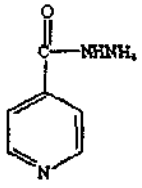
5. Comparative Study with Flonicamid (IKI-220) and Isoniazid Among Three Mouse Strains: In the final study (ISK Study # AN-2200), groups of 5 CD-1, B6C3F1 or C57 male mice were exposed to IKI-220 or the anti-tubercular drug isoniazid at concentrations of 0 or 2250 ppm nominally in the diet for 3 days. The animals were injected with BrdU, killed, and the lungs removed and fixed. Lung sections were immunohistochemically stained for BrdU and the labeling index determined. Additional lung sections were stained with a Clara cell-specific antibody. The labeling index (Table 30) was increased ($p \leq 0.01$) in CD-1 mice treated with IKI-220, while B6C3F1 and C57 mice treated with IKI-220 demonstrated labeling indices comparable to controls. In contrast, isoniazid caused increased ($p \leq 0.05$) labeling indices in all strains of mice. Examination of lung sections stained with the anti-Clara cell antibody showed that the terminal bronchioles of all 3 strains of mice contained similar percentages of Clara cells (79-81%) compared to control rats (35%) from the second study (AN-2130). This indicated different strains of mice exhibited variable sensitivity to the mitogenic effects of the test compound and isoniazid; however, there were no strain differences in the number of Clara cells of the terminal bronchioles of the three strains of mice.

Table 30. BrdU Index in the Mouse Lung in Response to Flonicamid and Isoniazid.

Group	Mouse Strain	Compound	Dose (ppm)	BrdU: Mean/1000 ± SD
1	CD-1	Control	0	3.4 ± 1.3
2	CD-1	Flonicamid	2250	10.6 ± 3.6**
3	CD-1	Isoniazid	2250	23.6 ± 2.7**
4	B6C3F1	Control	0	3.0 ± 0.5
5	B6C3F1	Flonicamid	2250	3.0 ± 1.4
6	B6C3F1	Isoniazid	2250	12.0 ± 3.3**
7	C57	Control	0	2.0 ± 0.8
8	C57	Flonicamid	2250	2.2 ± 0.7
9	C57	Isoniazid	2250	4.0 ± 1.6*

Table from page 32 of MRID46362201.
Bionomial test *p<0.05, **p<0.01

Note for Isoniazid (IND);



CAS number 54-85-3; Chemical name: isonicotinic acid hydrazide;
Molecular formula: $C_6H_7N_3O$; Molecular weight: 137.14

The Sponsor supplied some background information on isoniazid: it has been used as a drug to treat tuberculosis. It has been shown to increase the incidence of brochioloalveolar tumors in mice but not in rats (data not given). A comparative study between flonicamid and isoniazid confirmed that isoniazid was more potent in causing increased cell turnover in mouse lungs than was flonicamid (data not given). The IARC (International Agency for Research on Cancer) has classified isoniazid in Group 3 (cannot be classified as to its carcinogenicity to humans). A internet literature search provide the following regarding the carcinogenicity of isoniazid: IARC report on human carcinogenicity (inadequate) and IARC report of animal carcinogenicity (limited) -

rats, and failed to produce tumors in hamsters when given orally [International Agency for Research on Cancer (IARC) - Summaries & Evaluations - Isonicotinic Acid Hydrazide (Isoniazid) Supplement 7: 1987, p. 227.]. An article reported on the perinatal exposure: Tumorigenicity of isoniazid was studied in male and virgin Swiss mice. Isoniazid induced 50% lung tumors in males and 67% in females, respectively. Lung tumors were induced in animals of the F1 generation that were exposed during intrauterine life, lactation, and in the postweaning period. Also, the F2 generation from these F1 animals, which were exposed to isoniazid only during gestation and lactation, showed earlier and much higher tumor incidence than the parent generation. [Menon MM, Bhide SV. (1983) Perinatal carcinogenicity of isoniazid (INH) in Swiss mice. J Cancer Res Clin Oncol. 1983;105(3):258-61.]

The Sponsor states that isoniazid is mutagenic *in vitro* and questionably *in vivo* (presentation on July 27, 2004). This comparison between flonicamid and isoniazid shows that both chemicals produce tumors in mice, however one is non-mutagenic and the other is mutagenic, also it is not clear if the tumor type and cell type are the same, and further it does not provide enough information to allow us to make predictions from isoniazid to flonicamid for the human situation..

In regarding to using mouse lung data for extrapolation for human risk assessment, review (internet web search 12/01/04) of the literature reveals the following: The literature has stated that murine pulmonary adenomas have not only been used to provide insight into the molecular events in carcinogenesis but also have been used extensively to model human lung adenocarcinomas (Mason et al., 2000). Further, Hukkanen (2002) in his article concerning lung metabolism expressed the following: only limited comparison is possible based on current knowledge regarding to what extent lung metabolism in rodent and other laboratory animals reflect the situation in the human lung, because we still do not know enough about quantitative metabolism pathways in either human or animal tissue. Although similar cell types and that similar types of metabolism occurs in both human and mouse, there is still some uncertain in using extrapolations between mouse and human.

