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

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

MEMORANDUM

DATE: June 9, 2011

SUBJECT: Fluxapyroxad: Report of the Cancer Assessment Review Committee

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40 CFR: N/A

FROM: Jessica Kidwell, Executive Secretary
Cancer Assessment Review Committee
Health Effects Division (7509P)

Jessica Kidwell

THROUGH: Jess Rowland, Chair
Cancer Assessment Review Committee
Health Effects Division (7509P)

Jess Rowland

TO: Jessica Ryman, Toxicologist
RAB IV, Health Effects Division (7509P)

Olga Odiott
IB, RD (7505P)

The Cancer Assessment Review Committee met on April 6, 2011 to evaluate the cancer classification of fluxapyroxad in accordance with the *EPA's Final Guidelines for Carcinogen Risk Assessment* (March, 2005). Attached please find the final Cancer Assessment Document.

Rec'd in RD 6/14/2011 SH

CANCER ASSESSMENT DOCUMENT

EVALUATION OF THE CARCINOGENIC POTENTIAL OF

Fluxapyroxad

PC CODE 138009

Final
June 9, 2011

CANCER **A**SSESSMENT **R**EVIEW **C**OMMITTEE
HEALTH EFFECTS DIVISION
OFFICE OF PESTICIDE PROGRAMS

DATA PRESENTATION: Jess Rowland for JR
Jessica Ryman, Toxicologist

DOCUMENT PREPARATION: Jessica Kidwell
Jessica Kidwell, Executive Secretary

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

Gregory Akerman

Kit Farwell

Ray Kent 6/9/11

Nancy McCarroll Jess Rowland for NM

Karlyn Middleton

Jess Rowland, Chair Jess Rowland

P.V. Shah P.V. Shah

Yin-Tak Woo Jess Rowland for YTW

NON-COMMITTEE MEMBERS IN ATTENDANCE: (Signature indicates concurrence with the pathology report)

John Pletcher, Consulting Pathologist

OTHER ATTENDEES: Jack Fowle, Ivan Nieves, Abdallah Khasawinah, Becky Daiss, Debbie Smegal, PMRA Canada participants (on phone): Catherine Adcock, Martin Beauchamp, Olivier Tremblay, Jean Kim, Nihan Kavaslar, Leanne Yeung

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EXECUTIVE SUMMARY

On April 6, 2011, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of fluxapyroxad (BAS 700F).

Jessica Ryman of Risk Assessment Branch IV presented the chronic toxicity/carcinogenicity study in Wistar rats and the carcinogenicity study in C57BL/6 J Rj mice. Fluxapyroxad [BAS 700 F] was administered to Wistar rats at dietary dose levels of 0, 50, 250, 1500 and 3000 ppm (0/0, 2.1/2.7, 11/14, 68/82, or 145/182 mg/kg body weight/day in males/females) for 2 years to assess carcinogenicity. A satellite group of animals was sacrificed at one year to assess chronic toxicity. Fluxapyroxad [BAS 700 F] was administered to C57BL/6 J Rj mice in the diet for 18 months at doses of 0, 150, 750, 3000 and 6000 ppm (21, 107, 468 and 996 mg/kg body weight in males and 33, 158, 652 and 1307 mg/kg bw in females). A satellite group of animals was sacrificed at one year to assess chronic toxicity. In addition, mode of action data for the liver and thyroid follicular cell tumors were discussed, as well as mutagenicity data and structure activity relationships.

The CARC considered the following for a weight-of-evidence determination of the carcinogenic potential of fluxapyroxad.

Carcinogenicity

Rat

• *Liver Tumors*: Male Wistar rats had statistically significant trends for liver adenomas ($p<0.01$), carcinomas ($p<0.05$), and combined adenomas and/or carcinomas ($p < 0.01$). There were significant pair-wise comparisons of the 3000 ppm dose group with the controls for liver adenomas, carcinomas and combined adenomas and/or carcinomas, all at $p < 0.01$. There were also significant pair-wise comparisons of the 1500 ppm dose group with the controls for liver adenomas and combined adenomas and/or carcinomas, both at $p < 0.01$. The concurrent control incidences for the liver tumors were within the historical control ranges. The incidences of liver adenomas and combined at doses of ≥250 ppm exceeded the historical control ranges for these tumors.

Female rats had statistically significant trends for liver adenomas and combined adenomas and/or carcinomas, both at $p < 0.01$. There were significant pair-wise comparisons of the 3000 ppm dose group with the controls for liver adenomas at $p < 0.01$ and for liver combined adenomas and/or carcinomas at $p < 0.05$. The concurrent control incidences for the liver tumors were within the historical control ranges. The incidences of liver adenomas and combined at doses of ≥250 ppm exceeded the historical control ranges for these tumors.

While not statistically significant, the CARC also considered the liver tumors in males at 250 ppm and in females at 1500 ppm to be treatment-related and biologically relevant. The liver

tumors in both sexes are supported by precursor non-neoplastic lesions (i.e., dose-related increase in liver hypertrophy in both sexes and foci in males).

The CARC considered the liver tumors to be treatment-related in male rats at doses of ≥250 ppm (11 mg/kg/day) and in female rats at doses of ≥1500 ppm (82 mg/kg/day).

• *Thyroid Follicular Cell Tumors*: Male rats had statistically significant trends for thyroid follicular cell adenomas ($p<0.01$), carcinomas ($p<0.05$), and combined adenomas and/or carcinomas ($p < 0.01$). There were significant pair-wise comparisons of the 3000 ppm dose group with the controls for thyroid follicular cell combined adenomas and/or carcinomas at $p < 0.05$. The concurrent control incidences for the thyroid follicular cell tumors were within the historical control ranges. While not statistically significant, the CARC also considered the tumors at 1500 ppm to be treatment-related and biologically relevant. The thyroid follicular cell tumors in male rats are supported by precursor non-neoplastic lesions (i.e., dose-related increase in thyroid hypertrophy/hyperplasia). **The CARC considered the thyroid follicular cell tumors at doses of ≥1500 ppm to be treatment-related in male rats.**

• *Adequacy of Dosing*: Dosing at the high dose of 3000 ppm in male and female rats was considered adequate, but not excessive, for assessing carcinogenicity. This was based on decreases in body weights in both sexes (more pronounced in females), hematology and clinical chemistry changes, and non-neoplastic lesions in the liver and thyroid.

Mouse

• There were no treatment-related increases in tumors in either male or female mice.

• *Adequacy of Dosing*: The high dose of 3000 ppm was considered to be adequate, but not excessive, to assess carcinogenicity in both sexes of mice since the high dose in this study was the limit dose (996/1307 mg/kg/day in males/females). Decreases in absolute body weights in males at the limit dose did not exceed 13% during the study and did not exceed 8% at study termination. No other adverse effects were observed.

Mutagenicity: There is no concern for mutagenicity.

Structure Activity Relationship: Fluxapyroxad is a second-generation carboxamide of the pyrazole-carboxamide class. Other members of this class include bixafen, isopyrazam, and sedaxane. Isopyrazam caused thyroid follicular cell tumors in male rats, and liver and uterine tumors in female rats. Sedaxane caused liver adenomas and thyroid follicular cell tumors in male rats and uterine tumors in female rats. Data to support a MOA for liver and thyroid tumors were not provided for either of these chemicals. There is potential SAR concern for the trifluoro-2-aminobiphenyl moiety in fluxapyroxad because 2-aminobipenyl is an isomer of the well known human carcinogen, 4-aminobiphenyl. 3',4',5'-Trifluoro-2-aminobiphenyl is predicted to be a potential carcinogen by EPA's OncoLogic cancer expert system, although its potency is expected to be substantially lower than that of 4-aminobiphenyl. This is because the

2-position is not as favorable for metabolic activation of the amino group as the 4-position. 3',4',5'-Trifluoro-2-aminobiphenyl may be a potential degradation product or metabolite of fluxapyroxad via cleavage of the amide bond but there was no report of its presence in the submitter's rodent metabolism data.

Mode of Action: The CARC concluded that evidence with dose/time concordance was sufficient to support a mitogenic MOA for liver adenomas and carcinomas based on early changes in liver enzyme regulation and increased cell proliferation that lead to adenoma and then carcinoma formation. The CARC also concluded that there was sufficient evidence with dose/time concordance to support a non-genotoxic MOA for thyroid follicular adenomas based on early changes in liver enzyme regulation that lead to dysregulation of thyroid hormone homeostasis, thyroid follicular hypertrophy/hyperplasia, and thyroid follicular adenoma formation. The endpoint of changes in liver enzyme regulation provided a clear threshold for both of these tumors.

Classification and Quantification of Carcinogenic Potential

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC classified fluxapyroxad as "Not likely to be Carcinogenic to Humans" based on convincing evidence that carcinogenic effects are not likely below a defined dose range. This decision was based on the following considerations: **(i)** No treatment-related tumors were seen in male or female mice when tested at doses that were adequate to assess carcinogenicity (including the Limit Dose); **(ii)** Treatment-related liver tumors were seen in male rats at doses $\geq$ 250 ppm (11 mg/kg/day) and in female rats at doses $\geq$ 1500 ppm (82 mg/kg/day); **(iii)** Treatment-related thyroid follicular cell tumors were seen in male rats only at doses $\geq$ 1500 ppm; **(iv)** There is no mutagenicity concern from *in vivo* or *in vitro* assays; **(v)** The hypothesized mode of action (i.e, a non-genotoxic) for each tumor type (i.e., the liver and thyroid) was supported by adequate studies that clearly identified the sequence of key events, dose-response concordance and temporal relationship to the tumor types. The mode of action met the criteria established by the Agency.

The available data indicates the following: 1) a threshold of 250 ppm (11 mg/kg/day) for tumorigenesis; 2) provides sufficient evidence to support modes of action for all tumor types; 3) no concern for mutagenicity; and 4) a clear point of departure (POD) of 50 ppm (2.1 mg/kg/day), which is not expected to increase cell division, alter thyroid hormone homeostasis, or result in liver or thyroid tumors. Therefore, the Agency has determined that the quantification of risk using a non-linear approach (i.e., RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to fluxapyroxad.

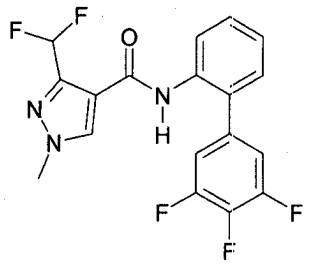
I. INTRODUCTION

On April 6, 2011, the Cancer Assessment Review Committee (CARC) of the Health Effects Division of the Office of Pesticide Programs met to evaluate the carcinogenic potential of fluxapyroxad (BAS 700F).

II. BACKGROUND INFORMATION

Fluxapyroxad (BAS 700F) is a new fungicidal active ingredient that is being registered for food use. This chemical is under global review with PMRA and Australia. HED has the lead on toxicology.

Fluxapyroxad (Figure 1) is a second generation carboxamide fungicide, and is of the same chemical class as isopyrazam and sedaxane, which were recently reviewed by the CARC. The pesticidal mode of action of fluxapyroxad is inhibition of succinate dehydrogenase in complex II of the mitochondrial respiratory chain. In fungi, this results in inhibition of spore germination, germ tubes, and mycelial growth. The mammalian mode of toxic action has not been identified. Fluxapyroxad is taken up by plants, where it is widely distributed and metabolized. Three impurities are produced during the manufacture of fluxapyroxad. Additionally, there are three metabolites of fluxapyroxad found in food crops and soil (M700F001, M700F002, and M700F048).



fluxapyroxad

Figure 1. Structure of fluxapyroxad, a second-generation carboxamides.

III. EVALUATION OF CARCINOGENICITY STUDIES

1. Combined Chronic Toxicity/Carcinogenicity Study in Rats

Buessen, R.; Strauss, V.; Groeters, A.; et al. (2009) BAS 700 F: Combined Chronic Toxicity/Carcinogenicity Study in Wistar Rats: Administration Via the Diet up to 24 Months. Project Number: 2009/1072490/OCR, 80C0683/05071/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Labor fuer Oekotoxicologie. 1779 p. MRID 47923591.

Survival data and liver and thyroid tumors were evaluated by SIMB, the results of which are presented below. For further details, see D376616, TXR 0055752, Memorandum from L. Brunsman to J. Kidwell (March 18, 2011).

A. Experimental Design

BAS 700 F (Batches COD – 000899 up to study day 454 and COD-001049 from study day 455 to termination; Purity: 99.7% and 99.2%, respectively) was administered to Wistar rats at dietary dose levels of 0, 50, 250, 1500 and 3000 ppm (0/0, 2.1/2.7, 11/14, 68/82, or 145/182 mg/kg bw/day in males/females) for 2 years to assess carcinogenicity. A satellite group of animals was sacrificed at one year to assess chronic toxicity.

B. Discussion of Survival Data

There were no survival disparities among the dose groups for male (Table 1) or female rats (Table 2).

Table 1. Fluxapyroxad – Wistar [Crl:WI (Han)] Rat Study (MRID 47923591)

Male Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose (ppm)	1-26	27-52	52[i]	53-78	79-106[f]	Total
0	0/60	0/60	10/60	1/50	5/49	6/50 (12%)
50	1/60	0/59	10/59	0/49	7/49	8/50 (16%)
250	0/60	1/60	10/59	0/49	5/49	6/50 (12%)
1500	0/60	0/60	10/60	1/50	4/49	5/50 (10%)
3000	0/60	1/60	10/59	3/49	5/46	9/50 (18%)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 104-106.
*Note: Time intervals were selected for display purposes only.

Table 2. Fluxapyroxad – Wistar [Crl:WI (Han)] Rat Study (MRID 47923591)

Female Mortality Rates[+] and Cox or Generalized K/W Test Results

	Weeks					
Dose (ppm)	1-26	27-52	52[i]	53-78	79-106[f]	Total
0	0/60	1/60	10/59	1/49	11/48	13/50 (26%)
50	0/60	0/60	10/60	3/50	7/47	10/50 (20%)
250	0/60	0/60	10/60	2/50	7/48	9/50 (18%)
1500	0/60	0/60	10/60	1/50	14/49	15/50 (30%)
3000	0/60	0/60	10/60	1/50	10/49	11/50 (22%)

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.
[i]Interim sacrifice at week 52.
[f]Final sacrifice at weeks 104-106.

