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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Isoxaflutole Qualitative Risk Assessment Based On CD-1 Mouse and CD(SD)BR VAF Plus Rat Dietary Studies

P.C. Code 123000

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Background

An oncogenicity study with Isoxaflutole in CD-1 mice was conducted by Pharmaco LSR, Eye, Suffolk, England, for Rhone-Poulenc Agriculture Limited, Ongar Research Station, Ongar, Essex, England, and issued October 16, 1995 (LSR Report No. 95/RHA509/0343; MRID No. 439048-07).

The study design allocated groups of 52 mice per sex to dose levels of 0, 25, 500, or 7000 ppm (0, 3.2, 64.4, or 977.3 mg/kg/day for males; 0, 4.0, 77.9, or 1161.1 mg/kg/day for females) of Isoxaflutole for 79 weeks. An additional 12 mice per sex per dose were designated for interim sacrifice at week 53. Twelve more mice per sex were designated for interim sacrifice at week 27 in the control and 7000 ppm dose groups.

A chronic oral toxicity and oncogenicity study with Isoxaflutole in CD(SD)BR VAF Plus rats was conducted by Life Science Research Limited, Eye, Suffolk, England, for Rhone-Poulenc Agriculture Limited, Ongar Research Station, Ongar, Essex, England, and issued October 27, 1995 (LSR Report No. 95/0499; MRID No. 439048-06).

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The study design allocated groups of 75 rats per sex to dose levels of 0, 0.5, 2, 20, or 500 mg/kg/day of Isoxaflutole for 105 weeks. An additional 10 rats per sex per dose were designated for interim sacrifice at week 53. An additional 10 rats per sex per dose were designated for a reversibility study in which the rats were dosed for 52 weeks, then held for an additional 8 week recovery phase, after which they were sacrificed. The animals of the reversibility study are not included in this analysis.

Survival Analyses

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Isoxaflutole in male or female mice. However, there were significant decreasing trends for mortality with increasing doses of Isoxaflutole in both male and female rats. See Tables 1 and 2 for mouse mortality test results, and Tables 6 and 7 for rat mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analyses

Two tables of statistical analyses for male mice have been provided, one excluding interim sacrifice animals, as is standard procedure, and one including ONLY 53-week interim sacrifice animals. This was done to illustrate the statistical significance at the 53-week interim sacrifice. Male mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 977.3 mg/kg/day dose group with the controls, for liver adenomas, carcinomas, and adenomas and/or carcinomas combined, all at $p < 0.01$. There were also significant increasing trends at $p < 0.01$, and significant differences in the pair-wise comparisons of the 977.3 mg/kg/day dose group with the controls at $p < 0.05$, for liver adenomas, and adenomas and/or carcinomas combined of the 53-week interim sacrifice group.

Female mice had significant increasing trends, and significant differences in the pair-wise comparisons of the 1161.1 mg/kg/day dose group with the controls, for liver adenomas, and adenomas and/or carcinomas combined, all at $p < 0.01$. There was also a significant increasing trend in liver carcinomas at $p < 0.01$.

Male rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 500 mg/kg/day dose group with the controls, for liver adenomas, and adenomas and/or carcinomas combined, and thyroid follicular cell adenomas and adenomas and/or carcinomas combined, all at $p < 0.01$. There was a

significant increasing trend at $p < 0.01$, and a significant difference in the pair-wise comparison of the 500 mg/kg/day dose group with the controls at $p < 0.05$, for liver carcinomas. There was also a significant difference in the pair-wise comparison of the 500 mg/kg/day dose group with the controls for thyroid follicular cell carcinomas at $p < 0.05$.

Female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 500 mg/kg/day dose group with the controls, for liver adenomas, carcinomas, and adenomas and/or carcinomas combined, all at $p < 0.01$

The statistical analyses of the mice were based upon the Exact trend test and the Fisher's Exact test for pair-wise comparisons. The statistical analyses of the male and female rats were based upon Peto's Prevalence Test since there were statistically significant negative trends for mortality with increasing doses of Isoxaflutole in both sexes. See Tables 3, 4 and 5 for mouse tumor analysis results. See Tables 8, 9 and 10 for rat tumor analysis results.

Table 1. Isoxaflutole - CD-1 Mouse Study

Male Mortality Rates* and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	Weeks 1-26	27[i]	27-52	53[i]	53-79[f]	Total
0	0/76	12/76	6/64	12/58	9/46	15/52 (29)
3.2	0/64	0/64	7/64	11/57	15/46	22/53 (42)
64.4	3/64	0/61	1/61	12/60	11/48	15/52 (29)
977.3	0/76	12/76	4/64	12/60	12/48	16/52 (31)

*Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifices at weeks 27 and 53.