The information submitted by the Sponsor was reviewed by Dr. John Pletcher of Pathology Associates (HED's CARC consulting pathologist) and his evaluation (12/8/2004, see Appendix 2 for full memo) used the weight of the evidence approach. Dr. Pletcher's evaluation: Cohen, Hardisty and McCarty's report entitled "FLONICAMID (IKI-220):Rationale for Regulation by Reference Dose" addresses the various EPA concerns in a reasoned and scientifically sound manner. I found their explanation for the slight and non-statistically significant increase observed in squamous cell carcinomas from the area of the nasal cavity persuasive; the J. Environ. Pathol. Toxicol. Oncol. (1994) article was very pertinent and germane. The argument that there exists a causal relationship between the occurrence of such tumor and chronic inflammation in the

nasolacrimal duct, the inflammation sometimes but not always being the result of dental malocclusion, makes very good sense and is difficult to refute. I believe that their conclusion concerning squamous cell carcinomas in the area of the nasal cavity in Wistar rats, that they are not treatment related, is sound. It should be noted that the fluoride in the Flonicamid compound is never released and is therefore not a cause of dental problems that could result in malocclusions in rodents. The occurrence of three granular cell tumors (GCT) in the high dose female Wistars was another concern; however, as correctly stated by Cohen et al., this is a benign neoplasm that is often only discovered microscopically and, therefore, is probably underreported in the historical data. To my knowledge, GCT's have never been identified as a chemically induced neoplasm. This fact, and the absence of any indication of a similar response in the males, persuades me to agree with Cohen et al. that the occurrence of GCT's in the Wistar study is incidental. Concerning the increase in bone marrow (femur) hematopoiesis in the Wistars, the increase in high dose males is clearly more apparent than real. The data showing the results of the evaluation of the early deaths and the terminal sacrifice males (and females) separately relieves any concerns I might have had. The additional studies presented by Cohen et al. to support their conclusion that Flonicamid is a carcinogen in CD-1 mice due to a particular susceptibility in the strain but does not produce cancer in humans (or rats) are scientifically valid and present a strong case, particularly in the absence of any demonstrable genotoxicity. These results will have to be considered in the "weight of evidence" process.

V. COMMITTEE'S ASSESSMENT OF THE WEIGHT-OF-THE-EVIDENCE

1. Carcinogenicity

Rat

- The incidence of nasolacrimal duct squamous cell carcinomas in male rats was 2/51 (4%), 3/50 (6%), 4/48 (8%), 2/52 (4%), 6/52 (12%) for the control, 50, 100, 200, and 1000 ppm dose groups, respectively. Although the high dose incidence was slightly outside the historical control range (0-10%), there were no significant trends or pair-wise comparisons of the dosed groups with the controls for nasolacrimal duct squamous cell carcinomas in male rats. The nasolacrimal duct tumor findings for males is confounded because the incidence fluctuates in the dose groups and the biological significance is questionable. There is a possible correlation between the incidence of inflammation and the fluctuating incidence of nasal tumors across dose groups. The sponsor has presented reasonable arguments regarding the origin of the tumors in relation to inflammation and malocculusion of incisor teeth. The CARC did not consider the nasolacrimal duct tumors to be treatment-related.

- The incidence of nasolacrimal duct squamous cell carcinomas in female rats was 0/52 (0%), 0/51 (0%), 0/52 (0%), 3/52 (6%) for the control, 200, 1000, and 5000 ppm dose groups, respectively. Female rats had a significant increasing trend in nasolacrimal duct squamous cell carcinomas at $p<0.05$, however, no significant differences in the pair-wise comparisons of the dosed groups with the controls were seen for this tumor type. This is considered to be a rare tumor in female rats and the incidence at the high dose is slightly above the historical control mean (0.8%) and range (0-4%). Unlike the male rats, the incidence of nasolacrimal duct tumors in female rats could not be clearly correlated with the inflammatory response due to the low incidence of both neoplastic and non-neoplastic lesions. The CARC considered the nasolacrimal duct squamous cell carcinomas to be possibly treatment related, but that a clear association with treatment could not be made.

- The incidence of cerebellar granular cell tumors was 3/52 (6%) in high dose females and showed a positive trend but no pair wise significance. These tumors occurred late in the study (≥94 weeks). The occurrence of these slow growing benign cerebellar granular cell tumors in females, although slightly outside of the historical controls range (0-4.35%), was not considered to be treatment related by the CARC. As stated by the Agency's reviewing pathologist "...this is a benign neoplasm that is often only discovered microscopically and, therefore, is probably underreported in the historical data. To my knowledge, granular cell tumors have never been identified as a chemically induced neoplasm. This fact, and the absence of any indication of a similar response in the males, persuades me to agree ... that the occurrence of GCT's in the Wistar study is incidental."
- The CARC considered dosing at the high dose in male (1000 ppm) and female (5000

ppm) rats to be adequate, but not excessive, for the assessment of carcinogenicity. This conclusion was based on overall decreased body weights, body weight gains (Weeks 0-104: 8%, male; 11% female), nephrotoxicity, hepatotoxicity, and ocular toxicity. Hyaline droplet deposition in renal proximal tubular cells, tubular basophilic changes, and kidney pelvic dilatation were observed starting at 1000 ppm. Also, cytoplasmic vacuolation and brown pigment (lipofuscin) deposition in the renal proximal tubular cells were observed at higher doses. Indications of hepatotoxicity in the high dose group was indicated by decreased triglycerides, increased total cholesterol, and increased relative liver weights, along with increased incidences of dark-colored livers with accentuated lobular pattern and slight centrilobular hepatocyte hypertrophy. Cataracts and retinal atrophy were seen in high dose females.