B. Neoplastic lesions

Male rats had statistically significant trends for liver adenomas and combined adenomas and/or carcinomas, and thyroid follicular cell adenomas and combined adenomas and/or carcinomas, all at $p < 0.01$. There were also statistically significant trends for liver carcinomas and thyroid follicular cell carcinomas, both at $p < 0.05$. There were significant pair-wise comparisons of the 3000 ppm dose group with the controls for liver adenomas, carcinomas and combined adenomas and/or carcinomas, all at $p < 0.01$, and for thyroid follicular cell combined adenomas and/or carcinomas at $p < 0.05$. There were also significant pair-wise comparisons of the 1500 ppm dose group with the controls for liver adenomas and combined adenomas and/or carcinomas, both at $p < 0.01$. The statistical analyses of the tumors in the male rats were based upon Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (Tables 3 and 5).

Female rats had statistically significant trends for liver adenomas and combined adenomas and/or carcinomas, both at $p < 0.01$. There were significant pair-wise comparisons of the 3000 ppm dose group with the controls for liver adenomas at $p < 0.01$ and for liver combined adenomas and/or carcinomas at $p < 0.05$. The statistical analyses of the tumors in the female rats were based upon Fisher's Exact Test for pair-wise comparisons and the Exact Test for trend (Table 4).

Table 3. Fluxapyroxad – Wistar [Crl:WI (Han)] Rat Study (MRID 47923591)

Male Liver Tumor Rates[+] and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)				
	0	50	250	1500	3000
Adenomas[a] (%)	0/50 (0%)	0/49 (0%)	4/49 (8%)	7/50 (14%)	15/49 (31%)
p =	0.00000**	1.00000	0.05628	0.00624**	0.00001**
Carcinomas[a] (%)	1/50 (2%)	0/49 (0%)	1/49 (2%)	3/50 (6%)	9/49 (18%)
p =	0.00003*	1.00000	0.74747	0.30865	0.00712**
Combined (%)	1/50 (2%)	0/49 (0%)	5/49 (10%)	10/50 (20%)	21[b]/49 (43%)
p =	0.00000**	1.00000	0.09757	0.00389**	0.00000**

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]All adenomas and carcinomas observed in final sacrifice animals, simultaneously in the 0, 250, 1500 and 3000 ppm dose groups.
[b]Three animals in the 3000 ppm dose group had both an adenoma and a carcinoma.
**p<0.01

Historical control:

Male liver adenomas: 2% avg (0-4%), based on 400 rats of this same strain
Male liver carcinomas: 1.5% avg (0-6%), based on 400 rats of this same strain
Male liver combined: 3.5% avg (0-8%), based on 400 rats of this same strain

Table 4. Fluxapyroxad – Wistar [Crl:WI (Han)] Rat Study (MRID 47923591)

Female Liver Tumor Rates[+] and
Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)				
	0	50	250	1500	3000
Adenomas (%)	0/49 (0%)	2/50 (4%)	0/50 (0%)	4/50 (8%)	7[a]/50 (14%)
p =	0.0005**	0.2525	1.0000	0.0612	0.0067**
Carcinomas (%)	1[b]/49 (2%)	1[b]/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
p =	0.1174	0.7576	1.0000	1.0000	1.0000
Combined (%)	1/49 (2%)	3/50 (6%)	0/50 (0%)	4/50 (8%)	7/50 (14%)
p =	0.0035**	0.3163	1.0000	0.1874	0.0317*

+Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.
[a]First adenoma observed at week 96, dose 3000 ppm.
[b]First carcinoma observed at week 105 in final sacrifice animals, simultaneously in the 0 and 50 ppm dose groups.
*p<0.05, **p<0.01

Historical control:

Female liver adenomas: 0.8% avg (0-6%), based on 400 rats of this same strain
Female liver carcinomas: 1.8% avg (0-6%), based on 400 rats of this same strain
Female liver combined: 2.5% avg (0-8%), based on 400 rats of this same strain

Table 5. Fluxapyroxad – Wistar [Crl:WI (Han)] Rat Study (MRID 47923591)

Male Thyroid Follicular Cell Tumor Rates[+]
and Fisher's Exact Test and Exact Trend Test Results

	Dose (ppm)				
	0	50	250	1500	3000
Adenomas (%)	3/60 (5%)	2/59 (3%)	4/59 (7%)	8/60 (13%)	9[a]/60 (15%)
p =	0.00556**	0.81257	0.49057	0.10217	0.06272
Carcinomas (%)	0/60 (0%)	0/59 (0%)	1[b]/59 (2%)	1[b]/60 (2%)	3[b]/60 (5%)
p =	0.01601*	1.00000	0.49580	0.50000	0.12185
Combined (%)	3/60 (5%)	2/59 (3%)	5/59 (8%)	9/60 (15%)	11[c]/60 (18%)
p =	0.00133**	0.81257	0.34907	0.06272	0.02171*

+Number of tumor bearing animals/Number of animals examined, excluding those that died before week 48.
[a]First adenoma observed at week 48, dose 3000 ppm.
[b]First carcinoma observed at week 105 in final sacrifice animals, simultaneously in the 250, 1500 and 3000 ppm dose groups.
[c]One animal in the 3000 ppm dose group had both an adenoma and a carcinoma.
*p<0.05, **p<0.01

Historical control:

Male thyroid follicular adenomas: 13% avg (4-28%), based on 400 rats of this same strain
Male thyroid follicular carcinomas: 2.3% avg (0-4%), based on 400 rats of this same strain
Male combined: 15% avg (0-30%), based on 400 rats of this same strain

C. Non-neoplastic lesions

Non-neoplastic lesions were observed in rats administered fluxapyroxad for 1 year (Tables 6-7) or 2 years (Tables 6, 8). These non-neoplastic lesions in the liver and thyroid provide supporting information for the tumorigenic effects. In the liver, only hypertrophy (microscopic) and pigmentary changes (microscopic and macroscopic) are observed at 1 year. However, at 2 years, a dose-related increase in cysts (males only), enlarged liver, foci, masses (in males only), and a prominent acinar pattern are observed macroscopically, while the additional microscopic changes of spongiosis hepatis and foci of cellular alteration are observed. Together, this indicates progressive liver changes. Likewise, in the thyroid, follicular cell hypertrophy/hyperplasia are observed in males and altered colloid are observed in both sexes at 1 year, with follicular hyperplasia observed in both sexes at 2 years. At two years, males also have enlarged thyroids and masses. Increases in thyroid weights at 1 year are also observed, as are increases in liver weights at 1 and 2 years (Tables 9-10).

Table 6 Incidence of selected gross pathological findings in rat administered BAS 700 F for 1 year (chronic toxicity group) or 2 years (carcinogenicity group animals)

Sex	Male					Female				
Dose [ppm]	0	50	250	1500	3000	0	50	250	1500	3000
	Interim Sacrifice (chronic toxicity group)									
Animals in group	10	10	10	10	10	10	10	10	10	10
Incisor - mandible, discoloration	-	-	**2**	**1**	**5**	-	-	-	-	**5**
Liver - Discoloration, dark brown	-	-	-	**1**	**7**	-	-	-	**1**	**5**
	Carcinogenicity Group									
Animals in group and examined	50	50	50	50	50	50	50	50	50	50
Abdominal cavity										
- Effusion	0	0	0	1	0	1	0	1	1	5
Liver										
- Cyst	**1**	**1**	-	**7**	**12**	**2**	**5**	**3**	**4**	**3**
- Discoloration, dark brown	**1**	-	-	**2**	**6**	-	**1**	-	**4**	**16**
- Enlarged	-	**1**	-	**1**	**7**	-	-	-	**1**	**4**
- Focus	**32**	**30**	**40**	**44**	**41**	**20**	**17**	**27**	**25**	**29**
- Mass	-	-	**3**	**8**	**16**	**1**	**2**	-	**6**	**5**
- Prominent acinar pattern	-	**1**	**1**	**8**	**17**	**1**	-	**1**	**8**	**18**
Bone (head)										
- Discoloration, bulla tymp	-	-	-	-	**31**	-	-	-	-	**31**
- Discoloration, dorsal skull	-	-	-	-	**16**	-	-	-	-	**21**
- Discoloration, frontal bone	-	-	-	-	**38**	-	-	-	-	**34**
Incisor										
- mandible , discoloration	-	-	2	32	39	-	-	1	36	39
- maxilla, discoloration	-	-	-	23	38	-	-	-	12	37
Thyroid gland										
- Enlarged	**1**	-	**2**	**2**	**10**	**1**	**1**	-	-	-
- Mass	**2**	-	**1**	**3**	**4**	**1**	**1**	**1**	**2**	**1**

Table 7 Incidence of non-neoplastic findings in rats administered BAS700 F 1 year

Sex	Males					Females				
Dose [ppm]	0	50	250	1500	3000	0	50	250	1500	3000
Animals in group	10	10	10	10	10	10	10	10	10	10
Femur # examined	10	10	10	10	10	10	10	10	10	10
- Perl's Prussian Blue stain	-	-	-	**10** *[1.9]*	**10** *[3.0]*	-	-	-	**10** *[2.9]*	**10** *[3.1]*
Liver # examined	10	10	10	10	10	10	9	10	10	10
- hypertrophy, centrilobular (zone 3)	2 *[1.0]*	-	1 *[1.0]*	**10** *[1.5]*	**9** *[1.7]*	-	-	**5** *[1.0]*	**10** *[2.3]*	**10** *[3.2]*
- pigment storage, diffuse	-	-	-	**3**	**6**	1	-	-	**8**	**9**
-Liver lymph node hyperplasia, lympho-reticul.	1	0	2	1	8	0	1	2	2	2
Thyroid # examined	10	10	10	10	10	10	9	10	10	10
- Hypertrophy/hyperplasia, follicular	3	5	5	**10**	**7**	-	-	-	1	-
- Altered colloid	6	7	8	10	9	1	1	3	**8**	**9**

[5] [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

Table 8 Incidence of non-neoplastic findings in rats administered BAS 700 F for 2 years

Sex	Males					Females				
Dose [ppm]	0	50	250	1500	3000	0	50	250	1500	3000
Animals in group	50	50	50	50	50	50	50	50	50	50
Femur # examined	50	50	50	50	50	50	50	50	50	50
- Perl's Prussian Blue stain	-	-	**35**** *[1.0]*	**50**** *[2.1]*	**50**** *[2.9]*	-	-	**33**** *[1.4]*	**50**** *[2.9]*	**50**** *[3.4]*
-inflammation, diffuse	3	0	*0*	*0*	*7*	0	0	*0*	*0*	*0*
Liver # examined	50	50	50	50	50	50	50	50	50	50
- hypertrophy, centrilobular (zone 3)	1 *[1.0]*	2 *[1.0]*	**30**** *[1.2]*	**43**** *[2.4]*	**43**** *[2.7]*	-	-	**28**** *[1.3]*	**41**** *[2.2]*	**44**** *[2.7]*
- pigment storage, diffuse	2	-	-	**9***	**14****	18	18	26	**33****	**47****
- spongiosis hepatis			1	**10****	**23****					
- Perl's Prussian Blue stain						42	38	30	**11**	**3**
- Focus of cellular alteration	43	47	42	44	40	46	46	47	24	27
- Basophilic tigroid	37	40	35	20	9	45	45	47	9	5
- Basophilic diffuse	7	8	2	5	1	4	1	-	-	1
- Basophilic (NOS)	3	3	5	7	**10***	5	2	4	3	2
- Clear cell	11	19	5	3	-	6	1	2	-	-
- Eosinophilic	33	36	39	38	35	10	10	19*	16	**23****
- Amphophilic	-	-	-	-	-	-	1	-	-	-
Parathyroid glands # examined	50	50	50	48	50	45	46	49	46	47
Hyperplasia, (multi)focal	8	7	6	6	12	1	2	3	1	3
Skull bones # examined	44	-	-	-	42	39	2	2	7	40
- Os frontale, hyperostosis	-	-	-	-	**41**	-	-	-	-	**32**
- Os parietale, hyperostosis	-	-	-	-	-	-	-	-	-	**5**
- Bully tympanica, hyperostosis	-	-	-	-	**4**	-	-	-	-	-
Thyroid # examined	50	50	50	50	50	50	50	50	50	50
- Hyperplasia, follicular	3	4	8	**17****	**14****	2	1	2	8*	4
- Altered colloid	40	44	46	48*	47*	30	33	37	41**	45**

$ [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence

* $p \leq 0.05$; ** $p \leq 0.01$ Comparison of all dose groups with the control group using FISHER'S EXACT test (one-sided) for the hypothesis of equal proportions

Table 9 Selected mean absolute and relative (± SD) organ weights of rats administered BAS 700 F for one year

Sex		Males (N=10)				Females (N=10)			
Organ weight	Dose [ppm]	Absolute weight	Δ%	Relative weight [%]	%	Absolute weight	Δ%	Relative weight [%]	Δ%
Terminal weight [g]	0	462.42 ±35.83				253.94 ±22.56			
	50	491.87 ±43.42	(6.4)			265.37 ±31.05	(4.5)		
	250	460.09 ±47.20	(-0.5)			252.55 ±21.82	(-0.5)		
	1500	461.06 ±48.66	(-0.3)			**232.3**** ±15.12	**(-8.5)**		
	3000	**407.32**** ±29.37	**(-11.9)**			**225.93 **** ±18.82	**(-11.0)**		
Liver [g]	0	9.290 ±0.679		2.017 ±0.197		5.376 ±0.480		2.119 ±0.117	
	50	9.717 ±0.820	(4.6)	1.981 ±0.148	(-1.8)	5.688 ±0.573	(5.8)	2.152 ±0.137	(1.5)
	250	9.621 ±1.112	(3.6)	2.093 ±0.140	(3.7)	**5.835*** ±0.473	**(8.5)**	**2.317**** ±0.161	**(9.3)**
	1500	**12.543**** ±2.108	**(35.0)**	**2.712**** ±0.237	**(34.4)**	**6.471**** ±0.540	**(20.4)**	**2.785**** ±0.141	**(31.4)**
	3000	**12.701**** ±1.385	**(36.7)**	**3.119**** ±0.281	**(54.7)**	**7.067**** ±0.540	**(31.5)**	**3.135**** ±0.200	**(47.9)**
Thyroid [mg]	0	28.2 ±4.94		0.006 ±0.001		18.2 ±3.5		0.007 ±0.001	
	50	29.0 ±5.6	(2.8)	0.006 ±0.001	(-3.3)	23.5** ±2.8	(29.1)	0.009** ±0.002	(26.0)
	250	29.5 ±4.0	(4.6)	0.006 ±0.001	(5.9)	20.6 ±4.7	(13.2)	0.008 ±0.002	(14.5)
	1500	31.0 ±4.4	(9.9)	0.007 ±0.001	(10.5)	19.5 ±2.4	(7.1)	0.008** ±0.001	(17.7)
	3000	31.75 ±4.5	(12.6)	**0.008** ±0.001**	**(25.3)**	22.0 ±4.5	(20.9)	0.010** ±0.002	(36.3)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may not calculate exactly due to rounding of figures

