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

JUL 27 1988

MEMORANDUM

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Rotenone  
FROM: Esther Rinde, Ph.D. *Esther Rinde 5/31/88*  
Scientific Mission Support Staff (TS-769c)  
TO: William Miller  
Product Manager #16  
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on Dec. 17, 1987 to discuss and evaluate the weight-of-the-evidence on Rotenone with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

William L. Burnam  
Robert Beliles  
Reto Engler  
Marion Copley  
Kerry Dearfield  
Judith Hauswirth  
Richard Levy  
Jack Quest  
Esther Rinde

*William L. Burnam*  
*Robert Beliles*  
*Reto Engler*  
*Marion Copley*  
*Kerry Dearfield*  
*Judith P. Hauswirth*  
*Richard Levy*  
*Jack A. Quest*  
*Esther Rinde*



D. Evaluation of Oncogenicity Evidence for Rotenone:

1. Rat - 2 year NTP Oncogenicity Study  
Testing Facility: Battelle Columbus Laboratories

Reference: Abdo, K. (1983) NIH Publication No. 86-2576;  
MRID # 40179801

Rotenone (Technical) was administered in the diet to groups of 50 male and 50 female F344/N rats at 0, 38 or 75 ppm for 2 years. Rats were housed five per cage.

The incidence of parathyroid adenomas in male rats at 75 ppm (HDT) was increased relative to concurrent controls: 4/44 (9.1%) vs 1/41 (2.4%) in control. The increase was not statistically significant; however, the incidence in treated rats greatly exceeded that in historical controls: 4/1314 (0.3%) with a range of 0/70 to 1/38 (2.6%); the concurrent control incidence was within this range.

Parathyroid Adenoma Incidence (%)			
	Dose		
	0	38	75 ppm
	1/41 (2.4)	0/44	4/44 (9)

Lesions were observed microscopically only (not grossly).

In female rats there was a statistically significant increase in combined subcutaneous tissue tumors at 38 ppm (5/50 vs 0/50 in controls, at  $p=0.013$  by Fisher Exact Test), which was not dose related (Table 1).

The NTP investigators concluded that the parathyroid tumors in the male rats may be related to administration of Rotenone; however, for the subcutaneous tumors of the female rats the evidence was equivocal "... because of a lack of a significant dose-related trend ... and because statistical significance was attained (only) by combining tumors of differing morphology ...".

The only non-neoplastic lesion reported to be significantly increased was focal hyperplasia in the anterior pituitary gland in high dose male rats (there were no increases in tumors at this site, however). There was a non-statistically significant increase in parathyroid hyperplasia at 75 ppm (9% vs 2%, in controls).

Mortality in treated rats was comparable to that of controls.

The Committee agreed that a dose level of 75 ppm (HDT) was adequate to evaluate the oncogenic potential of Rotenone, based on decreases in body weight gain seen at that dose in the 90 day study (8% in males, 13% in females).

Table 1

Summary of the incidences for subcutaneous tissue tumors reported to be statistically significantly increased in a treatment group.

<u>Observation</u>	<u>Dose (ppm)</u>		
	<u>0</u>	<u>38</u>	<u>75</u>
Fibromas			
Overall incidence	0/50	1/50	0/50
Neurofibroma			
Overall incidence	0/50	1/50	0/50
Sarcoma			
Overall incidence	0/50	1/50	1/50
Fibrosarcoma			
Overall incidence	0/50	1/50	2/50
Myxosarcoma			
Overall incidence	0/50	1/50	0/50
Combined incidence			
Intercurrent deaths	0/23	3/18	3/19
Terminal sacrifice	0/27	2/32	0/31
Overall	0/50	5/50*	3/50
<u>Week of first observation</u>	—	64	77

\*Statistically significantly different from controls ( $p=0.049$  in life table tests;  $p=0.013$  by Fisher Exact Test).

D. 2. Rat - 2 Year Hazleton Oncogenicity Study  
Testing Facility: Hazleton Labs. America, Inc.;  
Madison, Wisconsin

Reference: Tisdell, M. (1985) Study #6115-100;  
MRID #00156739

Rotenone (Technical) was administered in the diet to 40 male and 40 female Charles River Fisher 344 (CDG<sup>R</sup> F-344/Crl Br) rats for 2 years, at 0, 7.5, 37.5 or 75 ppm. Rats were individually caged.

There were no significant increases of any neoplastic lesions reported for either sex; however, the incidences of pituitary adenomas and mononuclear cell leukemia were decreased in both sexes.

Treatment-related decreases (small in magnitude) in blood total protein and albumin levels at 75 ppm (HDT) and increases in serum urea nitrogen levels at 37.5 and 75 ppm were observed in females; these were not considered to be biologically relevant.