Mouse (MRID 45854615)

- In male mice, the incidences (percent) of alveolar/bronchiolar adenomas, carcinoma, and combined adenomas and/or carcinomas for the control, 250, 750, and 2250 ppm dose groups, respectively, were as follows:

  Adenomas: 7/55 (13%), 25/59 (42%), 25/58 (43%), and 32/55 (58%)
  Carcinomas: 4/55 (7%), 6/59 (10%), 12/58 (21%), and 12/55 (22%)
  Combined: 10/55 (18%), 27/59 (46%), 29/58 (50%), and 35/55 (64%)

  Male mice had a significant increasing trend, as well as significant differences in the pair-wise comparisons of all the dosed groups with the control, for alveolar/bronchiolar adenomas, all at $p<0.01$. There was a significant increasing trend, as well as significant differences in the pair-wise comparisons of the 750 and 2250 ppm dose groups with the control, for alveolar/bronchiolar carcinomas, all at $p<0.05$. There was also a significant increasing trend, as well as significant differences in the pair-wise comparison of all the dosed groups with the control for combined alveolar/bronchiolar adenomas and/or carcinomas, all at $p<0.01$. The incidences of adenomas at dose levels ≥250 ppm were greater than the historical control mean (11.6%) and range (0-26%). The incidence of carcinomas was within the historical control mean (5.2%) and range (0-23.2%). **The CARC considered the alveolar/bronchiolar tumors to be treatment related.**

- In female mice, the incidences (percent) of alveolar/bronchiolar adenomas, carcinoma, and combined adenomas and/or carcinomas for the control, 250, 750, and 2250 ppm dose groups, respectively, were as follows:

  Adenomas: 9/56 (16), 20/57 (35), 29/57 (51), 24/56 (43)
  Carcinomas: 0/56 (0), 3/57 (5), 3/57 (5), 7/56 (13)
  Combined: 9/56 (16), 22/57 (39), 31/57 (54), 25/56 (45)

  Female mice had a significant increasing trend, at $p<0.05$, as well as significant differences in the pair-wise comparisons of the 250 ppm ($p<0.05$), 750 ppm ($p<0.01$), and 2250 ppm ($p<0.01$) dose groups with the control for alveolar/bronchiolar adenomas. There was a significant increasing trend, as well as a significant difference in the pair-

wise comparison of the 2250 ppm dose group with the control for alveolar/bronchiolar carcinomas, both at $p<0.01$. There was also a significant increasing trend, at $p<0.05$, as well as significant differences in the pair wise comparisons of all the dosed groups with the controls, for combined adenomas and/or carcinomas, all at $p<0.01$. The incidences of adenomas at dose levels ≥250 ppm were greater than the historical control mean (9.4%) and range (0-16%). The incidence of carcinomas at 2250 ppm (13%) was outside the historical control range (0-12%). **The CARC considered the alveolar/bronchiolar tumors to be treatment related.**

- The CARC concluded that dosing in both male and female mice was adequate and not excessive. This was based on inereased incidence of tissue masses/nodules in the lungs and microscopic findings in the liver, spleen, bone marrow, and lungs. In the liver at study termination, the high dose group demonstrated increased absolute and relative weights. Centrilobular hepatocellular hypertrophy was observed in all treated groups for males and high dose females, and incidence and severity increased with dose. In the spleen at terminal sacrifice, extramedullary hematopoiesis was noted in all treated doses for males and for doses 750 ppm and above for females. Increased pigment deposition in the spleen was also noted at the high dose. In the bone marrow decreased cellularity was observed in the femur of the 750 ppm and above treated males and in all treatment groups for females, and in the sternum of the high dose males and in females at doses 750 ppm and above. Pigment deposition was observed in the femur and sternum.

Mouse (MRID 46205801)

- In male mice, the incidences (percent) of alveolar/bronchiolar adenomas, carcinomas, and combined adenomas and/or carcinomas for the control, 10, 25, 80, or 250 ppm dose groups, respectively, were as follows:

  Adenomas: 8/46 (17), 11/48 (23), 12/45 (27), 11/46 (24), 21/48 (44)
  Carcinomas: 3/46 (7), 6/48 (13), 3/45 (7), 4/46 (9), 9/48 (19)
  Combined: 11/46 (24), 16/48 (33), 15/45 (33), 14/46 (30), 27/48 (56)

  Male mice had a significant increasing trend, as well as a significant difference in the pair-wise comparison of the 250 dose group with the control for alveolar/bronchiolar adenomas, both at $p<0.01$. There was a significant trend only for alveolar/bronchiolar carcinomas, at $p<0.05$. There was also a significant increasing trend, as well as a significant difference in the pair-wise comparison of the 250 ppm dose groups with the control, for combined alveolar/bronchiolar adenomas and/or carcinomas, both at $p<0.01$. The incidence (44%) of adenomas at the high dose (250 ppm) was outside the historical mean (19.9) and range (9.6-32). The incidence of carcinomas at the high dose was within the historical control mean(12.1%) and range (2-30.8). **The CARC considered the alveolar/bronchiolar tumors to be treatment related.**

