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

MEMORANDUM

AUG 5 1986

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Revisions to 4/11/86 Peer Review of Trifluralin

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769) *RBJ 8/5/86*

TO: Addressees

Attached please find Toxicology Branch response (7/31/86) to OGC comments regarding the Trifluralin Registration Standard and Peer Review. The revised pages 6 and 23 to the Peer Review (4/11/86) are the result of discussions between OGC (E. Gray), RD (C. Gray), SIS (J. Tice), and TB (R. Engler, W. Burnam, B. Jaeger). The revised pages 6 and 23, attached, should be inserted into your 4/11/86 copy of the Peer Review. They are non-substantial, in that they do not effect or alter the final decisions of the peer review panel. However, considering the distribution and potential dissemination of this document it is prudent to correct factual errors.

cc: T. Farber
W. Burnam
R. Engler
J. Quest
B. Fisher
L. Kasza
A. Barton
J. Tice
E. Gray
C. Gray
Caswell File #889

The information presented here in the NCI study indicate that tumors of the urinary tract occurred with similar or greater frequency in the control as the dosed groups. However, a short-coming of the data are the few number of rats examined in the treated groups which compromises any valid comparisons between the control and dose groups. The data on thyroid tumors presented below in b. are equivocal and fail to demonstrate any possible compound effect.

b. Tumors of the thyroid

| Dose (ppm) | 0 | | 3250 | | 6500 | |
|----------------|----|----|------|----|------|----|
| | M | F | M | F | M | F |
| Numbr of rats | 48 | 50 | 49 | 50 | 48 | 49 |
| Foll. Cell Ad. | 3 | 0 | 4 | 3 | 5 | 0 |
| Foll. Cell Ca. | 4 | 1 | 4 | 4 | 5 | 0 |
| | 7 | 1 | 8 | 7 | 10 | 0 |

5. Rat Oncogenicity Study of Trifluralin (Eli Lilly, 1980).

Fischer 344 rats (60 per sex per group) were administered Trifluralin in the diet for two years at concentrations of 0, 813, 3250 and 6500 ppm. The amount of NDPA present was determined to be less than 0.01 ppm.

Malignant neoplasms of the renal pelvis were increased in all dosed males, but significant in high dose only ($p < 0.05$). There was also an increase in benign urinary bladder neoplasms in mid and high dose females, and a significant ($p < 0.05$) increase in combined malignant and benign urinary bladder neoplasms in high dose females only. These data are summarized in Table 3.

H. Weight of Evidence Considerations:

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

1. Trifluralin produced an increased incidence of malignant neoplasms of the renal pelvis in all male rat dose groups, which was significant ($p < 0.05$) at the high dose.
2. Trifluralin produced a significant increase ($p < 0.05$) of combined malignant and benign urinary bladder neoplasms in high dose female rats; and an increase (not significant) of benign urinary bladder neoplasms in mid and high dose females.
3. Trifluralin produced an increase in follicular cell adenomas and carcinomas of the thyroid in male rats receiving > 3250 ppm in the diet. The increase became significant ($p < 0.05$) at the high dose.
4. Trifluralin demonstrated a species specific effect on the urothelium of the kidney and urinary bladder in rats, but not mice. Ingestion of trifluralin in the diet of rats was associated with a dose-related increase of: (a) chronic progressive nephropathy (CPN), (b) hyperplasia of the urothelium, (c) formation of microscopic renal calculi, and (d) BUN and plasma creatinine.
5. Trifluralin was not oncogenic in the B6C3F1 mouse at doses up to 4500 ppm for 2 years.
6. Trifluralin was not oncogenic in a long term Sprague-Dawley rat study at doses up to 2000 ppm.
7. There was no evidence of decrease time-to-tumor for neoplasms of the renal pelvis, urinary bladder or thyroid in the evidence examined from animals sacrificed moribund or which died prior to term.
8. There was no evidence of mutagenicity in rat dominant lethal, L5178Y mouse lymphoma, Ames Salmonella typhimurium, Saccharomyces cerevisiae, DNA repair assays, or SCE using chinese hamster cell.
9. Structure-activity relationship to other dinitroaniline pesticides demonstrated similar compound related effects on the renal pelvis and, at higher doses, effects on the thyroid and hemology. Metabolism data demonstrated a common urinary metabolite for trifluralin and ethalfluralin, which when isolated and administered separately, produced adverse effects on the urothelium and formation of calculi.



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JUL 31 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Trifluralin - Clarification of Toxicology Branch
Peer Review, dated 4/11/86.