Table 10 Selected mean absolute and relative organ weights (±SD) of rats administered BAS 700 F for two years (N in parenthesis: note no thyroid weights)

Sex		Males				Females			
Organ weight	Dose [ppm]	Absolute weight (N)	Δ%	Relative weight [%] (N)	Δ%	Absolute weight (N)	Δ%	Relative weight [%] (N)	Δ%
Terminal weight [g]	0	529.414 ±61.812 (44)				339.895 ±52.686 (37)			
	50	553.631 ±84.585 (42)	(4.6)			322.788 ±49.988 (40)	(-5.0)		
	250	541.936 ±65.441 (44)	(2.4)			**297.839**** ±39.689 (41)	**(-12.4)**		
	1500	514.005 ±60.542 (45)	(-2.9)			**281.314**** ±29.091 (35)	**(-17.2)**		
	3000	**477.800**** ±54.931 (41)	**(-9.7)**			**260.126**** ±36.269 (39)	**(-23.5)**		
Liver [g]	0	11.280 ±1.539 (44)		2.137 ±0.223 (44)		7.306 ±1.251 (37)		2.153 ±0.186 (37)	
	50	11.865 ±2.034 (42)	(5.2)	2.147 ±0.240 (42)	(0.5)	7.324 ±1.209 (40)	(0.2)	2.284 ±0.299 (40)	(6.1)
	250	**12.515**** ±2.028 (44)	**(10.9)**	**2.307**** ±0.231 (44)	**(8.0)**	7.194 ±1.220 (41)	(-1.5)	**2.418**** ±0.292 (41)	**(12.3)**
	1500	**14.958**** ±2.222 (45)	**(32.6)**	**2.953**** ±0.690 (45)	**(38.2)**	**8.253**** ±1.490 (35)	**(13.0)**	**2.937**** ±0.459 (35)	**(36.4)**
	3000	**16.368**** ±2.472 (41)	**(45.1)**	**3.448**** ±0.536 (41)	**(61.3)**	**8.645**** ±1.399 (39)	**(18.3)**	**3.329**** ±0.340 (39)	**(54.6)**
	3000	0.879** ±0.169 (41)	(-9.0)	0.186 ±0.038 (41)	(1.1)	0.551** ±0.107 (39)	(-25.7)	0.214 ±0.043 (39)	(-3.6)

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test, two sided)
# Values may not calculate exactly due to rounding of figures

D. Adequacy of Dosing for Assessment of Carcinogenicity

Dosing at the high dose of 3000 ppm in male and female rats was considered adequate, but not excessive, for assessing carcinogenicity. In males, body weight decreases of 6-12% were observed at 1 year and 8-10% at two years at the highest dose. This effect was more pronounced in females, with body weight decreases of 10% at 1 year and 23-24% at 2 years at the highest dose (see Tables 9-11). The highest dose was sufficient to cause non-neoplastic changes in the liver and thyroid and some changes in hematology (decreased prothrombin time) and clinical chemistry parameters (changes in liver enzymes) without causing increased death or clearly adverse effects. Together, the evidence indicates that 3000 ppm was a reasonable maximum tolerated dose (MTD) in both sexes.

Table 11 Mean body weight and body weight gain (±SD) of rats administered BAS 700 F for up to 2 years (chronic toxicity and carcinogenicity groups)

	Males					Females				
Dose level [ppm]	0	50	250	1500	3000	0	50	250	1500	3000
Body weight [g]										
- Day 0	176.9	176.2	176.6	174.9	173.3	134.8	135.5	134.3	133.2	132.5
±SD	± 8.7	± 9.2	± 10.-	± 9.3	± 8.6	± 8.7	± 7.2	± 6.6	± 6.9	± 7.6
(N)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)
- Day 91	394.1	400.7	397.0	392.7	**373.0****	239.5	236.7	234.3	**228.0****	**224.1****
±SD	± 34.8	± 33.6	± 39.1	± 29.9	± 29.0	± 18.4	± 16.2	± 15.3	± 13.7	± 15.1
(N)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)	(60)
Δ% (compared to control) #		1.7	0.8	-0.3	**-5.4**		-1.2	-2.1	**-4.8**	**-6.4**
- Day 371	496.9	510.2	506.2	497.5	**467.2****	284.4	276.4	**268.7****	**264.6****	**253.3****
±SD	± 56.0	± 57.2	± 54.2	± 38.1	± 45.6	± 30.5	± 26.5	± 21.0	± 21.3	± 19.0
(N)	(50)	(49)	(49)	(50)	(49)	(49)	(50)	(50)	(50)	(50)
Δ% (compared to control) #		2.7	1.9	0.1	**-6.0**		-2.8	**-5.5**	**-7.0**	**-10.9**
- Day 728	557.6	576.9	567.7	539.1	**502.2****	359.8	343.1	**317.1****	**301.3****	**277.7****
±SD	± 67.2	± 85.7	± 66.8	± 62.0	± 56.1	± 51.8	± 50.0	± 39.3	± 29.6	± 37.3
(N)	(45)	(42)	(44)	(45)	(41)	(37)	(41)	(41)	(37)	(40)
Δ% (compared to control) #		6.3	-1.0	0.6	**-8.4**		-4.7	**-11.9**	**-16.3**	**-22.8**

Values may not calculate exactly due to rounding of mean values

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnetr's test. two sided)

2. Carcinogenicity Study in Mice

Buesen, R.; Strauss, V.; Kuettler, K.; et al. (2010) BAS 700 F: Carcinogenicity Study in C57BL/6 J Rj Mice: Administration Via the Diet Over 18 Months (Including Amendment No. 1). Project Number: 2010/7003500/OCR, 87C0683/05082/OCR. Unpublished study prepared by BASFAktiengesellschaft, Experimental Toxicology and Ecology. 2182 p. **MRID 47923592.**

A. Experimental Design

BAS 700 F (Batch COD–000899, Purity 99.7% up to study day 349; Batch COD-001049, Purity 99.2% from day 350 to end of study) was administered to C57BL/6 J Rj mice in the diet for 18 months at doses of 0, 150, 750, 3000 and 6000 ppm (21, 107, 468 and 996 mg/kg bw in males and 33, 158, 652 and 1307 mg/kg bw in females). A satellite group of animals was sacrificed at one year to assess chronic toxicity.

B. Discussion of Survival and Tumor Data

The performing laboratory did not find any treatment-related changes in survival in either sex. There were also no treatment-related increases in tumor incidence in either sex. Therefore, no in-house statistical analyses were performed.

C. Non-neoplastic lesions

Non-neoplastic lesions of hepatocellular hypertrophy and macrovesicular fatty changes in the liver were observed in the 9 month satellite group and in the 18 month carcinogenicity group (Table 12). These changes were observed in both males and females and were considered adaptive (hypertrophy) and reversible (macrovesicular fat).

Table 12 Non-neoplastic changes in C57BL/6 J Rj mice administered fluxapyroxad for 9 months or 18 months

Sex	Male					Female				
Dose [ppm]	0	50	250	1500	3000	0	50	250	1500	3000
9 month interim sacrifice										
Animals in group	10	-	-	-	10	10	-	-	-	10
Liver # exam.	10	-	-	-	10	10	-	-	-	10
- Fatty change	10 *[2.3]*[$]				**10** ***[3.4]***	10 *[1.7]*				10 *[1.4]*
-macrovesicular	2				**10**					2
- Hypertrophy, peripheral	-	-	-	-	-	-	-	-	-	**3** ***[1.0]***
18 month terminal sacrifice										
- Hypertrophy, central	4 *[1.0]*[$]	2 *[1.0]*	2 *[1.0]*	2 *[1.5]*	**24**** ***[1.5]***	-	-	-	2 *[2.0]*	-
- Hypertrophy, peripheral	2 [1.0]	1 *[1.0]*	2 *[1.0]*	6 *[1.7]*	5 *[1.4]*	6 *[1.3]*	5 *[1.0]*	11 *[1.1]*	**15*** ***[1.3]***	**47**** ***[1.7]***
- Fatty change	36 *[2.0]*	40 *[2.1]*	**40** ***[2.4]***	**40** ***[2.6]***	**42** ***[2.6]***	33 *[2.3]*	39 *[2.2]*	**41** ***[2.3]***	**32** ***[2.8]***	**41** ***[2.4]***
- macrovesicular	17	22	**30****	**36****	**36****	14	12	**26***	**26***	**29***

[$][ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence of the respective finding.

D. Adequacy of Dosing for Assessment of Carcinogenicity

The high dose of 3000 ppm was considered to be adequate, but not excessive, to assess carcinogenicity in both sexes since the high dose in this study was the limit dose (996/1307 mg/kg/day in males/females). Decreases in absolute body weights in males at the limit dose did not exceed 13% during the study and did not exceed 8% at study termination (Table 13). No other adverse effects were observed, indicating that the limit dose was not excessive.

Table 13 Mean body weights and body weight gain (±SD) of mice administered BAS 700 F about 18 months

Dose level [ppm]	Males (N=10) 0	50	250	1500	3000	Females (N=10) 0	50	250	1500	3000
	Satellite Group									
Body weight [g]										
- Day 0	22.2 ±1.4				22.0 ±0.7	19.0 ±0.8				18.7 ±0.9
- Day 91	32.5 ±2.1				**30.3*** ±1.7 (-6.8)	25.8 ±2.7				25.2 ±1.3
- Day 175	36.9 ±3.1				**34.1*** ±1.4 (-7.6%)	30.6 ±5.2				28.9 ±3.7
- Day 273	41.9 ±4.3				39.6 ±3.1	36.1 ±6.3				31.7 ±4.4
	Main (Carcinogenicity) Group (N=50/sex/dose unless otherwise noted)									
Body weight [g]										
- Day 0	22.5 ±1.0	22.3 ±1.1	22.5 ±1.0	22.1 ±0.9	22.3 ±1.0	19.1 ±0.8	18.8 ±0.8	18.8 ±1.0	18.7 ±1.0	18.6* ±0.7 (-2.6%)
- Day 91	33.3 ±3.1	33.5 ±2.4	33.7 ±2.9	**31.4**** ±2.6 (-5.7%)	**30.5**** ±1.9 (-8.4%)	26.1 ±2.6	25.8 ±2.7	25.9 ±2.8	25.2 ±2.3	25.1 ±1.7
- Day 175	38.6 ±4.1	38.8 ±3.2	39.3 ±3.8	**35.1**** ±3.8 (-9.1%)	**33.9**** ±3.0 (-12%)	30.4 ±4.3	29.4 ±4.9 (N=49)	30.1 ±5.3	29.5 ±4.7	28.6 ±3.2
- Day 371	44.8 ±5.6	45.2 ±4.9 (N=48)	45.3 ±4.6 (N=48)	**42.2*** ±4.7 (-7.9%) (N=48)	**39.0**** ±3.9 (-13%)	36.4 ±6.0 (N=49)	36.3 ±6.5 (N=49)	36.9 ±6.0 (N=48)	35.5 ±6.9 (N=50)	34.3 ±4.8 (N=50)
- Day 546	40.5 ±7.0 (N=41)	43.0 ±7.8 (N=45)	42.3 ±7.4 (N=40)	39.7 ±7.3 (N=40)	**37.5** ±6.4 (-7.4%) (N=45)	34.9 ±8.3 (N=40)	34.9 ±6.1 (N=42)	36.9 ±7.3 (N=37)	35.7 ±8.1 (N=41)	36.0 ±6.6 (N=47)

* $p \leq 0.05$; ** $p \leq 0.01$ (Dunnett's test,. two sided)

Values may not calculate exactly due to rounding of mean figures

IV. TOXICOLOGY

1. Metabolism

A. *Mammalian metabolism of fluxapyroxad*

Pharmacokinetic and metabolism studies with radiolabeled fluxapyroxad in rats show that plasma levels of radiolabel scaled with dose, indicating that uptake was not saturated up to dose levels of 500 mg/kg. There were no sex differences observed in the rate or extent of absorption. The time to maximum plasma levels was dependent upon on dose, and ranged from 1 hour (at 5 mg/kg) to 24 hours (at 500 mg/kg). Radioactivity was widely distributed in both sexes with a similar pattern: the highest concentrations were found in the gut contents and stomach contents. However, lower concentrations were found in numerous other organs/tissues, including the **liver**, **thyroid**, adrenal glands, kidney, pancreas, testes/uterus, and brain. For both males and females, radioactivity declined in all tissues over time. The time course of the amount of radioactivity found in urine and feces indicated the excretion occurred predominantly within three days after

dosing. Bile duct cannulation experiments showed that the bile was a major route of excretion. The main biotransformation steps of BAS 700 F in rats are hydroxylation at the biphenyl ring system, N-demethylation at the pyrazole ring system, loss of a fluorine atom at the biphenyl ring system, and conjugation with glucuronic acid or with glutathione derivatives.

B. Plant and soil metabolites of fluxapyroxad

There are three metabolites of fluxapyroxad found in food crops and soil: M700F001, M700F002, and M700F048. These metabolites were screened for genotoxicity, as described below. Additionally, an OECD 417 toxicokinetic study was performed for M700F002 and M700F048. Both of these metabolites were systematically bioavailable and observed in the blood and bone marrow after oral exposure at the limit dose. No separate toxicokinetic studies were performed for M700F001.

2. Mutagenicity

Genotoxicity studies were performed on fluxapyroxad, plant and soil metabolites (M700F001, M700F002, and M700F048) and impurities (EPA Reg. Nos 5356469, 5410775, and 5425764). Genotoxicity studies were also performed on an artificial batch, in which the a.i. was absent.