- In female mice, the incidences (percent) of alveolar/bronchiolar adenomas, carcinoma,

and combined adenomas and/or carcinomas for the control, 10, 25, 80, or 250 ppm dose groups, respectively, were as follows:

Adenomas: 10/46 (22), 8/47 (17), 11/39 (28), 14/38 (37), 13/41 (32)
Carcinomas: 1/49 (2), 4/48 (8), 2/41 (5), 3/42 (7), 3/45 (7)
Combined: 10/49 (20), 12/48 (25), 12/41 (29), 16/42 (38), 16/45 (36)

Female mice had a significant increasing trend, as well as significant differences in the pair-wise comparisons of the 80 and 250 ppm dose groups with the control, for combined alveolar/bronchiolar adenomas and/or carcinomas, all at $p<0.05$. The incidence of adenomas (32%) at the high dose was outside the historical mean (13.7%) and range (3.8-26.9%). The incidence of carcinomas (7%) at the high dose was within the historical control mean (8%) and range (2-15.7%). **The CARC considered the alveolar/bronchiolar tumors to be treatment related.**

- Dosing at the high dose in male and female mice was considered to be adequate, and not excessive, based on lung masses and terminal bronchiole epithelial cell hyperplasia/hypertrophy in both sexes.

2. Mutagenicity

There is no mutagenicity concern for flonicamid. Flonicamid technical did not cause mutations in the bacterial reverse mutation or mouse lymphoma tests with or without metabolic activation, chromosome damage in the mouse micronucleus or *in vitro* cytogenetics tests with and without metabolic activation. Similarly, there was no increase in DNA damage in the comet assay or in an *in vivo* rat unscheduled DNA synthesis (UDS) study.

3. Structure Activity Relationship

There was no SAR information that was useful in the weight-of-the-evidence analysis.

4. Mode of Action

The registrant has presented plausible data showing that the lung tumors in CD-1 mice fed flonicamid are due to mitogenesis, a non-linear, non-genotoxic mode of action for which a threshold has been established. The Agency has reviewed and evaluated the data submitted and concluded that the data support this MOA. This conclusion is based on the following:

1) *In vivo* and *in vitro* mutagenicity studies confirm that flonicamid is not mutagenic.

2) The BrdU labeling index studies demonstrated the following:

- There was a *dose-response concordance* between the lung tumors and cell proliferation. The threshold level is between 80 and 250 ppm;

- *A temporal relationship* was demonstrated, supporting the MOA. The proliferative response was identified as early as 3 days and was present through 28 days of administration;

- The early mitogenic effect is *reversible;*

- The data support direct mitogenesis as a cause for the increased proliferation rather than cytotoxicity and regeneration. There was *no evidence of necrosis* by light microscopy or by transmission electron microscopy.

- A clear species difference was observed between mice and rats in the incidence of lung tumors and the BrdU Index studies. No tumors were seen in the lungs of rats. The flonicamid induced increase in the BrdU Index appears to be related to the different sensitivity of strains of mice, with the CD-1 mice being a relatively sensitive strain.

The Agency cannot, however, dismiss human relevancy. Although the sponsor has shown that Clara cells are involved in the toxicological response to treatment with the test compound, they have not ruled out if other cell types are involved in the tumorgenic response to treatment. Further, although Clara cells are more numerous in the mouse than in humans, Clara cells are present in the human lung and have been shown to be responsive to the metabolic activity of xenobiotics.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA Draft Guidelines for Carcinogen Risk Assessment (July 1999), the CARC classified Flonicamid into the category **"Suggestive Evidence of Carcinogenicity, but Not Sufficient to Assess Human Carcinogenic Potential"**. This classification was based on the following weight-of-the-evidence considerations:

(i) Flonicamid is not mutagenic.

(ii) The treatment-related CD-1 mouse lung tumors (benign and malignant) which occurred in both sexes were due to an established mitogenic mode of action that occurred in a susceptible mouse strain with a high background. A clear species difference was observed between mice and rats in the incidence of lung tumors and the BrdU Index studies. No tumors were seen in the lungs of rats. The flonicamid induced increase in the BrdU Index appears to be related to the different sensitivity of strains of mice, with the CD-1 mice being a relatively sensitive strain.

(iii) The only other tumor response was nasolacrimal duct tumors which occurred in female rats at the high dose which were considered to be possibly treatment-related, but a clear association with treatment could not be made. Unlike male rats, the nasal tumor response in females could not be clearly associated with spontaneous inflammation due to the low incidence of both the neoplastic and non-neoplastic lesions.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The quantification of human cancer risk is not recommended.