A significant dose-related decrease in food consumption in mid- and high-dose group female rats was also noted, which correlated with lower terminal body weight of these rats, as did reduced organ weights and changes in organ-to-body weight ratios; decreases in food consumption also correlated (inversely) with increases in serum urea nitrogen. These changes suggest a nutritional imbalance which could be related to the reduced food consumption<sup>1</sup>.

Non-neoplastic: Increased incidences of angiectasis and hemorrhage in adrenals of high-dose males and females and increased mineralization of the kidneys in high dose females; other non-neoplastic lesions (chronic progressive nephropathy and hepatocellular degeneration) were decreased in females at the HDT (75 ppm). The significance of the adrenal lesions could not be determined because the investigators did not include histopathology for the low and mid-dose groups.

The highest dose level of 75 ppm was adequate to evaluate the oncogenic potential of Rotenone, based on decreases in body weight gain seen at that dose in an NTP 90 day study (8% in males, 13% in females).

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<sup>1</sup> Decreased food consumption was not reported in male rats of this study; in either male or female rats in the NTP study, or in the mouse or hamster studies.

D. 3. Mouse - 2 year NTP Oncogenicity Study  
Facility: Battelle Columbus Labs.

Reference: Abdo, K. (1983) NIH Publication #86-2576;  
MRID #40179801.

Rotenone (Technical) was administered in the diet to groups of 50 male and 50 female B6C3F1 mice at 0,600 or 1200 ppm for 2 years.

In male mice there were statistically significant decreases in hepatocellular carcinoma, adenoma and combined carcinoma/adenoma at 1200 ppm (HDT) at  $p < .01$ . In female mice, no significant increases or decreases in tumor incidence were reported (Table 2).

There were no significant increases or decreases in non-neoplastic lesions in either sex.

Mortality in treated male mice decreased with dose; no significant effects on mortality were noted in females.

The Committee concluded that the study may not have been conducted at a high enough dose for evaluating the oncogenic potential of Rotenone in either sex, based on the absence of any non-neoplastic lesions or clinical signs of toxicity, and a weight gain decrement that was not dose-related. It was however pointed out that the HDT (1200 ppm) was more than 1/2 of the MTD (1900 ppm) as determined from the subchronic study, based on body weight decrements of >20% at that dose.

Since it has been shown that Rotenone inhibits the mono function oxidase (MFO) system in fish, it was suggested that the decreased incidence of tumors in treated mice (compared to controls), might be explained by this mechanism. That is, if traces of carcinogen requiring activation by MFO were in the laboratory diet (and contributing to the background tumor incidence in controls), in the Rotenone-treated mice there might be an inhibitory effect and thus, the lower incidence. However, it has not been demonstrated that Rotenone inhibits MFO in the rodent, nor could other mechanisms be ruled out.

Table 2

Summary of the incidences of liver tumors reported to be associated with administration of rotenone\*  
In the B6C3F1 Male Mouse

<u>Observation</u>	<u>Dose (ppm)</u>		
	<u>0</u>	<u>600</u>	<u>1200</u>
Hepatocellular Adenomas			
Intercurrent deaths	2/13	2/13	0/3
Terminal sacrifice	5/29	7/36	1/47
Overall	7/47†	9/49	1/50††
Week of first observation	89	88	104
Hepatocellular Carcinoma			
Intercurrent deaths	2/13	1/13	0/3
Terminal sacrifice	4/29	2/36	0/47
Overall	6/47*	3/49	0/50**
Week of first observation	76	87	---
Hepatocellular Adenomas and Carcinoma			
Intercurrent deaths	4/13	3/13	0/3
Terminal sacrifice	8/29	9/36	1/47
Overall	12/47†	12/49	1/50††
<u>Week of first observation</u>	<u>76</u>	<u>87</u>	<u>---</u>

†Statistically significant negative trend (p<0.001; life table analysis)

††Statistically significantly decreased incidence (p<0.001; pairwise comparison, life table analysis)

\*Statistically significant negative trend (p=0.002; life table analysis)

††Statistically significantly decreased incidence (p<0.002; pairwise comparison, life table analysis)

There were no significant increases or decreases in the incidences of tumors in female mice given rotenone in the diet according to the report.

D. 4. 18 Month Hamster Study  
Facility: Battelle's Columbus Lab.

Reference: Leber, A. and Persing, R. (1979) NTIS  
EPA 600/1-79-04a; MRID 00143256

Rotenone (95% pure) was fed in the diet at 0, 125, 250, 500 or 1000 ppm to groups of 50 male and 50 female Syrian Golden Hamsters for 18 months.

In both sexes, there was an increase in the incidence of adrenal cortical carcinoma at 1000 ppm (HDT). In females there was also an increase in adenoma at 125 ppm. The incidence of hyperplasia was increased only in males.

These increases were neither statistically significant by pair-wise comparison, nor were there any statistically significant trends for any of the lesions.