A. Fluxapyroxad

Fluxapyroxad was tested in a battery of standard genotoxicity tests *in vitro* and *in vivo* (Table 15). These studies demonstrate that fluxapyroxad has no genotoxic potential. There was no indication of gene mutation either in the presence or absence of metabolic activation in both the bacterial reverse mutation and mammalian gene mutation tests. The *in vitro* chromosome aberration test and the *in vivo* mouse micronucleus test were both negative and, thus, a clastogenic potential may be excluded. Additionally, an *in vivo* study was performed to determine if fluxapyroxad induced unscheduled DNA synthesis in rat hepatocytes. No evidence of increased nuclear grain counts or cells undergoing DNA repair was observed.

Table 14. Genotoxicity panel and unscheduled DNA synthesis study for fluxapyroxad		
Gene Mutation 870.5100 *In vitro* Bacterial Gene Mutation	47923572 (2008) Acceptable, guideline 0, 20, 100, 500, 2500, 5000 µg/plate ±S9	Not mutagenic in the reverse mutation assay in *Salmonella. typhimurium* or *Escherichia coli* with or without metabolic activation.
Gene Mutation 870.5300 *In vitro* Mammalian Cells Gene Mutation (Chinese Hamster Ovary Cells)	47923579 (2007) Acceptable, guideline 0-100 µg/ml ±S9	Does not induce forward mutations in CHO cells with or without metabolic activation.
Cytogenetics 870.5375 *In vitro* Mammalian Cytogenetics Chromosomal Aberration Assay-human peripheral blood lymphocytes	47923577 (2008) Acceptable, guideline 0-400 µg/ml ±S9	Does not cause clastogenic effects in V79 cells with or without metabolic activation.
Cytogenetics-other 870.5395 *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus-mouse	47923584 (2006) Acceptable, guideline 0, 500, 1000, 2000 mkd	Did not lead to any increase in micronucleated polychromatic erythrocytes.
Non-OPPTS guideline, OECD 486 Unscheduled DNA Synthesis Test-mammalian liver cells (rat)	47923589 (2009) Acceptable, non-guideline 0, 1000, 2000 mkd	Does not induce unscheduled DNA synthesis.

B. Metabolites M700F001, M700F002, and M700F048

The M700F001, M700F002, and M700F048 metabolites of fluxapyroxad were tested in a battery of standard genotoxicity tests *in vitro* and *in vivo* (Tables 15-17). There was no indication of gene mutation either in the presence or absence of metabolic activation in both the bacterial reverse mutation and mammalian gene mutation tests for any of these metabolites. The *in vitro* chromosome aberration test and the *in vivo* mouse micronucleus test were both negative for M700F001 and M700F002 indicating no clastogenic potential. M700F048 demonstrated an increase in chromosomal aberrations *in vitro* in the presence of metabolic activation and long incubation times. However, no evidence of increased micronuclei was observed in mice treated with M700F048 in the mammalian erythrocyte micronucleus test. Therefore, M700F048 was considered unlikely to result in clastogenic effects *in vivo*. An *in vivo* study was also performed

to determine if fluxapyroxad induced unscheduled DNA synthesis in hepatocytes. No evidence of increased nuclear grain counts or cells undergoing DNA repair was observed.

Table 15. Genotoxicity panel for M700F001		
Gene Mutation 870.5100 *In vitro* Bacterial Gene Mutation	47923609 (2009) Acceptable, guideline	Not mutagenic.
Gene Mutation 870.5300 *In vitro* Mammalian Cells Gene Mutation (Chinese Hamster Ovary Cells)	47923611 (2009) Acceptable, guideline	Not mutagenic.
Cytogenetics 870.5375 *In vitro* Mammalian Cytogenetics Chromosomal Aberration Assay-human peripheral blood lymphocytes	47923610 (2009) Acceptable, guideline	Not clastogenic.
Cytogenetics-other 870.5395 *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus-mouse	47923612 (2009) Acceptable, guideline O, 500, 1000, 2000 mkd	Did not lead to any increase in micronucleated polychromatic erythrocytes.

Table 16. Genotoxicity panel for M700F002		
Gene Mutation 870.5100 *In vitro* Bacterial Gene Mutation	47923617 (2007) Acceptable, guideline	Not mutagenic.
Gene Mutation 870.5300 *In vitro* Mammalian Cells Gene Mutation (Chinese Hamster Ovary Cells)	47923619 (2008) Acceptable, -guideline	Not mutagenic.
Cytogenetics 870.5375 *In vitro* Mammalian Cytogenetics Chromosomal Aberration Assay-human peripheral blood lymphocytes	47923618 (2008) Acceptable, -guideline	Not clastogenic.
Cytogenetics-other	47923620 (2009)	Did not lead to any increase in polychromatic erythrocytes.

870.5395 *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus-mouse	Acceptable, guideline O, 375, 750, 1500 mkd	

Table 17. Genotoxicity panel and unscheduled DNA synthesis study for M700F048		
Gene Mutation 870.5100 *In vitro* Bacterial Gene Mutation	47923625 (2009) Acceptable, guideline	Not mutagenic.
Gene Mutation 870.5300 *In vitro* Mammalian Cells Gene Mutation (Chinese Hamster Ovary Cells)	47923627 (2009) Acceptable, guideline	Not mutagenic.
Cytogenetics 870.5375 *In vitro* Mammalian Cytogenetics Chromosomal Aberration Assay-human peripheral blood lymphocytes	47923626 (2009) Acceptable, guideline	Clastogenic with metabolic activation.
Cytogenetics-other 870.5395 *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus-mouse	47923628 (2009) Acceptable, guideline 0, 500, 1000, 2000 mkd	Did not lead to any increase in micronucleated polychromatic erythrocytes.
Non-OPPTS guideline, OECD 486 Unscheduled DNA Synthesis Test-mammalian liver cells (rat)	47923629 (2009) Acceptable, non-guideline 0, 1000, 2000 mkd	Does not induce unscheduled DNA synthesis.

C. Impurities

Three impurities produced during the manufacture fluxapyroxad and an artificial batch lacking the a.i. were tested in a battery of standard genotoxicity tests *in vitro* and *in vivo*. These studies demonstrate that none of these impurities have genotoxic potential. Additionally, for the artificial batch, an *in vivo* rat study was performed to determine if fluxapyroxad induced unscheduled DNA synthesis in hepatocytes. No evidence of increased nuclear grain counts or cells undergoing DNA repair was observed (data not shown).

3. Structure-Activity Relationship

Fluxapyroxad is a second-generation carboxamide of the pyrazole-carboxamide class. Other members of this class include bixafen, isopyrazam, and sedaxane (Figure 2). PubMed searches of a) pyrazole carboxamide AND tumor, and b) pyrazole carboxamide AND cancer revealed no indications that this class of chemicals is tumorigenic and indicated that they have actually been pursued as anti-cancer chemotherapeutic agents. The searches did indicate, however, induction of apoptosis in hepatocytes and possible activation of cannabinoid receptors.

Isopyrazam was classified by the CARC as "Likely to be Carcinogenic to Humans" based on the presence of thyroid follicular cell tumors in male rats, and liver and uterine tumors in female rats (TXR No. 0055619). Sedaxane was recently classified by the CARC as "Likely to be Carcinogenic to Humans" based on liver adenomas and thyroid follicular cell tumors in male rats and uterine tumors in female rats (3/16/2011 CARC meeting). Data to support a MOA for liver and thyroid tumors were not provided for either of these chemicals.

Unlike isopyrazam and sedaxane, fluxapyroxad does not cause uterine tumors in females. There is substantial data to support MOAs for the liver and thyroid tumors caused by fluxapyroxad. These MOAs are described in detail in section *5. Mode of Action Studies*.

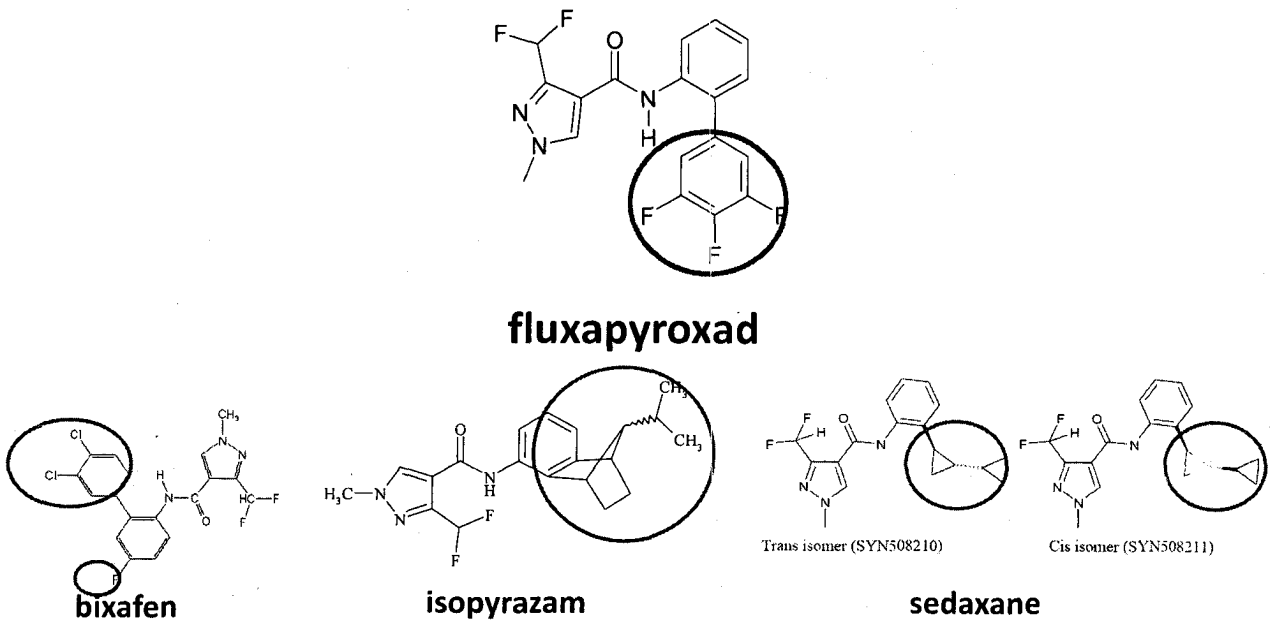
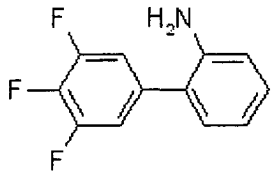
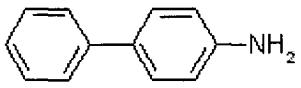


Figure 2. Structural similarities between fluxapyroxad and the second-generation carboxamides isopyrazam and sedaxane. The different side chains are circled in blue. The molecule core remains unchanged.

A potential SAR concern for the trifluoro-2-aminobiphenyl moiety in fluxapyroxad was raised by one of the CARC because 2-aminobipenyl is an isomer of the well known human carcinogen, 4-aminobiphenyl. 3',4',5'-Trifluoro-2-aminobiphenyl is predicted to be a potential carcinogen by EPA's OncoLogic cancer expert system, although its potency is expected to be substantially lower than that of 4-aminobiphenyl. This is because the 2-position is not as favorable for metabolic activation of the amino group as the 4-position. 3',4',5'-Trifluoro-2-aminobiphenyl may be a potential degradation product or metabolite of fluxapyroxad via cleavage of the amide bond but there was no report of its presence in the submitter's rodent metabolism data.



3',4',5'-trifluoro-2-aminobiphenyl



4-aminobiphenyl

4. Subchronic and Chronic Toxicity

The primary target organ for fluxapyroxad exposure via the oral route is the liver. Liver toxicity in response to fluxapyroxad was observed in rats, dogs, and mice, with species differences in the sensitivity, development, and manifestations of liver toxicity. In rats, adaptive effects of hepatocellular hypertrophy and increased liver weights and changes in liver metabolism were first observed. As the dose or duration of exposure to fluxapyroxad increased, clinical chemistry changes related to liver function were observed, followed by hepatocellular necrosis, which was considered a clearly adverse effect. Liver toxicity was also observed in mice and dogs. In subchronic studies in mice, increased liver weights were observed along with fatty changes in the liver, with few observations of hepatocellular hypertrophy. At higher doses, liver necrosis was also observed. Higher doses were required to achieve adverse liver effects in the mouse than in the rat. Liver effects in subchronic studies in dogs were limited to increased liver weights at high doses. However, in chronic studies, increased liver weights were observed in conjunction with fibrosis and cirrhosis at doses lower than those that caused adverse liver effects in rats and mice.

In rats only, changes in liver metabolism resulted in secondary toxicity in the thyroid. Fluxapyroxad administration via the oral route results in toxic effects in the thyroid. These thyroid effects are secondary to the effects on the liver. Thyroid effects in rats were observed throughout the toxicity database and included changes in serum thyroid hormone levels, enlarged thyroid, increased thyroid weights, and/or microscopic effects of thyroid follicular hypertrophy and hyperplasia. These effects showed a clear threshold, were evident within weeks and were observed out to two years. Although these effects were observed in male and female rats, males were usually more sensitive.

Supplementary, mechanistic studies were provided to characterize the effects of fluxapyroxad on the thyroid. A perchlorate discharge assay showed that fluxapyroxad treatment resulted in increased uptake of radiolabeled iodine by the thyroid similar to that observed with phenobarbital. (Phenobarbitol is a hepatic enzyme inducer that is known to increase thyroid hormone clearance by the liver, resulting in indirect stimulation of thyroid hormone synthesis via a compensatory stimulation of the thyroid gland). Other mechanistic studies showed that fluxapyroxad caused increased liver weights and induction of Phase I and Phase II metabolizing enzymes similar to phenobarbital. T_4-metaboilizing Phase II enzymes, in particular, were induced by fluxapyroxad at doses lower than those that cause increased serum levels of TSH, decreased serum levels of T_4 and thyroid follicular hypertrophy and hyperplasia. The effects of fluxapyroxad on the thyroid were also shown to be reversible following discontinuation of treatment with fluxapyroxad.

5. Mode of Action Studies

A. Mitogenic MOA for Liver Tumors

i. Overview of Proposed MOA

The proposed MOA for liver tumors provided by the Registrant is that fluxapyroxad works through a mitogenic MOA whereby cell proliferation in the liver progresses to adenomas and carcinomas. This occurs in the context of other changes (Key Events) in the liver that are commonly associated with these effects (e.g. enzyme induction, hepatocellular hypertrophy, increased liver weights, enlarged liver, and non-neoplastic alterations in the liver at the gross and microscopic level). These Key Events and their dose-response and temporal associations are discussed in detail below and are also summarized in Tables 18-19.