[f]Final sacrifice at week 79.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Isoxaflutole - CD-1 Mouse Study

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

Dose (mg/kg/day)	Weeks 1-26	27[i]	27-52	53[i]	53-79[f]	Total
0	0/76	12/76	1/64	12/63	8/51	9/52 (17)
4.0	0/64	0/64	2/64	11/62	12/51	14/53 (26)
77.9	1/64	0/63	4/63	11/59	9/48	14/53 (26)
1161.1	0/76	12/76	1/64	12/63	5/51	6/52 (12)

[*]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifices at weeks 27 and 53.

[f]Final sacrifice at week 79.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 3. Isoxaflutole - CD-1 Mouse Study

Male Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values) EXCLUDING 53-Week Interim Sacrifice Animals

	Dose (mg/kg/day)			
	0	3.2	64.4	977.3
Adenomas (%)	9/47 (19)	10/50 (20)	9[a]/48 (19)	27/49 (55)
p =	0.000**	0.560	0.584	0.000**
Carcinomas (%)	4/47 (9)	5/50 (10)	8/48 (17)	17/49 (35)
p =	0.000**	0.540	0.188	0.002**
Combined (%)	13/47 (28)	15/50 (30)	14[c]/48 (29)	38[d]/49 (78)
p =	0.000**	0.488	0.526	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 47. Also excludes week 27 and week 53 interim sacrifice animals.

[a]First liver adenoma, excluding 53-week interim sacrifice animals, observed at week 55, dose 64.4 mg/kg/day.

[b]First liver carcinoma observed at week 47, dose 977.3 mg/kg/day.

[c]Three animals in the 64.4 mg/kg/day dose group had both an adenoma and a carcinoma.

[d]Six animals in the 977.3 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. See Table 4 for separate analysis of 53-week interim sacrifice animals.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 4. Isoxaflutole - CD-1 Mouse Study

Male Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values) 53-Week Interim Sacrifice Animals ONLY

	Dose (mg/kg/day)			
	0	3.2	64.4	977.3
Adenomas (%)	2/12 (17)	1/11 (9)	0/12 (0)	7/12 (58)
p =	0.001**	0.534[n]	0.239[n]	0.045*
Carcinomas (%)	0/12 (0)	0/11 (0)	1/12 (8)	0/12 (0)
p =	0.745	1.000	0.500	1.000
Combined (%)	2/12 (17)	1/11 (9)	1/12 (8)	7/12 (58)
p =	0.002**	0.534[n]	0.500[n]	0.045*

[+]Number of tumor bearing animals/Number of animals examined, including ONLY those that were sacrificed at week 53.

[n]Negative change from control.

Note: ONLY 53-week interim sacrifice animals are included in this analysis.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Isoxaflutole - CD-1 Mouse Study

Female Liver Tumor Rates[+] and Exact Trend Test and Fisher's Exact Test Results (p values) EXCLUDING 53-Week Interim Sacrifice Animals

	Dose (mg/kg/day)			
	0	4.0	77.9	1161.1
Adenomas (%)	0/51 (0)	1/50 (2)	1/48 (2)	15[a]/51 (29)
p =	0.000**	0.495	0.485	0.000**
Carcinomas (%)	0/51 (0)	0/50 (0)	0/48 (0)	4[b]/51 (8)
p =	0.004**	1.000	1.000	0.059
Combined (%)	0/51 (0)	1/50 (2)	1/48 (2)	18[c]/51 (35)
p =	0.000**	0.495	0.485	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before week 54. Also excludes week 27 and week 53 interim sacrifice animals.

[a]First liver adenoma observed at week 77, dose 1161.1 mg/kg/day.

[b]First liver carcinoma observed at week 60, dose 1161.1 mg/kg/day.

[c]One animal in the 1161.1 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 6: Isoxaflutole - CD(SD)BR VAF Plus Rat Study

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

Dose (mg/kg/day)	Weeks 1-26	27-52	53[i]	53-78	79-106[f]	Total
0	1/85	1/84	10/83	12/73	27/61	41/75 (55)**[n]
0.5	1/85	1/84	10/83	16/73	32/57	50/75 (67)
2.0	1/85	1/84	10/83	6/73	33/67	41/75 (55)
20.0	0/85	3/85	10/82	6/72	29/66	38/75 (51)
500.0	2/85	0/83	10/83	4/73	23/69	29/75 (39)*[n]

[t]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

[n]Negative trend or negative change from control.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 7. Isoxaflutole - CD(SD)BR VAF Plus Rat Study

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

Dose (mg/kg/day)	Weeks 1-26	27-52	53[i]	53-78	79-105[f]	Total
0	1/82	0/81	7/81	12/74	27/62	40/75 (53)[**n]
0.5	0/84	2/84	9/82	17/73	33/56	52/75 (69)[*]
2.0	1/85	2/84	10/82	14/72	33/58	50/75 (67)
20.0	0/85	4/85	10/81	20/71	29/51	53/75 (71)[*]
500.0	0/85	1/85	10/84	7/74	19/67	27/75 (36)[*n]

[+]Number of animals that died during interval/Number of animals alive at the beginning of the interval.