		Incidence (%) of Adrenal Lesions <sup>1</sup>		
		Dose		
		0	125	1000 ppm
Cortical Cell:				
Adenoma	M	8/30 (27)	8/32 (25)	8/32 (25)
	F	5/27 (19)	7/26 (27)	6/33 (18)
Carcinoma	M	0/30	0/32	1/32 (3)
	F	0/27	0/26	2/33 (6)
Hyperplasia	M	11/30 (37)	15/32 (47)	19/32 (59)
	F	13/27 (48)	12/26 (46)	15/33 (45)

<sup>1</sup>Overall incidence, including animals which were sacrificed or died during the study.

No histopathology was submitted for hamsters fed 250 or 500 ppm. Individual animal or historical data were not available.

There was high mortality in female controls during the final 5 months of the study which may have been related to enteric infections, which were prevalent in this group early in the study. Results in females were thus considered to be compromised. Mortality in males appeared to be comparable in each group.



E. Additional Toxicology Data on Rotenone:

1. Metabolism

In a series of experiments in the rat with single or repeated low dose (.01 mg/kg) and single high dose oral (5 mg/kg), and a single low IV dose (.01 mg/kg), almost all of the Rotenone was excreted in the feces with 80-90% of the administered label recovered within 48 hours after dosing. The route of dosing had no apparent effect on excretion and females excreted label at a slower rate than did males. Enterohepatic circulation is suggested from the IV dosing experiments. Metabolites in feces were characterized as polar compounds (not conjugated with aryl sulfatase or glucuronide) but were not specifically identified.

2. Mutagenicity:

Rotenone was negative in *Salmonella typhimurium*, both with and without activation.

Rotenone was negative in *Saccharomyces cerevisiae* for mutation, mitotic recombination and mitotic gene conversion, both with and without activation.

In the Mouse Spot Test, Rotenone was negative: there was no toxicity or somatic mutations in embryonic melanocytes of pregnant mice at 0.05-1 mg/kg. There were also no somatic mutations at a dose of 1000 mg/kg, where toxicity was demonstrated.

In the mouse lymphoma assay, there was a very large increase in mutant frequency without activation at concentrations up to 4 ug/ml (the assay was not run with activation).

A detailed report on in vitro SCE and cytogenetics has been requested from the NTP. Preliminary results indicated a negative response in both assays.

There was no available information in OPP files at this time on tests for aneuploidy or numerical chromosome aberrations.

Overall evaluation: Incomplete, based on absence of adequate data in structural chromosomal aberrations area. Mouse lymphoma results indicate a mutagenicity concern, based on the magnitude of the increase observed.

There were two in vivo structural aberration assays submitted that had been reviewed as acceptable - a rat bone marrow aberrations study, which Dr. Dearfield feels did not have acceptable levels of toxicity (none reported at HDT), and a mouse micronucleus assay, which was performed with an inadequate protocol by today's standards (negative results would give no real information). [K. Dearfield]

E. 3. Structure-Activity Correlations

A search of NLM ELHILL data bases did not identify any compounds with carcinogenic activity which are structurally related to Rotenone or its metabolites.

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Rotenone to be of importance in a weight-of-evidence determination of oncogenic potential.

I. NTP Rat Study

1. In a 2-year NTP study in the F344/N rat, Rotenone in the diet was associated with a (non-statistically significant) increased incidence of parathyroid adenomas in male rats at 75 ppm (HDT).

The incidence in treated males 4/44 (9%) vs 1/41 (2.4%) in concurrent controls, greatly exceeded that in historical controls: 4/1314 (0.3%), with a range of 0/70 to 1/38 (2.6%).

2. In female rats, there was also a statistically significant increase in combined subcutaneous tissue tumors at 38 ppm with  $p=0.013$  which was not dose-related. There was however no increase in individual tumor types (significance was only attained by combining tumors of different morphology).

3. The study was conducted at sufficiently high dose to satisfy MTD requirements; usual NTP protocol inadequacies were noted (eg: Gang-caging).

II. Hazleton Rat Study

1. In a second 2 year Hazleton Study in the F344 rat, Rotenone in the diet (administered at the same levels as in the NTP study) was not associated with statistically significant increases in any neoplastic lesions.

There were however decreases in the incidence of pituitary adenomas and mononuclear cell leukemia.

2. There was a significant dose-related decrease in food consumption in female rats, correlating with reduced organ weights and changes in organ/body weight ratios, and terminal body weights.

#### F. Weight of Evidence (continued)

The observed decrease in food consumption (which inferred reduced palatability) in this study, was not observed in the NTP study. This discrepancy could not be fully explained; however, it was suggested that since the NTP animals had been group-caged (and these were singly caged) differences in the ability to monitor food consumption may have been a factor.