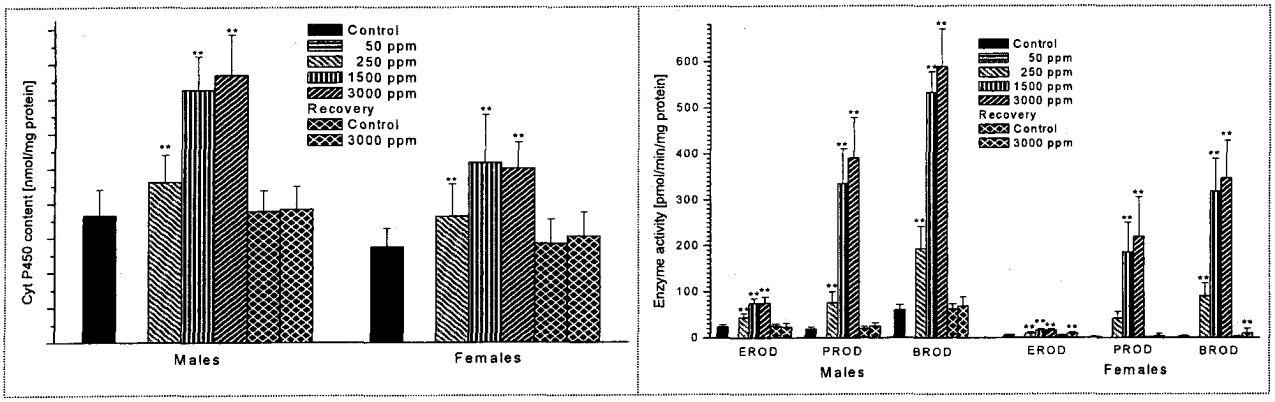
ii. Key Events

- Liver enzyme induction

Fluxapyroxad administration for 14 days caused increases in total liver P450 and induction of PROD/BROD, which are associated with CYP-2B in particular. This occurs at doses of 250 ppm and above, consistent with doses causing liver tumors in males. Total liver P450s and PROD/BROD levels return to levels equal to control (or slightly higher) in a 4 week recovery group, indicating near to complete reversibility of this effect (MRID 47923593).

ENZYME INDUCTION DATA FROM MRID 47923593:

Figure 5.5/1 Induction of total Cyt P450 content and of selected Phase I enzyme activities (N=10/dose/sex)



ENZYME INDUCTION DATA FROM MRID 47923593:

Table 5.5/1 Mean (±SD) cytochrome P450 content and selected Phase I enzyme activities from MRID 47923593

Cyt.P450						
	Sex	Males (N=10)		Females (N=10)		
Allocation	Dose [ppm]	Mean [nmol/mg Prot.]	Rel. value [%]	Mean [nmol/mg Prot.]		Rel. value [%]
Main group	0	0.730 ± 0.150		0.548 ± 0.108		
	250	**0.923 ± 0.155**	**126****	**0.726 ± 0.185**		**132***
	1500	**1.453 ± 0.191**	**199****	**1.036 ± 0.274**		**189****
	3000	**1.538 ± 0.232**	**211****	**1.002 ± 0.151**		**183****
Recovery group	0	0.755 ± 0.118		0.567 ± 0.144		
	3000	0.766 ± 0.134	101	0.609 ± 0.139		107
EROD						
	Sex	Males (N=10)		Females (N=10)		
Allocation	Dose [ppm]	Mean [pmol/min/ mg Prot.]	Rel. value [%]	Mean [pmol/min/mg Prot.]		Rel. value [%]
Main group	0	23.84 ± 4,28		5.57 ± 1.34		
	250	**43.67 ± 9.13**	**183****	**9.26 ± 2.10**		**166****
	1500	**73.93 ± 10.64**	**310****	**15.27 ± 3.39**		**274****
	3000	**74.35 ± 13.71**	**312****	**15.31 ± 3.04**		**275****
Recovery group	0	24.88 ± 4.66		4.81 ± 1.42		
	3000	23.06 ± 7.53	93	**9.14 ± 2.65**		**190****
PROD						
	Sex	Males (N=10)		Females (N=10)		
Allocation	Dose [ppm]	Mean [pmol/min/mg Prot.]	Rel. value [%]	Mean [pmol/min/mg Prot.]		Rel. value [%]
Main group	0	19.11 ± 3.84		1.75 ± 0.84		
	250	**75.14 ± 24.15**	**393****	**41.54 ± 13.82**		**2375****
	1500	**335.30 ± 75.51**	**1755****	**185.55 ± 64.59**		**10608****
	3000	**390.64 ± 87.67**	**2044****	**219.34 ± 87.30**		**12540****
Recovery group	0	19.83 ± 3.82		1.50 ± 0.81		
	3000	24.04 ± 7.41	121	3.96 ± 4.39		265
BROD						
	Sex	Males (N=10)		Females (N=10)		
Allocation	Dose [ppm]	Mean [pmol/min/mg Prot.]	Rel. value [%]	Mean [pmol/min/mg Prot.]		Rel. value [%]
Main group	0	59.27 ± 11.40		2.74 ± 1.87		
	250	**193.33 ± 48.14**	**326****	**89.80 ± 28.24**		**3277****
	1500	**533.11 ± 44.81**	**899****	**318.71 ± 71.06**		**11632****
	3000	**589.21 ± 81.23**	**994****	**347.67 ± 81.06**		**12688****
Recovery group	0	62.26 ± 11.12		1.93 ± 1.85		
	3000	68.12 ± 19.90	109	**9.06 ± 10.19**		**470****

** p < 0.01 (Wilcoxon test wit Bonferoni-Holm-Adjustment)

- Cell proliferation

Cell proliferation in the liver was also measured by labelling with BrdU (MRIDs 47923596-8). A dose and time-related increase in cell proliferation was observed. Cell proliferation was increased in all liver zones (1, 2, and 3), but was highest in Zone 3, which is the centrilobular region of the liver. At doses ≥ 1500 ppm, increased cell proliferation was maximal for males Days 3-7 and negligible by day 28. For females, increased cell proliferation was observed at doses ≥ 250 ppm from Days 3-91. Cell proliferation was maximal on Day 7 and declined Days 28 and 91, but still remained significant. Treatment for 28 days with fluxapyroxad followed by a 28 day recovery period resulted in insignificant cell proliferation at all dose levels (MRIDs 47923598, 47923596, 47923597). This recovery study was likely done to show reversibility. *However, a caveat is that peak cell proliferation was at Day 7 in both males and females, not Day 28. By Day 28, cell proliferation is essentially zero in males and is about 30% of the maximum in females.*

ZONE 3 CELL PROLIFERATION DATA FROM MRID 47923598:

Figure 5.5/2 S-Phase response in the liver of rats after treatment with BAS 700 F for 1, 3, 7 and 14 days

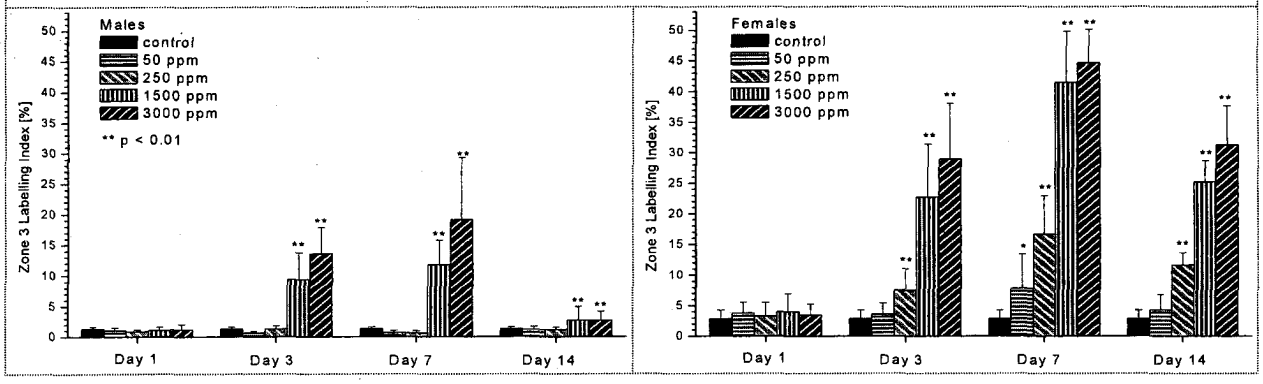
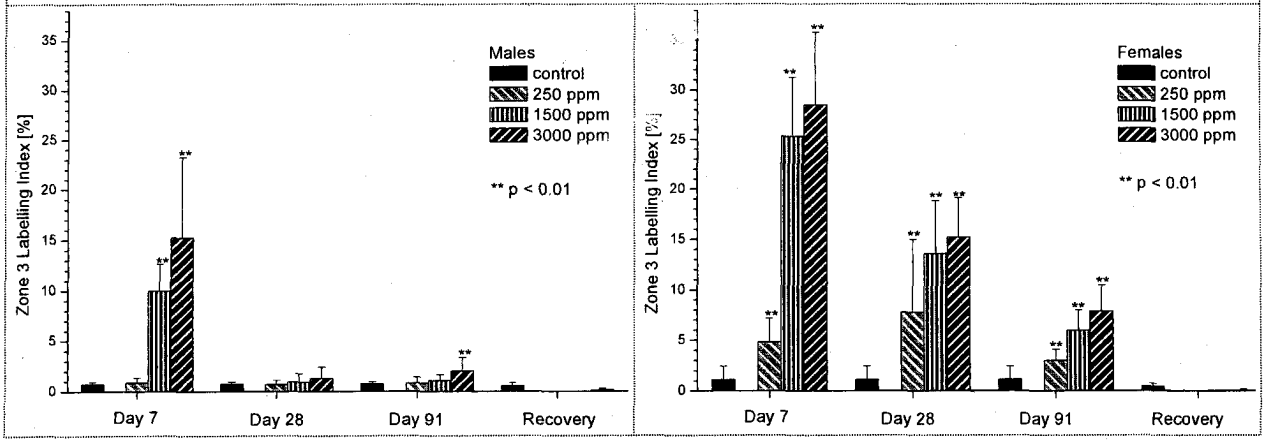


Figure 5.5/3 S-Phase response in the liver of rats after treatment with BAS 700 F for 7, 28 and 91 days including a 28-day recovery period after 28-days of treatment



- Hepatocellular hypertrophy, increased liver weights, and enlarged liver

Increased liver weight, enlarged liver, and hepatocellular hypertrophy were observed over time and the reversibility of these effects was also examined. After 3 days, enlarged livers and increased absolute/relative liver weights were observed in males and females at doses ≥ 1500 ppm. By 14 days, these effects (with the exception of enlarged liver) were present in both sexes at doses ≥ 250 ppm. Hepatocellular hypertrophy was evident by Day 7 at doses of ≥ 250 ppm and males and at 3000 ppm in females (MRID 47923598) but the dose-response for this effect was not as robust as in a second study, which also examined reversibility (MRID 47923593). In this study, a clear dose-related increase in hepatocellular hypertrophy was observed in males at ≥ 250 ppm and females at ≥ 1500 ppm, with increases in absolute/relative liver weights in both sexes at ≥ 250 ppm after two weeks after 14 days of fluxapyroxad administration. In a 4 week recovery group, hepatocellular hypertrophy was not observed and absolute/relative liver weights remained increased in males only.

ENLARGED LIVER DATA FROM MRID 47923598:

Table 5.5/2 Incidence of selected gross pathological findings in rat administered BAS 700 F for up to 14 days

Sex	Male				Female			
Dose [ppm]	0	250	1500	3000	0	250	1500	3000
	14 days of treatment							
Animals in group	10	10	10	10	10	10	10	10
Liver								
- Enlarged	-	-	**10**	**10**	-	-	**5**	**10**
	7 days of treatment							
Liver								
- Enlarged	-	-	**10**	**10**	-	-	**4**	**10**
	3 days of treatment							
Liver								
- Enlarged	-	-	**6**	**7**	-	-	**2**	**7**
	1 day of treatment							
Liver								
- Enlarged	-	-	-	-	-	-	-	-

LIVER WEIGHT DATA FROM MRID 47923598:

Table 5.5/3 Liver weights after 1, 3, 7 and 14 days of treatment with BAS 700 F (mean ± SD)

Sex		Males (N=10)				Females (N=10)			
Organ weight	Dose [ppm]	Absolute weight	% of control	Relative weight [%]	% of control	Absolute weight	% of control	Relative weight [%]	% of control
		14 days of treatment							
Liver [g]	0	11.08 ±1.048		3.285 ±0.211		6.944 ±0.824		3.201 ±0.198	
	50	11.179 ±0.977	(101)	3.363 ±0.168	(102)	6.763 ±0.677	(97)	3.242 ±0.274	(101)
	250	**12.056*** ±0.753	(109)	**3.641*** ±0.259	(111)	7.057 ±0.562	(102)	3.309 ±0.231	(103)
	1500	**15.338**** ±0.958	(138)	**4.523**** ±0.239	(138)	**8.532**** ±0.639	(123)	**4.093**** ±0.178	(128)
	3000	**15.979**** ±1.507	(144)	**4.86 **** ±0.348	(148)	**9.909**** ±0.706	(143)	**4.617**** ±0.259	(144)
		7 days of treatment							
Liver [g]	0	11.08 ±1.048		3.285 ±0.211		6.944 ±0.824		3.201 ±0.198	
	50	10.876 ±0.689	(98)	3.231 ±0.154	(98)	7.351 ±0.655	(106)	3.362 ±0.215	(105)
	250	11.415 ±0.881	(103)	3.447 ±0.123	(105)	7.638 ±0.883	(110)	3.432 ±0.306	(107)
	1500	**14.108**** ±0.921	(127)	**4.152**** ±0.144	(126)	**8.259**** ±0.918	(119)	**3.887**** ±0.271	(121)
	3000	**14.875**** ±1.782	(134)	**4.502**** ±0.331	(137)	**9.067**** ±0.493	(131)	**4.214**** ±0.124	(132)
	3000	**23.5**** ±3.659	(128)	**0.007**** ±0.001	(131)	18.9 ±1.792	(107)	0.009 ±0.001	(108)
		3 days of treatment							
Liver [g]	0	11.08 ±1.048		3.285 ±0.211		6.944 ±0.824		3.201 ±0.198	
	50	10.513 ±1.124	(95)	3.113 ±0.162	(95)	6.947 ±0.669	(100)	3.185 ±0.195	(99)
	250	11.755 ±1.23	(106)	3.454 ±0.258	(105)	7.077 ±0.611	(102)	3.266 ±0.229	(102)
	1500	**13.293**** ±1.134	(120)	**3.927**** ±0.226	(120)	**7.911*** ±0.725	(114)	**3.654**** ±0.256	(114)
	3000	**13.738**** ±1.248	(124)	**4.126**** ±0.2	(126)	**8.855**** ±0.792	(128)	**4.178**** ±0.323	(131)