VIII BIBLIOGRAPHY

MRID No.	CITATION
45854615	Ridder, W.E. and M. Watson. (2003) An oncogenicity study in mice with IKI-220 technical. Toxicology and Pharmacology, Ricerca Biosciences, LLC, Painesville, OH. Laboratory Document #: 011885-1, January 3, 2003.
45854616.	Nomura, M. (2003) IKI-220: Discussion on lung finding observed in mouse oncogenicity study. Safety Science Research Laboratory, Central Research Institute, Ishihara Sangyo Kaisha, Ltd., Japan. Laboratory Document #: AN-2203, January 21, 2003.
45863801	Kuwahara, M. (2002) IKI-220 technical: combined chronic toxicity and carcinogenicity study in rats. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project Study ID: IET 98-0142, December 12, 2002.
46205801	Nagaoka, T. (2004) Dietary carcinogenicity study of IKI-220 technical in mice. Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan. Laboratory Study No.: SBL40-50, January 22, 2004.
46242202	Historical Control Data: Nagaoka, T. (2004) Response to EPA review and submission of supplemental data addendum to report: Historical data for Dietary carcinogenicity study of IKI-220 technical in mice. Shin Nippon Biomedical Laboratories, Ltd., Kagoshima, Japan. Laboratory Study No.: SBL40-50 - ADDENDUM, January 22, 2004 & April 2, 2004 - ADDENDUM..
46275601	Historical Control Data: Kuwahara, M. (2002) Response to EPA review and submission of supplemental data to report: Historical control data for IKI-220 technical: combined chronic toxicity and carcinogenicity study in rats. Document No.: IET 98-0142 SUPP.1, December 12, 2002 & May 18, 2004 - Supplement.
46323701	Historical Control Data: Kuwahara, M. (2002) Response to EPA review and submission of supplemental data to report: Historical control data (RCC LTD., Itingen, Switzerland) for IKI-220 technical: combined chronic toxicity and carcinogenicity study in rats. Document No.: IET 98-0142 SUPP.2, December 12, 2002 & June 25, 2004 - Supplement 2.
46362201	Rationale: Cohen, SM, Hardisty, JF, and McCarty, JD (2004) Flonicamid

(IKI-220): Rationale for Regulation by Reference Dose. FMC Corporation, Agricultural Products Group, Princeton, NJ. Document #: Flonicamid 04-04, August 31, 2004.

46362202 Re-Read: Kuwahara, M. (2004) IKI-220: Combined chronic toxicity and carcinogenicity study in rats histopathological examination of nasal cavity. Laboratory Project ID: IET 04-0067, August 19, 2004.

46362205 Additional Historical Control Data in Rats for Nasal Cavity: Kuwahara, M. (2004) IKI-220: Combined chronic toxicity and carcinogenicity study in rats - To provide historical control data in rats. Laboratory Project ID: IET 98-0142, August 27, 2004. (Data from the same and/or similar studies submitted in MRID 46275601)

46415501 Kuwahara, M. (2004) IKI-220 Technical: Combined Chronic Toxicity and Carcinogenicity Study in Rats (MRID # 4563801) with Amendments 1 and 2. Project Number: IET/98/0142. Unpublished study prepared by Institute of Environmental Toxicology. 2052 p. Receipt date: 01-Dec-2004

Giknis, MLA and Clifford, CB (2000) Spontaneous Neoplastic Lesions in the Crl:CD-1 (ICR)BR Mouse. Charles River Laboratories, March 2000.

Hukkanen, J, Pelkonen, O, Hakkola, J, and Raunio, H. (2002) Expression and Regulation of Xenobiotic-Metabolizing Cytochrome P450 (CYP) Enzymes in Human Lung. Critical Reviews in Toxicology, 32(5):391-411.

Kauffman, SL. (1981) Histogenesis of the Papillary Clara Cell Adenoma. American Journal of Pathology, 103:174-180.

Kidwell, J. (2004). Flonicamid: Qualitative Risk Assessment Based on CD-1 Mouse and Wistar Rat Dietary Studies. November 22, 2004. TXR No. 0052829.

Maronpot RR, Boorman GA, and Gaul BW (eds.).(1999) Pathology of the Mouse. Cache River Press, Vienna IL, 1999.

Mason, RJ, Kalina, M, Nielsen, LD, Malkinson, AM, and Shannon JM. (2000) Surfactant Protein C Expression in Urethane-Induced Murine Pulmonary Tumors. American Journal of Pathology, 156:175-182.

Nakashima, N (2004) Historical Histopathological Data Male and Female CD-1 Mice- Studies of 78 Weeks Duration Studies Terminated between August 1990 and June 2003. Unpublished data prepared by the Institute of Environmental Toxicology.

Schoevers, EJ, Kuijpers, MHM, Woutersen, RA, and Feron, VJ. (1994) Spontaneous squamous cell carcinoma of nasal and paranasal structures in the CpdPWU (Wistar random) rat: Naslachrymal duct as major site of origin. J Environ Pathol Toxicol Oncol 1994; 13(1):49-57.

Appendix 1

Cerebellum Granular Cell -- Benign
Table addressing the incidence of cerebellum granular cell (benign) tumors seen in the rat study (MRID 45863801) and further discussed in MRID 48362203.

Kuwahara, M. (2004) IKI-220 Technical: Combined Chronic Toxicity and Carcinogenicity Study in Rats. To provide discussion of questions concerning the bone/bone marrow effects and cerebellar granular tumors. The Institute of Environmental Toxicology, Ibaraki, Japan. Laboratory Project ID IET 98-0142, August 16, 2004. MRID 46362203.

The observation of three high dose female rats with cerebellum granular cell tumors is considered to be incidental and unlikely due to treatment with flonicamid. Note only one tumor was seen in males, which was in the 200 ppm treated group. Incidences for the highest treated dose groups are slightly above historical controls.

Table: Evaluating: Cerebellum Granular Cell -- Benign

First tumor occurred at 94 weeks in dose group 3. Excludes animals that died before week 54. Also excludes both interim sacrifice groups. No tumors were observed in any interim sacrifice animals.