3. There were reported increases in the incidences of angiectasis and hemorrhage of adrenals in both sexes of high dose animals, and increases in mineralization of kidneys in high dose females.

Significance of adrenal lesions could not be determined because histopathology for low- and mid-dose groups were not included.

4. The high dose was adequate for evaluating the oncogenic potential of Rotenone; this protocol was deemed to be better<sup>2</sup> than the NTP study; however, it was incomplete, because histopathology for low- and mid-dose group rats was missing.

#### III. NTP Study in the Mouse

1. In a 2 year NTP study in the B6C3F1 mouse, Rotenone in the diet (up to 1200 ppm) was not associated with increases of any tumor type in either sex.

2. There were however, decreases in hepatocellular carcinoma, adenoma and combined carcinoma/adenoma at 1200 ppm in male mice.

3. Mortality also decreased with dose in males.

4. The study may not have been conducted at a high enough dose, but it was conducted at a dose of more than 1/2 of the MTD predicted from the subchronic study.

#### IV. Hamster Study

1. In an 18 month study in the Syrian Golden Hamster, Rotenone in the diet at doses up to 1000 ppm, was associated (in both sexes) with a non-statistically significant increase in the incidence of adrenal cortical cell carcinoma at 1000 ppm only. In females, there was also an increase in adrenal adenoma at 125 ppm. Increases were not dose-related.

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<sup>2</sup> Hazleton caged animals individually and provided accurate records on: body and organ weights, food consumption and clinical chemistry.

F. Weight of Evidence (continued)

2. There was also a non-statistically significant increase in adrenal cortical cell hyperplasia in males.
3. Individual animal or historical data was not available and histopathology was not submitted for 2 dose groups (250 and 500 ppm).
4. It was concluded that the tumor response was neither dose- nor treatment-related. The results in females were further compromised because of high mortality in controls. The study was judged inadequate because of the poor survival, incomplete reporting of results and undetermined dose adequacy.

V. Additional Evidence

1. Evidence for mutagenicity was mainly negative or incomplete; however, a mutagenicity concern was raised by the mouse lymphoma test results.
2. There were no supporting data from structurally-related analogs.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

Committee members present at the Dec. 17 meeting could not reach a consensus as to the classification of Rotenone, nor was there a majority vote. A draft was subsequently distributed to the full Committee and comments solicited, with individual members providing a rationale for their vote. A summary for each position follows:

Category C was proposed, based on the increased incidence of parathyroid adenoma in male rats at 75 ppm, in the NTP study. The incidence in treated animals (9%) was not statistically significantly increased over that in concurrent controls (2.4%), however, it greatly exceeded the historical control incidence (0.3%) and the tumor type is considered to be rare [NTP]. Although the Hazleton study, which used the same strain of rat at the same dose levels, failed to reproduce this finding, it was pointed out that there were differences between the 2 studies in the way the animals were housed and in their food consumption (see Section F, Part II). The overall weight of evidence for this interpretation is "limited".

## G. Classification (contd.)

Category D was proposed, based on the parathyroid tumors in the NTP study, which were not statistically significantly increased; occurred in only one sex and one species; at only one dose; were benign only. It was pointed out that, since the Hazleton study did not reproduce these results, the data could not be interpreted as showing either the presence or absence of a carcinogenic effect; thus, the weight of evidence is "inadequate".

Category E was proposed, based on the lack of statistical significance in the NTP rat study; the absence of neoplastic effects at other doses, in the other sex, in other species or in other studies with the same species, and because additional evidence from mutagenicity assays or SAR was not supportive<sup>3</sup> of a higher classification. It was also argued that the parathyroid tumors are not compound-related, because if the NTP study represented the true tumor frequency (9%), the likelihood of observing an incidence of zero in the Hazleton study would be less than 3% [R. Levy]. The overall weight of evidence for this interpretation is "no evidence".

Category D received the most votes (there were 4 votes cast for Category C; 7 for Category D; and 2 for Category E). Further support for this classification was offered by Dr. Slaughter, who pointed out that the NTP historical base appears to be incomplete, since from the numbers it does not seem that a sufficient number of glands were examined (a rat has a minimum of 2 parathyroid glands). The NTP data does not provide information on how many glands were necropsied and thus the true incidence cannot be determined for either the study, or for the historical data base. Thus the data from this study cannot be interpreted as either showing the presence or absence of a tumor response.

The classification of Rotenone was determined by a majority of the Peer Review Committee to be Category D (Inadequate Evidence). In view of the strong positive response in the mouse lymphoma assay, it was recommended that an adequate rat in vivo cytogenetics assay be requested. Additional testing could also be requested, depending on these results and any other information that becomes available. It was also recognized that the classification of Rotenone may change as new data is developed.

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<sup>3</sup> There was concern raised for mutagenicity based on the strong response in the mouse lymphoma assay, which was not corroborated in any other in vitro or in vivo assays.