LIVER WEIGHT DATA FROM MRID 47923598:

Table 5.5/3 Liver weights after 1, 3, 7 and 14 days of treatment with BAS 700 F (mean ± SD)

Sex		Males (N=10)				Females (N=10)			
Organ weight	Dose [ppm]	Absolute weight	% of control	Relative weight [%]	% of control	Absolute weight	% of control	Relative weight [%]	% of control
		1 days of treatment							
Liver [g]	3000	337.81 ±12.45	(100)			211.01 ±8.746	(98)		
	0	11.08 ±1.048		3.285 ±0.211		6.944 ±0.824		3.201 ±0.198	
	50	10.422 ±0.908	(94)	**3.029*** ±0.208	(92)	7.226 ±0.529	(104)	3.312 ±0.197	(103)
	250	10.896 ±1.003	(98)	3.172 ±0.236	(97)	6.965 ±0.628	(100)	3.195 ±0.184	(100)
	1500	10.529 ±0.903	(95)	3.148 ±0.151	(96)	7.017 ±0.583	(101)	3.263 ±0.251	(102)
	3000	11.18 ±0.729	(101)	3.311 ±0.203	(101)	6.577 ±0.461	(95)	3.115 ±0.134	(97)

HEPATOCELLULAR HYPERTROPHY DATA FROM MRID 47923598:

Table 5.5/4 Incidence of selected histopathological lesions in rats administered BAS 700 F for up to 14 days

Sex	Males				Females			
Dose [ppm]	0	250	1500	3000	0	250	1500	3000
# examined	10	10	10	10	10	10	10	10
14 days of treatment								
Liver								
- hypertrophy, centrilobular	-	2 *[2.0]*	10 *[2.5]*	7 *[2.3]*	-	-	10 *[2.5]*	10 *[3.0]*
7 days of treatment								
Liver								
- hypertrophy, centrilobular	-	1 *[1.0]*	7 *[2.3]*	4 *[2.0]*	-	-	-	4 *[2.3]*
3 days of treatment								
Liver								
- hypertrophy, centrilobular	-	-	-	-	-	-	-	8 *[2.0]*
1 day of treatment								
Liver								
- hypertrophy, centrilobular	-	-	-	-	-	-	-	-

[ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4) and massive/severe (Grade 5). The mean severity is the sum of the gradings divided by the incidence.

HEPATOCELLULAR HYPERTROPHY DATA FROM MRID 47923593:

Table 5.5/5 Incidence of selected macro- and histopathological lesions in rats administered BAS700 F for 14 days from 47923593

Sex		Males				Females			
Dose [ppm]		0	250	1500	3000	0	250	1500	3000
Animals in group		10	10	10	10	10	10	10	10
Macropathology/ Main Group									
Liver	# examined	10	10	10	10	10	10	10	10
- enlargement		-	-	-	10 *[3.9]*	-	-	-	10 *[3.2]*
Histopathology/ Main Group									
Liver	# examined	10	10	10	10	10	10	10	10
- Centrilobular hypertrophy		1 *[1.0]*	6 *[1.2]*	9 *[1.7]*	10 *[2.4]*	-	2 *[1.0]*	10 *[1.9]*	10 *[2.6]*

$ [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked/severe (Grade 4) and massive/extreme (Grade 5). The mean severity is the sum of the gradings divided by the incidence

- Non-neoplastic alterations in the liver (macro/microscopic)

Non-neoplastic lesions in the liver provide supporting information for the tumorigenic effects (See Tables 6-8). Only pigmentary changes (microscopic and macroscopic) are observed at 1 year. However, at 2 years, a dose-related increase in non-neoplastic lesions (e.g. cysts (males only), and microscopic and macroscopic foci) are observed at ≥1500 ppm in both sexes (MRID 47923591).

- Liver tumors

Adenomas are observed at lower doses in male rats (≥1500 ppm) than carcinomas (3000 ppm) (see Tables 3-4). These tumors were not observed until 2 years. At 2 years, three male rats had both adenomas and carcinomas. Females, which are not as sensitive to the tumorigenic effects of fluxapyroxad did not demonstrate a treatment-related increase in carcinomas and demonstrated adenomas only at 3000 ppm. Together these data indicate a dose-related and time-related progression of adenomas to carcinomas for which males are more sensitive.

iii. Dose-response

The dose-response for the Key Events leading up to (and including) liver tumor formation are summarized in Table 18. Males are clearly the more sensitive sex, with adenoma formation at ≥1500 ppm. At 3000 ppm, males have adenomas and carcinomas. For females, adenomas do not form until 3000 ppm and there are no treatment-related increases in carcinomas. Several of the underlying Key Events (liver enzyme induction, cell proliferation, hepatocellular hypertrophy/increased liver weight/enlarged liver) occur at doses lower than tumor formation (≥250 ppm).

iv. Temporal Association

The temporal associations for the Key Events leading up to (and including) liver tumor formation are summarized in Table 19. Changes in the liver (enzyme induction, cell proliferation, hepatocellular hypertrophy/increased liver weight/enlarged liver) occur within days. Many of these effects (hepatocellular hypertrophy/increased liver weight/enlarged liver) persist and are observed in conjunction with tumors. Cell proliferation, however, appears to be an early event (within 90 days). Mild non-neoplastic changes in the liver (e.g. pigmentary changes) are observed at 1 year. However, by 2 years, several non-neoplastic changes are observed in conjunction with adenomas and carcinomas.

Table 18 Dose-Response Association of Key Events of Liver Tumorigenesis

Sex	Male					Female				
Dose [ppm]	0	50	250	1500	3000	0	50	250	1500	3000
Liver enzyme induction			X	X	X			X	X	X
Cell proliferation in liver				X	X			X	X	X
Hepatocellular hypertrophy			X	X	X				X	X
Increased liver weight			X	X	X			X	X	X
Enlarged liver				X	X				X	X
Non-neoplastic changes in liver (cysts, macro/micro foci)				X	X				X	X
Adenoma				X	X					X
Carcinoma					X					
Adenoma/carcinoma					X					
Adenoma & carcinoma same animal					X					

Table 19 Temporal Association of Key Events of Liver Tumorigenesis

Sex	Male					Female				
Dose [ppm]	≤ 7 Days	≤ 14 Days	≤ 28 Days	≤ 1 Year	≤ 2 Years	≤ 7 Days	≤ 14 Days	≤ 28 Days	≤ 1 Year	≤ 2 Years
Liver enzyme induction	X	X				X	X			
Cell proliferation in liver	X	X		Low at 91 days		X	X	X	Low at 91 days	
Hepatocellular hypertrophy	X	X	X	X	X	X	X	X	X	X
Increased liver weight	X	X	X	X	X	X	X	X	X	X
Enlarged Liver	X	X	X	X	X	X	X	X	X	X
Non-neoplastic changes in liver (cysts, macro/micro foci)					X					X
Adenomas					X					X
Carcinomas					X					
Adenoma/ carcinoma					X					
Adenoma & carcinoma same animal					X					

v. Strength, consistency, and specificity

The effects underlying the Key Events in this MOA are robust and many are replicated in different studies, supporting strength and consistency. The liver zone of origin for tumors was not known and the zones of enzyme induction were not confirmed. However, P450s are known to be highly expressed in the centrilobular regions (Zone 3). Cell proliferation occured in all zones of the liver but was highest in Zone 3. Also, hepatocellular hypertrophy occured in the centrilobular region (Zone 3). Together, the Key Events indicate high specificity for tumorigenesis in the liver.

vi. Biological plausibility and coherence

The proposed MOA is considered biologically plausible and coherent, as it is a known MOA for liver tumors in rodents.

vii. Alternative MOAs

- Genotoxicity leading to tumors. There is no evidence of genotoxicity in the fluxapyroxad database. Therefore, any genotoxic MOA would have to be via an reactive oxygen species (ROS)-mediated mechanism associated with direct ROS generation by the a.i. or from inflammation. There is no evidence that fluxapyroxad directly generates ROS and there is no evidence of inflammatory effects in the liver.
- Activation of PPARα leading to tumors. There was no microscopic evidence of peroxisome proliferation.
- Acute cellular toxicity leading to cell death and compensatory cell proliferation. Cell proliferation was observed within 3 days, and so cell death would have to occur earlier

for this MOA to be operative. Histopathological examination of the liver was performed on Day 1 of oral administration, and no evidence of liver necrosis, inflammation, or other signs of acute injury were reported.

viii. Human relevance

The key events in the non-genotoxic mitogenic MOA are plausible in humans. One would anticipate the downstream events of increased cell proliferation and tumor response if a dose was reached that produced similar liver perturbations. The MOA is applicable to all populations.

ix. Conclusion

The identified key events with corresponding dose and temporal concordance adequately support the proposed non-genotoxic mitogenic MOA for liver adenomas and carcinomas in rats. This MOA is based on early changes in liver enzyme regulation and increased cell proliferation that lead to microscopic and macroscopic foci and then to adenoma and carcinoma formation. The endpoint of changes in liver enzyme regulation provides a clear threshold for liver tumors.

B. *Non-genotoxic MOA for Thyroid Tumors*

i. Overview of Proposed MOA

The proposed MOA for thyroid follicular adenomas and carcinomas is that fluxapyroxad induces liver microsomal enzymes leading to increased metabolism (turnover) of thyroid hormones T3/T4, resulting in lower circulating levels of T3/T4. The decrease in thyroid hormones results in a compensatory increase in pituitary thyroid stimulating hormone (TSH) production. Sustained elevated plasma TSH levels promote hypertrophy/hyperplasia in the thyroid which, over time, progresses to tumors. These Key Events and their dose-response and temporal associations are discussed in detail below and are also summarized in Tables 20-21.

ii. Key Events

- Liver enzyme induction

Fluxapyroxad administration for 14 days caused increases in total liver P450 and induction of PROD/BROD, which are associated with CYP-2B in particular. This occurs at doses of 250 ppm and above (MRID 47923593). Also after 14 days, increased T4-UDP-GT is observed in males and females at ≥1500 ppm. This increase is 57-59% in males and 176-183% in females. This is accompanied by increases in Phase I liver enzymes in both sexes at ≥250 ppm. The increases in T4-UDP-GT are reversible 4 weeks after cessation of oral exposure (MRID 47923593).

T4-UDP-GT DATA FROM MRID 47923593:

Table 5.5/6 Mean (±SD) enzyme Activity of selected Phase II enzymes in rats administered BAS 700 F for 14 days

T4-UDP-GT								
	Sex	Males (N=10)			Females (N=10)			
Allocation	Dose [ppm]	Mean Increase of T4-GT (% control)			Mean Increase of T4-GT (% control)			
Main group	0	100.00	±		100.00	±		
	250	110.45	±	29	181.12	±	134	
	1500	**152.12****	**±**	**57**	**237.74****	**±**	**176**	
	3000	**158.47****	**±**	**59**	**268.41****	**±**	**183**	
Recovery group	0	100.00	±		100.00	±		
	3000	106.61	±	31	115.76	±	52	

** p < 0.01 (Wilcoxon test wit Bonferoni-Holm-Adjustment)

LIVER ENZYME INDUCTION DATA PREVIOUSLY PRESENTED ON p 25-26

- Increased excretion of thyroid hormones and compensatory changes in thyroid hormone levels

T4 levels are decreased ca. 15-20% and TSH levels are increased ca. 70% in males by 14 days of oral administration of fluxapyroxad at 3000 ppm (MRID 47923594). Increases in TSH by 14 days at 3000 ppm in males are reversible 4 weeks after cessation of treatment (MRID 47923593).

T4 AND TSH DATA FROM MRID 47923594:

Table 5.5/7 Mean (±SD) hormone levels in rats administered BAS 700 F for 28 days (second analysis)

Dose [ppm]	0	50	250	1500	3000
Sampling day	**Thyroxine (T_4) [nmol/L]**				
	Males (N=10)				
7	51.90 ± 8.61	59.87 ± 8.37	55.52 ± 9.56	58.04 ± 8.17	48.90 ± 8.13
14	65.22 ± 9.73	73.89 ±11.44	68.28 ±14.14	67.86 ±13.60	**52.33**** ± 6.43 (-19%)
21	62.87 ± 7.82	71.94 ±11.54	72.27 ±11.75	66.50 ±11.03	54.64 ± 8.63 (-13%)
28	70.50 ± 8.94	74.79 ±13.06	69.32 ± 6.84	71.17 ±14.80	**60.44*** ± 7.33 (-14%)
	Thyroid Stimulating Hormone (TSH) [µg/mL]				
	Males (N=10)				
-3	5.38 ± 1.65	ND	ND	ND	5.22 ± 2.46
3	6.08 ± 1.58	ND	ND	ND	6.29 ± 2.16
7	5.88 ± 1.60	5.44 ± 1.22	4.59 ± 1.34	6.06 ± 1.75	6.56 ± 2.39
14	5.47 ± 0.93	5.62 ± 2.68	4.95 ± 1.47	6.35 ± 3.08	**9.26*** ± 5.70 (+69%)
21	5.39 ± 1.49	5.65 ± 1.92	5.81 ± 2.55	8.00 ± 3.61	9.09 ± 6.55 (+68%)
28	4.40 ± 1.78	6.16 ± 3.24	5.39 ± 1.28	7.38 ± 3.32	6.88 ± 4.40 (+56%)
	Females (N=10)				
-3	3.96 ± 0.75	ND	ND	ND	3.86 ± 1.56
3	3.83 ± 0.71	ND	ND	ND	4.17 ± 1.17
7	4.11 ± 0.62	3.41 ± 1.13	4.07 ± 0.71	4.23 ± 0.78	3.84 ± 1.56
14	4.28 ± 0.77	3.56* ± 0.67	4.02 ± 0.97	4.92 ± 1.05	4.83 ± 1.25
21	4.49 ± 0.68	3.45**± 0.74	4.11 ± 0.72	4.67 ± 0.74	5.14 ± 1.64
28	4.25 ± 0.90	3.44 ± 1.08	3.92 ± 0.53	4.43 ± 1.30	4.46 ± 1.01

* $p \leq 0.05$; ** $p \leq 0.01$ (Kruskal-Wallis and Wilcoxon-test. two sided)
ND: not determined

A perchlorate discharge assay showed that after 14 days of exposure to 3000 ppm fluxapyroxad, pulsing with ^{125}I, and discharge with perchlorate, a significant amount of ^{125}I remained, similar to a phenobarbital control. This indicated that the effects of fluxapyroxad on the thyroid were indirect by interfering with hormone synthesis, and not direct by interfering with thyroid hormone biosynthesis (as observed for the PTU control, MRID 47923595).