[i]Interim sacrifice at week 53.

[f]Final sacrifice at week 105.

[n]Negative trend or negative change from control.

( )Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 8. Isoxaflutole - CD(SD)BR VAF Plus Rat Study

Male Liver Tumor Rates[†] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)				
	0	0.5	2.0	20.0	500.0
Adenomas (%)	2/41 (5)	3/42 (7)	5/49 (10)	6[a]/46 (13)	14/54 (26)
p =	0.001**	0.204	0.117	0.076	0.004**
Carcinomas (%)	5[b]/58 (9)	1/53 (2)	4/62 (6)	2/64 (3)	17/68 (25)
p =	0.000**	-	-	-	0.011*
Combined (%)	7/58 (12)	4/53 (8)	8[c]/62 (13)	8/64 (12)	31/68 (46)
p =	0.000**	-	0.457	0.494	0.000**

[†]Number of tumor bearing animals/Number of animals examined, excluding those that died before the observation of the first tumor; also excludes week 53 interim sacrifice animals.

[a]First liver adenoma observed at week 53, dose 500.0 mg/kg/day, in an interim sacrifice animal. Second liver adenoma observed at week 97, dose 20.0 mg/kg/day, in an animal that died on study.

[b]First liver carcinoma observed at week 85, dose 0 mg/kg/day, in an animal that died on study.

[c]One animal in the 2.0 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There was one liver adenoma in the interim sacrifice group at the 500.0 mg/kg/day dose.
Significance of trend denoted at control.
Significance of pair-wise comparison with control denoted at dose level.
If *, then $p < 0.05$. If **, then $p < 0.01$.

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Table 9. Isoxaflutole - CD(SD)BR VAF Plus Rat Study

Male Thyroid Follicular Cell Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)				
	0	0.5	2.0	20.0	500.0
Adenomas (%)	3/66 (5)	1/60 (2)	5[a]/69 (7)	7/68 (10)	15/69 (22)
p =	0.000**	-	0.271	0.127	0.005**
Carcinomas (%)	0/53 (0)	1/46 (2)	2/59 (3)	1/58 (2)	3[b]/62 (5)
p =	0.113	0.117	0.159	0.169	0.042*
Combined (%)	3/66 (5)	2/60 (3)	7/69 (10)	8/68 (12)	17[c]/69 (25)
p =	0.000**	-	0.120	0.081	0.002**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before the observation of the first tumor; also excludes week 53 interim sacrifice animals.

[a]First thyroid follicular cell adenoma observed at week 70, dose 2.0 mg/kg/day.

[b]First thyroid follicular cell carcinoma observed at week 91, dose 500.0 mg/kg/day.

[c]One animal in the 500.0 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no thyroid follicular cell tumors in any interim sacrifice animals.

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

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Table 10. Isoxaflutole - CD(SD)BR VAF Plus Rat Study

Female Liver Tumor Rates[+] and Peto's Prevalence Test Results (p values)

	Dose (mg/kg/day)				
	0	0.5	2.0	20.0	500.0
Adenomas (%)	4/66 (6)	2/59 (3)	1/60 (2)	0/55 (0)	29[a]/69 (42)
p =	0.000**	-	-	-	0.000**
Carcinomas (%)	0/70 (0)	0/71 (0)	1/69 (1)	0/66 (0)	24[b]/73 (33)
p =	0.000**	-	0.118	-	0.000**
Combined (%)	4/70 (6)	2/71 (3)	2/69 (3)	0/66 (0)	46[c]/73 (63)
p =	0.000**	-	-	-	0.000**

[+]Number of tumor bearing animals/Number of animals examined, excluding those that died before the observation of the first tumor.

[a]First liver adenoma observed at week 76, dose 500.0 mg/kg/day.

[b]First liver carcinoma observed at week 61, dose 500.0 mg/kg/day.

[c]Seven animals in the 500.0 mg/kg/day dose group had both an adenoma and a carcinoma.

Note: Interim sacrifice animals are not included in this analysis. There were no liver tumors in any interim sacrifice animals.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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