DOSE (ppm)	0.000	200.0	1000.	5000.
	0/ 52 (0) P=0.0297*	0/ 20 (0) P = 1.00000	1/ 29 (3) P = 0.35802	3/ 52 (6) P = 0.12136

(Table: Email from Jessica Kidwell, 12/8/04).

Historical Controls for Cerebellum Granular Cells - MRID 46362203, page 7

Historical Control Incidence of Cerebellar Granular Cell Tumors	Male	Female
Brain (Number examined)	2.735	2.682
Number of tumors observed	58	27
Incidence (Range)	0% - 7.14%	0% - 4.35%
Incidence (Mean)	2.12%	1.01%

Appendix 2

MEMORANDUM

DATE: December 8, 2004

TO: William Burnam, HED/EPA

CC: Jessica Kidwell, HED/EPA

FROM: John M. Pletcher, Pathology Associates (CARC consulting pathologist)

SUBJECT: Flonicamid data from the chronic toxicity and carcinogenicity studies

I have completed my review of the reports you provided on the combined toxicity and carcinogenicity studies in rats concerning the above referenced insecticide.

Cohen, Hardisty and McCarty's report entitled "FLONICAMID (IKI-220):Rationale for Regulation by Reference Dose" addresses the various EPA concerns in a reasoned and scientifically sound manner. I found their explanation for the slight and non-statistically significant increase observed in squamous cell carcinomas from the area of the nasal cavity persuasive; the J. Environ. Pathol. Toxicol. Oncol. (1994) article was very pertinent and germane. The argument that there exists a causal relationship between the occurrence of such tumor and chronic inflammation in the nasolacrimal duct, the inflammation sometimes but not always being the result of dental malocclusion, makes very good sense and is difficult to refute. I believe that their conclusion concerning squamous cell carcinomas in the area of the nasal cavity in Wistar rats, that they are not treatment related, is sound. It should be noted that the fluoride in the Flonicamid compound is never released and is therefore not a cause of dental problems that could result in malocclusions in rodents. The occurrence of three granular cell tumors (GCT) in the high dose female Wistars was another concern; however, as correctly stated by Cohen et al., this is a benign neoplasm that is often only discovered microscopically and, therefore, is probably underreported in the historical data. To my knowledge, GCT's have never been identified as a chemically induced neoplasm. This fact, and the absence of any indication of a similar response in the males, persuades me to agree with Cohen et al. that the occurrence of GCT's in the Wistar study is incidental. Concerning the increase in bone marrow (femur) hematopoiesis in the Wistars, the increase in high dose males is clearly more apparent than real. The data showing the results of the evaluation of the early deaths and the terminal sacrifice males (and females) separately relieves any concerns I might have had. The additional studies presented by Cohen et al. to support their conclusion that Flonicamid is a carcinogen in CD-1 mice due to a particular susceptibility in the strain but does not produce cancer in humans (or rats) are scientifically valid and present a strong case, particularly in the absence of any demonstrable genotoxicity. These results will have to be considered in the "weight of evidence" process.

Appendix 3

ISK BIOSCIENCES CORPORATION Document No Flonicamid 04-04 (from pages 40-44 of the company's document)

XIV. Discussion of Mode of Action of Flonicamid

A. Mitogenesis as the Mode of Action and Human Relevance Flonicamid produced an increased incidence of preneoplastic and neoplastic lesions of the lungs of CD-1 mice in both males and females. There was no other tissue affected at a statistically significant level in the mouse. In addition, the rat two-year bioassay was negative for significant increased incidences of any tumor, including any increased incidence of preneoplastic or neoplastic lesions of the lung. This was true in both male and female rats. Questions regarding some of the specific lesions identified in treated and control rats are addressed in other sections of this document.

B. Mitogenesis as the Mode of Action Flonicamid is a non-DNA reactive chemical that produced a statistically increased incidence of tumors at one site in one species, in both sexes. The key issue is an assessment of the potential carcinogenicity of flonicamid to humans, based on these findings and the additional experimental information regarding mode of action that has been generated. During the past two years, a working group sponsored by the United States Environmental Protection Agency and Health Canada, organized through the International Life Sciences Institute-Risk Science Institute (ILSI/RSI) has been developing a framework to be used for the evaluation of the human relevance of mode of action, hazard identification data generated in animals that can then be used in the process of risk assessment. Given the large amounts of data that have been generated regarding flonicamid, it is appropriate to evaluate this chemical utilizing this framework. The framework was published in detail in the November, 2003 issue of Critical Reviews in Toxicology (Cohen *et al.*, 2003 and Meek *et al.*, 2003). A summary has been published in Toxicological Sciences in the past few months (Cohen *et al.*, 2004). The fundamentals of this approach are to address three questions utilizing all data available, not only with respect to the chemical itself, but what is known about the overall mode of action in both animals and in humans, and then to present an overall assessment of the conclusions and implications for risk assessment. It is important to ask the relevant question: Is the weight of evidence sufficient to establish the mode of action (MOA) of flonicamid in animals? To address this question, the framework developed by the IPCS and the EPA for an evaluation of the mode of action for chemical carcinogens is utilized (Sonich-Mullin *et al.*, 2001). It is based partly on the Bradford Hill criteria for causality, which were developed initially for use in assessing the validity of epidemiologic studies regarding human etiology. This mode of action framework is based on defining measurable key events, beginning with administration of the chemical to the ultimate production of tumors. It is to be distinguished from the more detailed, molecularly defined mechanism of action.