PERCHLORATE DISCHARGE DATA FROM MRID 47923595:

Table 5.5/8: Thyroid weights and ^{125}I counts in male rats administered $KClO_4$ prior to sacrifice

Group (N=6)		01	11	21	31
Treatment		Control + $KClO_4$	BAS 700 F + $KClO_4$	PTU + $KClO_4$	PB + $KClO_4$
Thyroid weight [g]	Mean	0.013	0.015	**0.050****	**0.017***
	SD	0.002	0.004	0.009	0.002
	□%		15	285	31
Blood count [cpm]	Mean	3950	4014	**5850****	4215
	SD (g)	313	563	785	774
	□%		2	48	7
Thyroid count [cpm]	Mean	96736	**191522****	**32382****	**195589****
	SD	34054	46444	3833	47277
	□%		98	-67	102
Specific Blood count [cpm/g]	Mean	3891	3944	**5765****	4180
	□%		1	48	7
Specific Thyroid count [cpm/g]	Mean	7557512	**12382902****	**653073****	**11561918****
	□%		64	-91	53
Ratio of specific thyroid/blood counts	Mean	1937	**3231****	**117****	2867
	SD	493	659	23	876
	□%		67	-94	48

Table 5.5/9: Thyroid weights and ^{125}I counts in female rats administered $KClO_4$ prior to sacrifice

Group (N=6)		01	11	21	31
Treatment		Control + $KClO_4$	BAS 700 F + $KClO_4$	PTU + $KClO_4$	PB + $KClO_4$
Thyroid weight [g]	Mean	0.009	0.010	**0.041****	0.010
	SD	0.002	0.002	0.006	0.002
	□%		11	356	11
Blood count [cpm]	Mean	5266	5593	**9338****	**6551***
	SD (g)	462	1192	985	958
	□%		6	77	24
Thyroid count [cpm]	Mean	101068	**164599***	87107	155325
	SD	41125	45492	29636	48609
	□%		63	-14	54
Specific Blood count [cpm/g]	Mean	5172	5473	**9081****	**6365***
	□%		6	76	23
Specific Thyroid count [cpm/g]	Mean	10848075	**16795840***	**2130613****	14816334
	□%		55	-80	37
Ratio of specific thyroid/blood counts	Mean	2171	3221	**234****	2460
	SD	891	1022	61	925
	□%		48	-89	13

- Thyroid hypertrophy/hyperplasia/increased thyroid weights

Support that these changes in thyroid hormone regulation lead to increased cell proliferation in the thyroid is provided by histopathology, which shows a dose-related increase in thyroid follicular hypertrophy/hyperplasia and altered colloid in males at ≥250 ppm after 14 days of exposure. Females were less sensitive, showing thyroid follicular hypertrophy/hyperplasia at 3000 ppm only. These histopathological effects are decreased in incidence or completely reversed 4 weeks following cessation of oral exposure (MRID 47923593).

THYROID FOLLICULAR HYPERTROPHY/HYPERPLASIA DATA FROM MRID 47923593:

Table 5.5/10 Incidence of selected macro- and histopathological lesions in rats administered BAS700 F for 14 days

Sex	Males				Females			
Dose [ppm]	0	250	1500	3000	0	250	1500	3000
Animals in group	10	10	10	10	10	10	10	10
Thyroid gland # examined	10	10	10	10	10	10	10	10
- Follicular hypertrophy/hyperplasia	-	2 *[1.5]*	4 *[1.8]*	5 *[2.2]*	-	-	-	4 *[1.0]*
- altered colloid	-	1 *[1.0]*	1 *[2.0]*	2 *[1.0]*	-	-	-	-
-Histopathology/ Recovery Group								
Thyroid gland # examined	10			10	10			10
- Follicular hypertrophy/hyperplasia	2 *[1.0]*			3 *[1.0]*	-			-
- altered colloid	1 *[1.0]*			1 *[1.0]*	-	-	-	-

$ [ ] mean severity grading; histopathological findings were graded minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked/severe (Grade 4) and massive/extreme (Grade 5). The mean severity is the sum of the gradings divided by the incidence

Thyroid weights were also examined. Significant increases in absolute/relative thyroid weights were observed in males as early as 3 days at ≥50 ppm (MRID 47923598) and in females as early as 14 days at 3000 ppm (47923593). An attempt was made to show reversibility of thyroid weights at Day 28, but thyroid weights in treated males with no recovery period actually decreased. The Day 28 data in this particular study were considered spurious, since increased thyroid weights were also observed throughout the toxicity database for fluxapyroxad.

COMPILED THYROID WEIGHT DATA FROM MRIDs 4792358, 4792356, 4792353:

Sex		Males (N=10)				Females (N=10)			
Organ weight	Dose [ppm]	Absolute weight	% of control	Relative weight [%]	% of control	Absolute weight	% of control	Relative weight [%]	% of control
28 days of treatment (MRID 4792356)									
Thyroid [mg]	0	31.8 ±8.337		0.007 ±0.002		21.5 ±3.44		0.009 ±0.001	
	250	23.4* ±5.562	(74)	0.005* ±0.001	(75)	23.8 ±1.549	(111)	0.009 ±0.001	(110)
	1500	25.3* ±3.529	(80)	0.006 ±0.001	(80)	24.8 ±3.91	(115)	0.01 ±0.002	(122)
	3000	27.7 ±3.466	(87)	0.006 ±0.001	(89)	24.0 ±3.887	(112)	0.01 ±0.002	(118)
28 days of treatment + 28 days recovery (MRID 4792356)									
Thyroid [mg]	0	23.8 ±4.392		0.005 ±0.001		22.8 ±3.676		0.009 ±0.001	
	250								
	1500								
	3000	20.0 ±2.867	(84)	0.005 ±0.001	(89)	20.3 ±2.71	(89)	0.008 ±0.001	(92)
14 days of treatment (MRID 4792358)									
Thyroid [mg]	0	18.4		0.005 ±0.001		17.6 ±1.578		0.008 ±0.001	
	50	22.5* ±4.1483	(122)	0.007* ±0.001	(124)	16.6 ±1.838	(94)	0.008 ±0.001	(98)
	250	23.5** ±.3.136	(128)	0.007** ±0.001	(131)	18.5 ±3.779	(105)	0.009 ±0.002	(107)
	1500	25.5** ±4.696	(139)	0.008** ±0.001	(138)	17.9 ±2.283	(102)	0.009 ±0.001	(105)
	3000	24.5** ±4.327	(133)	0.007** ±0.001	(137)	18.8 ±2.251	(107)	0.009 ±0.001	(107)
(MRID 4792353)									
Thyroid [mg]	0	0.022 ±0.003		0.006 ±0.001		0.016 ±0.001		0.007 ±0.001	
	3000	0.024 ±0.004	(8.2)	0.006 ±0.001	(10.4)	0.020* ±0.004	(21.0)	0.009** ±0.001	(22.1)
(MRID 4792353)									
Thyroid glands ±SD	0	0.021 ±0.002		0.006 ±0.001		0.017 ±0.003		0.008 ±0.001	
	250	0.022 ±0.003	(7.7)	0.007 ±0.001	(8.3)	0.017 ±0.002	(-2.4)	0.008 ±0.001	(0.7)
	1500	0.025** ±0.003	(19.7)	0.008* ±0.001*	(18.9)	0.018 ±0.004	(8.3)	0.009 ±0.002	(9.6)
	3000	0.023 ±0.004	(12.0)	0.007* ±0.001	(13.3)	0.019 ±0.003	(9.5)	0.009 ±0.002	(14.2)

COMPILED THYROID WEIGHT DATA FROM MRIDs 4792358, 4792356, 4792353:

Sex		Males (N=10)				Females (N=10)			
Organ weight	Dose [ppm]	Absolute weight	% of control	Relative weight [%]	% of control	Absolute weight	% of control	Relative weight [%]	% of control
		7 days of treatment (MRID 4792358)							
Thyroid [mg]	0	18.4 ±2.875		0.005 ±0.001		17.6 ±1.578		0.008 ±0.001	
	50	22.8* ±3.425	(124)	0.007** ±0.001	(124)	19.3 ±2.497	(110)	0.009 ±0.001	(108)
	250	23.6** ±4.195	(128)	0.007** ±0.001	(131)	19.0 ±2.582	(108)	0.009 ±0.001	(105)
	1500	23.4** ±3.688	(127)	0.007* ±0.001	(126)	18.5 ±2.173	(105)	0.009 ±0.001	(107)
	3000	23.5** ±3.659	(128)	0.007** ±0.001	(131)	18.9 ±1.792	(107)	0.009 ±0.001	(108)
		(MRID 4792356)							
0	31.8 ±8.337		0.007 ±0.002		21.5 ±3.44		0.009 ±0.001		
250	24.7* ±3.057	(78)	0.006 ±0.001	(78)	15.2** ±3.584	(71)	0.006** ±0.001	(72)	
1500	24.4* ±3.534	(77)	0.005 ±0.001	(76)	20.5 ±3.136	(95)	0.009 ±0.001	(100)	
3000	22.7** ±3.743	(71)	0.005 ±0.001	(74)	19.0 ±1.944	(88)	0.008 ±0.001	(93)	
		3 days of treatment (MRID 4792358)							
Thyroid [mg]	0	18.4 ±2.875		0.005 ±0.001		17.6 ±1.578		0.008 ±0.001	
	50	23.2** ±3.938	(126)	0.007* ±0.001	(126)	15.5 ±2.991	(88)	0.007 ±0.001	(87)
	250	23.4** ±3.026	(127)	0.007** ±0.001	(127)	16.9 ±2.644	(96)	0.008 ±0.001	(96)
	1500	23.6** ±2.297	(128)	0.007** ±0.001	(128)	16.7 ±3.199	(95)	0.008 ±0.001	(94)
	3000	21.1* ±2.846	(115)	0.006* ±0 001	(116)	15.9 ±2.685	(90)	0.007 ±0.001	(92)

COMPILED THYROID WEIGHT DATA FROM MRIDs 4792358, 4792356, 4792353:

Sex		Males (N=10)				Females (N=10)			
Organ weight	Dose [ppm]	Absolute weight	% of control	Relative weight [%]	% of control	Absolute weight	% of control	Relative weight [%]	% of control
		1 days of treatment (MRID 4792358)							
Thyroid [mg]	0	18.4 ±2.875		0.005 ±0.001		17.6 ±1.578		0.008 ±0.001	
	50	20.0 ±3.162	(109)	0.006 ±0.211	(107)	19.2 ±1.751	(109)	0.009 ±0.001	(108)
	250	21.6 ±2.119	(117)	0.006 ±0.001	(115)	17.3 ±2.71	(98)	0.008 ±0.001	(97)
	1500	21.6 ±3.134	(117)	0.006 ±0.001	(119)	17.8 ±2.486	(101)	0.008 ±0.001	(101)
	3000	21.7 ±2.908	(118)	0.006 ±0.001	(118)	18.7 ±3.129	(106)	0.009 ±0.002	(109)

- Thyroid follicular adenomas

An increase in thyroid follicular adenomas and combined adenomas/carcinomas was observed only in males and only at the high dose (see Table 5). Importantly, adenomas were observed temporally before carcinomas, with one high dose animal having both adenomas and carcinomas. Together, these data indicate that thyroid follicular adenomas form first and progress to thyroid follicular carcinomas.

iii. Dose response

The dose-response for the Key Events leading up to (and including) liver tumor formation are summarized in Table 20. While both males and females are equisensitive to liver enzyme induction at ≥250 ppm and increased excretion of thyroid hormone at ≥1500 ppm, males are more sensitive to compensatory changes in thyroid hormone levels. These compensatory changes do not occur until 3000 pp and were absent in females. The increases in thyroid follicular hypertrophy/hyperplasia occured at lower doses than changes in thyroid hormone levels in both sexes. The reason for this is unclear (perhaps histopathology was a more sensitive endpoint that blood levels of hormones in this case). In any case, thyroid follicular hypertrophy/hyperplasia occured at doses much lower than those that cause increased adenomas and adenomas/carcinomas in males.

Table 20 Dose-Response Association of Key Events of Thyroid Follicular Adenomas/Carcinomas

Sex	Male					Female				
Dose [ppm]	0	50	250	1500	3000	0	50	250	1500	3000
Liver enzyme induction			X	X	X			X	X	X
Increased excretion of T4-UDP-GT				X	X				X	X

Changes in thyroid hormone levels (↑TSH ↓T4)					X					
Thyroid follicular hypertrophy/hyperplasia			X	X	X					X
Adenoma					X					
Adenoma/carcinoma					X					

iv. Temporal Association

The temporal associations for the Key Events leading up to (and including) thyroid follicular adenomas and adenomas/carcinomas is summarized in Table 21. The earliest events (within weeks) are liver enzyme induction, increased excretion of thyroid hormone, and changes in thyroid hormone (T4 and TSH) levels. Thyroid follicular hypertrophy/hyperplasia are also observed by two weeks and persist out to 1 and 2 years. Adenomas and adenomas/carcinoma increase for males only, with adenomas occuring at 1 year.

Table 21 Temporal Association of Key Events of Thyroid Follicular Adenomas/Carcinomas

Sex	Male					Female				
Dose [ppm]	≤ 7 Days	≤ 14 Days	≤ 28 Days	≤ 1 Year	≤ 2 Years	≤ 7 Days	≤ 14 Days	≤ 28 Days	≤ 1 Year	≤ 2 Years
Liver enzyme induction	X	X				X	X			
Increased excretion of T4-UDP-GT		X					X			
Changes in thyroid hormone levels (↑TSH ↓T4)		X								
Thyroid follicular hypertrophy/hyperplasia		X	X	X	X		X	X	X	X
Adenoma				X	X					
Adenoma/carcinoma					X					

v. Strength, consistency, and specificity

The effects underlying the Key Events in this MOA are statistically robust and many are replicated in different studies, which supports strength and consistency. The studies underlying the Key Events in the MOA were conducted using thyroid tissue or plasma from orally dosed animals and the thyroid hormones measured are specific to the thyroid. Therefore, this MOA is considered to have adequate strength, consistency, and specificity.

vi. Biological plausibility and coherence

The proposed MOA is considered biologically plausible and coherent, as it is a known MOA for thyroid tumorigenesis in rodents.

vii. Alternative MOAs

There are two alternative MOAs for thyroid tumors. The first is genotoxicity leading to thyroid tumors. There is no evidence of genotoxicity in the fluxapyroxad database. Therefore, any genotoxic MOA would have to be via an ROS-mediated mechansim associated with direct ROS generation by the a.i. or from inflammation. There is no evidence that fluxapyroxad directly generates ROS and there is no evidence of inflammatory effects in the thyroid. The second alternative MOA is direct action of fluxapyroxad on the thyroid to modulate thyroid hormone biosynthesis. However, the results for the perchlorate discharge assay rule out direct effects of fluxapyroxad on the thyroid.

i. Human relevance

While the established mode of action of fluxapyroxad for follicular thyroid carcinogenesis in animals is qualitatively plausible in humans, it is quantitatively implausible based on differences in thyroid physiology (also reflected in differences in incidence data) between rats and humans. "*Quantitatively*, if humans develop cancer through thyroid-pituitary disruption, it appears that humans are less sensitive to the carcinogenic effects than are rodents. Rodents show significant increases in cancer with thyroid-pituitary disruption; humans show little, if any" (USEPA 1998).

i. Conclusion

There is sufficient evidence with dose/time concordance to support a non-genotoxic MOA for thyroid follicular adenomas. This MOA is based on early changes in liver enzyme regulation that lead to dysregulation of thyroid hormone homeostasis, thyroid follicular hypertrophy/hyperplasia, and thyroid follicular adenoma formation. The endpoint of changes in liver enzyme regulation provides a clear threshold for thyroid tumors.

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

The Committee considered the following for a weight-of-evidence determination of the carcinogenic potential of fluxapyroxad.

1. Carcinogenicity

Rat

• *Liver Tumors*: Male Wistar rats had statistically significant trends for liver adenomas ($p<0.01$), carcinomas ($p<0.05$), and combined adenomas and/or carcinomas ($p < 0.01$). There were significant pair-wise comparisons of the 3000 ppm dose group with the controls for liver adenomas, carcinomas and combined adenomas and/or carcinomas, all at $p < 0.01$. There were also significant pair-wise comparisons of the 1500 ppm dose group with the controls for liver adenomas and combined adenomas and/or carcinomas, both at $p < 0.01$. The concurrent control incidences for the liver tumors were within the historical control ranges. The incidences of liver adenomas and combined at doses of ≥250 ppm exceeded the historical control ranges for these tumors.

Female rats had statistically significant trends for liver adenomas and combined adenomas and/or carcinomas, both at $p < 0.01$. There were significant pair-wise comparisons of the 3000 ppm dose group with the controls for liver adenomas at $p < 0.01$ and for liver combined adenomas and/or carcinomas at $p < 0.05$. The concurrent control incidences for the liver tumors were within the historical control ranges. The incidences of liver adenomas and combined at doses of ≥250 ppm exceeded the historical control ranges for these tumors.

While not statistically significant, the CARC also considered the liver tumors in males at 250 ppm and in females at 1500 ppm to be treatment-related and biologically relevant. The liver tumors in both sexes are supported by precursor non-neoplastic lesions (i.e., dose-related increase in liver hypertrophy in both sexes and foci in males).

The CARC considered the liver tumors to be treatment-related in male rats at doses of ≥250 ppm (11 mg/kg/day) and in female rats at doses of ≥1500 ppm (82 mg/kg/day).

• *Thyroid Follicular Cell Tumors*: Male rats had statistically significant trends for thyroid follicular cell adenomas ($p<0.01$), carcinomas ($p<0.05$), and combined adenomas and/or carcinomas ($p < 0.01$). There were significant pair-wise comparisons of the 3000 ppm dose group with the controls for thyroid follicular cell combined adenomas and/or carcinomas at $p < 0.05$. The concurrent control incidences for the thyroid follicular cell tumors were within the historical control ranges. While not statistically significant, the CARC also considered the tumors at 1500 ppm to be treatment-related and biologically relevant. The thyroid follicular cell tumors in male rats are supported by precursor non-neoplastic lesions (i.e., dose-related increase in thyroid hypertrophy/hyperplasia). **The CARC considered the thyroid follicular cell tumors at doses of ≥1500 ppm to be treatment-related in male rats.**

• *Adequacy of Dosing*: Dosing at the high dose of 3000 ppm in male and female rats was considered adequate, but not excessive, for assessing carcinogenicity. This was based on decreases in body weights in both sexes (more pronounced in females), hematology and clinical chemistry changes, and non-neoplastic lesions in the liver and thyroid.

Mouse

• There were no treatment-related increases in tumors in either male or female mice.

• *Adequacy of Dosing*: The high dose of 3000 ppm was considered to be adequate, but not excessive, to assess carcinogenicity in both sexes of mice since the high dose in this study was the limit dose (996/1307 mg/kg/day in males/females). Decreases in absolute body weights in males at the limit dose did not exceed 13% during the study and did not exceed 8% at study termination. No other adverse effects were observed.

2. Mutagenicity: There is no concern for mutagenicity.

3. Structure Activity relationship: Fluxapyroxad is a second-generation carboxamide of the pyrazole-carboxamide class. Other members of this class include bixafen, isopyrazam, and sedaxane. Isopyrazam caused thyroid follicular cell tumors in male rats, and liver and uterine tumors in female rats. Sedaxane caused liver adenomas and thyroid follicular cell tumors in male rats and uterine tumors in female rats. Data to support a MOA for liver and thyroid tumors were not provided for either of these chemicals. There is potential SAR concern for the trifluoro-2-aminobiphenyl moiety in fluxapyroxad because 2-aminobipenyl is an isomer of the well known human carcinogen, 4-aminobiphenyl. 3',4',5'-Trifluoro-2-aminobiphenyl is predicted to be a potential carcinogen by EPA's OncoLogic cancer expert system, although its potency is expected to be substantially lower than that of 4-aminobiphenyl. This is because the 2-position is not as favorable for metabolic activation of the amino group as the 4-position. 3',4',5'-Trifluoro-2-aminobiphenyl may be a potential degradation product or metabolite of fluxapyroxad via cleavage of the amide bond but there was no report of its presence in the submitter's rodent metabolism data.

4. Mode of Action: The CARC concluded that evidence with dose/time concordance was sufficient to support a mitogenic MOA for liver adenomas and carcinomas based on early changes in liver enzyme regulation and increased cell proliferation that lead to adenoma and then carcinoma formation. The CARC also concluded that there was sufficient evidence with dose/time concordance to support a non-genotoxic MOA for thyroid follicular adenomas based on early changes in liver enzyme regulation that lead to dysregulation of thyroid hormone homeostasis, thyroid follicular hypertrophy/hyperplasia, and thyroid follicular adenoma formation. The endpoint of changes in liver enzyme regulation provided a clear threshold for both of these tumors.

VI. CLASSIFICATION OF CARCINOGENIC POTENTIAL

In accordance with the EPA's Final Guidelines for Carcinogen Risk Assessment (March, 2005), the CARC classified fluxapyroxad as "Not likely to be Carcinogenic to Humans" based on convincing evidence that carcinogenic effects are not likely below a defined dose range. This decision was based on the following considerations:

(i) No treatment-related tumors were seen in male or female mice when tested at doses that were adequate to assess carcinogenicity (including the Limit Dose);

(ii) Treatment-related liver tumors were seen in male rats at doses $\geq$ 250 ppm (11 mg/kg/day) and in female rats at doses $\geq$ 1500 ppm (82 mg/kg/day);

(iii) Treatment-related thyroid follicular cell tumors were seen in male rats only at doses $\geq$ 1500 ppm;

(iv) There is no mutagenicity concern from *in vivo* or *in vitro* assays;

(v) The hypothesized mode of action (i.e, a non-genotoxic) for each tumor type (i.e., the liver and thyroid) was supported by adequate studies that clearly identified the sequence of key events, dose-response concordance and temporal relationship to the tumor types. The mode of action met the criteria established by the Agency.

VII. QUANTIFICATION OF CARCINOGENIC POTENTIAL

The available data indicates the following: 1) a threshold of 250 ppm (11 mg/kg/day) for tumorigenesis; 2) provides sufficient evidence to support modes of action for all tumor types; 3) no concern for mutagenicity; and 4) a clear point of departure (POD) of 50 ppm (2.1 mg/kg/day), which is not expected to increase cell division, alter thyroid hormone homeostasis, or result in liver or thyroid tumors. Therefore, the Agency has determined that the quantification of risk using a non-linear approach (i.e., RfD) will adequately account for all chronic toxicity, including carcinogenicity, that could result from exposure to fluxapyroxad.

VIII. BIBLIOGRAPHY

47923567 Kamp, H.; Strauss, V.; Groeter, S.; et al. (2009) BAS 700 F: Repeated Dose 90-Day Oral Toxicity Study in Wistar Rats: Administration in the Diet. Project Number: 2007/1005069/OCR, 50C0683/05064/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 448 p.

47923568 Kamp, H.; Strauss, V.; Groeters, S.; et al. (2009) BAS 700 F: Repeated Dose 90-Day Oral Toxicity Study in C57BL/6 J Rj Mice Administration in the Diet. Project Number: 2007/1018641/OCR, 51C0683/05070/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 292 p.

47923572 Schulz, M.; Landsiedel, R. (2008) BAS 700 F: Salmonella typhimurium/Escherichia coli Reverse Mutation Assay (Standard Plate Test and Preincubation Test). Project Number: 2008/1028479/OCR, 40M0683/054184/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 52 p.

47923573 Schulz, M.; Landsiedel, R. (2009) BAS 700 F: Salmonella typhimurium / Escherichia coli Reverse Mutation Assay (Standard Plate Test and Preincubation Test). Project Number: 2009/1080768/OCR, 40M0683/054204/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 52 p.

47923577 Schulz, M.; Landsiedel, R. (2008) BAS 700 F: In vitro Chromosome Aberration Assay in V79 Cells. Project Number: 2007/1023153/OCR, 32M0683/054166/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 92 p.

47923580 Schulz, M.; Landsiedel, R. (2009) BAS 700 F: In Vitro Gene Mutation Test in CHO Cells (HPRT Locus Assay). Project Number: 2009/1078663/OCR, 50M0683/054205/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 57 p.

47923584 Schulz, M.; Landsiedel, R. (2006) Cytogenetic Study in vivo with LS 5094351 in the Mouse Micronucleus Test After Two Oral Administrations. Project Number: 2006/1032708/OCR, 26M0683/054160/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 51 p.

47923591 Buessen, R.; Strauss, V.; Groeters, A.; et al. (2009) BAS 700 F: Combined Chronic Toxicity/Carcinogenicity Study in Wistar Rats: Administration Via the Diet up to 24 Months. Project Number: 2009/1072490/OCR, 80C0683/05071/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Labor fuer Oekotoxicologie. 1779 p.

47923592 Buesen, R.; Strauss, V.; Kuettler, K.; et al. (2010) BAS 700 F: Carcinogenicity Study in C57BL/6 J Rj Mice: Administration Via the Diet Over 18 Months (Including Amendment No. 1). Project Number: 2010/7003500/OCR, 87C0683/05082/OCR.

Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 2182 p.

47923590 Schulz, M.; Landsiedel, R. (2009) BAS 700 F: In Vivo Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes. Project Number: 2009/1078661/OCR, 80M0683/054198/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 49 p.

47923593 Buessen, R.; Strauss, V; Fabian, E.; et al. (2009) BAS 700 F: Enzyme Induction in Liver of Wistar Rats: Administration in the Diet Over 2 Weeks and Recovery Period of About 4 Weeks. Project Number: 2009/1072495/OCR, 48C0683/05098/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 228 p.

47923594 Buesen, R.; Strauss, V.; Fabian, E.; et al. (2009) BAS 700 F: Thyroid Hormone Study: Repeated Dose Oral Toxicity Study in Wistar Rats: Administration Via the Diet for 4 Weeks. Project Number: 2009/1072497/OCR, 48C0683/05115/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 279 p.

47923595 Buesen, R.; Fabian, E.; Ravenzwaay, B. (2009) BAS 700 F: Thyroid Function Test in Wistar Rats Using Perchlorate Discharge as a Diagnostic Test: Administration Via the Diet Over 2 Weeks. Project Number: 2009/1072501/OCR, 48C0683/05110/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 103 p.

47923596 Buesen, R.; Kauffmann, W.; Fabian, E.; et al. (2010) BAS 700 F: S-Phase Response Study in Wistar Rats Administration in the Diet for 7, 28 and 91 Days. Project Number: 2009/1072498/OCR, 40C0683/05099/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 425 p.

47923597 Buesen, R.; Kaufmann, W.; Fabian, E.; et al. (2010) BAS 700 F: S-Phase Response Study in Wistar Rats: Administration in the Diet for 7, 28 and 91 Days. Project Number: 2009/1072499/OCR, 40C0683/05111/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 207 p.

47923598 Buesen, R.; Kaufmann, W.; Fabian, E.; et al. (2010) BAS 700 F: S-Phase Response Study in Wistar Rats: Administration via the Diet for 1, 3, 7 and 14 Days. Project Number: 2009/1072500/OCR, 40C0683/05114/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 502 p.

47923599 Buesen, R.; Fabian, E.; Groeters, S.; et al. (2010) BAS 700 F: Enzyme Induction in Liver of Wistar Rats: Administration in the Diet Over 2 Weeks. Project Number: 2009/1072496/OCR, 48C0683/05113/OCR. Unpublished study prepared by BASF Aktiengesellschaft, Experimental Toxicology and Ecology. 155 p.

Memo D376616, TXR 0055752, Memorandum from L. Brunsman to J. Kidwell (March 18, 2011).

USEPA. 1998. EPA/630/R-97/002. Assessment of Thyroid Follicular Cell Tumors. Washington, DC.

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