The mode of action of flonicamid has been defined as follows: 1. Ingestion of the chemical (oral) 2. Distribution to the lung 3. Mitogenic stimulation of Clara cells 4. Increased proliferation leading to hyperplasia, adenomas, and ultimately carcinomas.

C. Dose Response Bromodeoxyuridine (BrdU) labeling index has been used to assess the proliferation rate of a variety of cell types based on the incorporation of BrdU during the "S" (DNA synthesis) phase of the cell cycle. The specific data for the proliferation studies for flonicamid are detailed elsewhere in this report and are summarized here. Utilizing BrdU labeling index, cell proliferation was clearly demonstrated with an increase in bronchiolar Clara cells in the high dose compared to controls at 3, 7, and 28 days following dietary administration of flonicamid. In addition, there was a clear dose response with a No Observed Effect Level (NOEL) of 80 ppm in the diet. In the original mouse 18 month carcinogenicity study in CD-1 mice, doses of 250,

750, and 2250 ppm were evaluated, and there was an increased incidence of proliferative lesions in the lung at all of these doses. Thus, there was no NOEL in this original carcinogenicity study. The short term labeling index study evaluating increased proliferation defined a NOEL of 80 ppm; therefore a second 18 month carcinogenicity bioassay was performed utilizing a dose of 250 ppm to overlap with the previous study, but also utilizing lower doses (10, 25, 80, 250 ppm) so that a NOEL could be defined. In the second oncogenicity study, a NOEL of 80 ppm was observed, which was identical to the NOEL observed for the BrdU labeling index. Thus, there is complete concordance between the dose-response for increased labeling index compared to carcinogenicity. Although complete concordance between the doses for early and late events is not essential, it is essential that the NOEL in the short term, precursor-related study (BrdU labeling index) is the same or lower than that for the carcinogenicity endpoint. This has clearly been attained for flonicamid.

D. Temporal Relationships The BrdU labeling index studies combined with the long-term histopathology clearly demonstrate the temporal relationship supporting the mode of action that has been proposed. An increased rate of proliferation was identified as early as three days after administration, the earliest time an evaluation was performed. At this time, there was not only an increase in proliferation rate, but there were morphologic changes indicating an increase in cell size and metabolic activity of the Clara cells. The increased proliferative rate was present through 28 days of administration. The question could be raised that this has not been followed to subsequent time points; however, clear evidence of increased proliferation was present based on the histopathology observance of increased incidences of hyperplasia. Hyperplasia by definition is an increase in cell number and nearly always is associated with an increase in proliferation rate. For the mouse lung, hyperplasia is associated with an increase in proliferative rate. Even if it were not, the fact that hyperplasia is present is a clear indication that there is an increase in cell proliferation, since the critical parameter is the increase in number of cells that are proliferating, not the rate at which they are proliferating.

Another relevant issue regarding temporality is the potential reversibility of the early changes. This was examined both morphologically and utilizing BrdU labeling index by administering flonicamid for 28 days followed by 28 days of recovery, with interim sacrifices beginning one week after termination of the chemical in the diet. A rapid reversal occurred to normal levels of the BrdU labeling index along with a reversal to normal of the morphologic changes of the bronchiolar Clara cells. Thus, the early mitogenic effect is reversible. Although this has not been corroborated through a full carcinogenicity bioassay, based on what is known of this tumor model in CD-1 mice, this would be highly likely (see below).

E. Strength and Consistency There are considerable data supporting the sequence of events for lung tumors in this strain of mice, from mitogenesis to hyperplasia to adenoma to carcinoma, not only for flonicamid, but based on numerous studies with other chemicals. Two long-term oncogenicity studies were conducted, and the observations at the overlapping dose of 250 ppm were very similar, even more so than is typical for long-term bioassays. In addition, the observations at 3, 7, and 28 days, as well as the reversibility study, are highly consistent, with reproducible changes in labeling index and morphology. In addition, the dose response is highly consistent between the short term changes (BrdU labeling index and morphology) and long-term tumor production. In addition, the data strongly support a role for direct mitogenesis as a cause for the increased proliferation rather than cytotoxicity and regeneration. Not only was there no evidence of an increase in exfoliated cells in the respiratory tract as an indicator of necrosis, there was no evidence of cell necrosis by either light microscopy or, more sensitively, by transmission electron microscopy, even at times when there was obvious increased proliferation. The fact that flonicamid is non-DNA reactive (and more broadly, non-genotoxic) clearly leaves increased mitogenesis as the most plausible mode of action. In addition, there was no morphologic evidence of changes in apoptosis, either increased or decreased, nor is that a typical change in the mouse lung pulmonary epithelium.

F. Biological Plausibility and Coherence The mouse lung tumor model has been studied for more than four decades. It is very clear that certain strains have a marked increase in susceptibility compared to others. The strain A mouse is particularly susceptible, with extensive work by Dr. M. Shimkin (1975) and his colleagues through the 50's, 60's, and 70's trying to develop this as a screening assay for carcinogens. However, it has been essentially abandoned as a screening assay because the incidence of lung tumors is so high, with nearly all of the animals ultimately developing tumors. Frequently, the only distinction that could be made between the test chemical and the controls were the number and/or size of the lung tumors rather than the incidence of lung tumors. The CD-1 mouse was originally derived from the strain A mouse, so it is not surprising that it also has a very high level of spontaneous lung tumors. Based on the literature, this can range up to 78 percent, depending on specific conditions of the assay. Because it is so high, it is not unusual for chemicals to produce a statistically significant increase in lung carcinomas.

In addition, there has been significant confusion over the years as to the proper classification of these tumors. Several decades ago, many of these lesions were routinely classified as carcinoma, regardless of their various features. Over the past few decades, specific criteria have been established for hyperplasia, adenoma, and carcinoma in the mouse as well as in the rat, so that there is more precise definition of the lesion and better classification (Boorman, 1985; Boorman and Eustis, 1990; Dixon, 1999; Kauffamn 1985; Kauffman and Sato, 1985; Dungworth *et al.* 2001; Schwartz *et al.* 1994; IARC 1992). The CD-1 mouse has a considerably higher spontaneous rate of lung lesions than does the B6C3F1 mouse strain, which has a higher incidence than the C57BL mouse. The rat also has a very low spontaneous incidence compared to the mouse, and the morphology of the lesions differs somewhat between the two species. Nevertheless, in the rat, it appears that the sequence of events is also increased proliferation leading to hyperplasia, adenoma, and ultimately carcinoma. This was not observed in rats administered flonicamid. There has also been difficulty regarding the specific pathogenesis and histogenesis of these lesions, particularly as it relates to the human. This is discussed below. The sequence of events for mouse lung tumors has clearly been delineated by a number of investigators as predominantly arising from Clara cells and undergoing the sequence of increased proliferation followed by hyperplasia, leading to adenomas and ultimately carcinomas. More importantly, there also is evidence that if you do not have the 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 the recent cloning and identification of a specific gene, the pulmonary adenoma susceptibility 1 (*Pas1*) locus (Manenti, *et al.* 2004) identifying the increased susceptibility of the CD-1 strain compared to other strains.

G. Alternative Modes of Action As described elsewhere in this report, flonicamid has undergone an extensive battery of *in vitro* and *in vivo* genotoxicity studies, even broader than the narrow definition of DNA reactivity. They have been consistently negative, clearly indicating that the lung tumors in mice are not being produced by a genotoxic, or more specifically, DNA-reactive mode of action. An increase in cell proliferation can be produced by either an increase in cell births or decrease in cell deaths (Cohen, 1998, Cohen *et al.*, 2003, 2004). A decrease in cell deaths can be produced by either inhibiting apoptosis or cell differentiation, two cell death processes. There is no evidence for such changes indicative of effects on apoptosis or differentiation in the mouse lung tumor model in general, nor with flonicamid specifically.

Increased cell birth can be produced either by direct mitogenesis or by cytotoxicity followed by regeneration. As described above, based on exfoliation, light microscopy, and electron microscopy, there is no evidence of cytotoxicity or cell necrosis following flonicamid administration, leaving increased mitogenesis as the most plausible mode of action for producing increased cell proliferation. As with other processes leading to an

increase in cell proliferation, the dose response is non-linear. Whether or not a threshold can be definitively identified at this point cannot be determined, although based on the findings with flonicamid and other mouse studies with other chemicals, a threshold is highly likely.

H. Level of Confidence Given the breadth, depth, and consistency of the data, lack of genotoxicity, and lack of cytotoxicity, there is a strong level of confidence in the described mode of action and specific key events.

I. Uncertainties and Data Gaps The evidence is strong supporting a mitogenic mode of action. As always, additional experiments could be done, but it is unlikely that they would alter the conclusion of this mode of action. Further corroboration could be accomplished by examining the long-term administration of flonicamid in the B6C3F1 or C57BL mouse, extending the lack of changes in proliferation at the earlier times. However, based on studies with flonicamid and other chemicals, the likelihood of a disparity arising from such a study is remote. Also, the rat did not show early proliferative changes and did not develop lung tumors. Obviously, this is a well-defined mode of action, but specific molecular changes and mechanism of action have not been defined. The specific cellular target of flonicamid leading to the mitogenic response has not been identified. For that matter, it has not been identified for other chemicals in this model, either. Whether it is the parent compound or a specific metabolite that produces the mitogenic response also has not been definitively determined. However, administration of the major metabolites of flonicamid to the CD-1 mouse did not produce an increase in cell proliferation. However, this does not exclude the possibility that one (or more) of these metabolites actually is the ultimate form of flonicamid that produces the mitogenic effect, as there may have been metabolic and kinetic differences producing the metabolites at the target site (bronchiolar Clara cell) after flonicamid administration compared to direct oral administration of that metabolite. In summary, the high level of confidence in a non-genotoxic, mitogenic mode of action for flonicamid in production of lung neoplastic lesions in the mouse is strongly supported by the data. Although additional research is certainly possible, the mode of action is well defined and the additional research would focus more on mechanism rather than mode of action.

13544

R105855

Chemical:	**Flonicamid**
PC Code:	**128016**
HED File Code	**21200 CARC Reports**
Memo Date:	**02/24/2005**
File ID:	**TX0052013**
Accession Number:	**412-05-0094**

HED Records Reference Center
04/12/2005