TO: Carol V. Gray
Registration Division (TS-767C)

FROM: R. Bruce Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

RBJ 7/31/86

WJZ 7/31/86

Toxicology Branch received some comments from OGC (Ed Gray) regarding the weight-of-evidence considerations and consistency of Trifluralin oncogenic classification with other peer review documents or with Agency policy. Toxicology Branch recognizes some valid criticisms raised by OGC but reiterates its conclusion that Trifluralin be classified as a Group C - possible human carcinogen rather than a B2 - probable human carcinogen, with the stipulation that a Q* is also required.

The following comments are not intended to replace the 4/11/86 Trifluralin Peer Review but to support and clarify the conclusions delineated in the that document.

The Proposed Guidelines for Carcinogen Risk Assessment (FR, Vol. 49, No. 227, November 23, 1984, pgs 46294-46301) outlines the criteria when assessing the evidence for carcinogenicity. A copy of page 46300 of this document is appended and should be referred to. These guidelines specify that "sufficient" evidence of carcinogenicity is demonstrated when "there is an increased incidence of malignant tumors or combined malignant and benign tumors:

(a) in multiple species or strains;"

TB COMMENT: There was no evidence of oncogenicity in multiple species or strains of rodents tested. Trifluralin was positive in both sexes of one strain of rat (Fischer 344). It was negative in two other strains of rat (Sprague-Dawley and Osborne-Mendel). In the EPA PD2/3, CAG concluded that the 1966 Sprague-Dawley rat study "showed no evidence of carcinogenicity and that the study was an adequate basis

for safety evaluation." In the 1978 NCI Osborne-Mendel rat study CAG concluded that "at the dose levels used in this experiment , the results are inadequate to demonstrate that trifluralin is a carcinogen in Osborne-Mendel rats." Trifluralin was negative in the 1980 B6C3F1 mouse study.

"or (b) in multiple experiments (preferably with different routes of administration or using different dose levels);"

TB COMMENT: There was no evidence in multiple experiments since only one experiment in rats was positive (1980 Fischer 344). An additional 2-year oncogenicity study in the Fischer 344 rat has been submitted by IPICHI and is currently undergoing evaluation by our contractor.

"or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset."

TB COMMENT: (1) The site or type of tumor in the Eli Lilly 1980 rat study (i.e. transitional epithelial cell tumors of the renal pelvis and urinary bladder) is unusual and statistically significant based on historical control evidence provided by Eli Lilly and on the study results. In a personal conversation with Dr. Wm. Busey (EPL, 7/29/86) it was explained that criteria used to judge a "rare" event or finding is that the spontaneous background frequency in control animals be less than 1%. Eli Lilly historical control data from 24 studies demonstrate 0% for kidney transitional cell cancer and 0-3.45% (males) for kidney transitional cell adenomas. Incidence in the 1980 study was 0% (control), 3.3% (low dose), 5.1% (mid dose), and 10% (high dose) for transitional cell cancer in males. The high dose incidence of 10% for combined benign/malignant kidney tumors compared to the historical controls of 0-3.45% is statistically significant but not "rare" in the experience of this laboratory (3 out of 24 studies demonstrated the occurrence of transitional cell adenomas of the kidney in control males). Furthermore, the CAG Guidelines note that "benign and malignant tumors will be combined unless the benign tumors are not considered to have the potential to progress to the associated malignancies of the same morphological type." Therefore, the benign and malignant transitional cell tumors of the kidney were combined in our evaluation of the evidence.

(2) There was no indication that the age of onset for transitional cell renal tumors was reduced in dosed versus control animals. It is recognized, however, that none of the studies incorporated interim or serial sacrifices into their protocol for demonstrating this specific response. Nonetheless, the evidence examined from the moribund animals sacrificed or which died prior to term did not demonstrate that age of onset was triggered for this event. Furthermore, tumors did not evidently effect longevity as the majority of such tumors were not discovered until terminal sacrifice, survival was unaffected by treatment and there was no difference between control and dosed animals for total number of tumor bearing animals (all types).

The CAG Guidelines define "limited" evidence of carcinogenicity is indicated when: "(a) the studies involve a single species, strain, or experiment;"

TB COMMENT: All of these criteria were triggered by the data for trifluralin.

"or (b) the experiments are restricted by inadequate dosage levels, inadequate duration of exposure to the agent, inadequate period of follow-up, poor survival, too few animals, or inadequate reporting;"

TB COMMENT: None of these criteria were triggered by the studies considered relevant for demonstrating the oncogenic potential or absence of oncogenicity for trifluralin.

"or (c) an increase in the incidence of benign tumors only."

TB COMMENT: This was true only for the female rats in the 1980 Eli Lilly study (i.e. benign urinary bladder tumors).

Additional comments: TB recognizes that the classification as a "Group C - possible human carcinogen" is a borderline judgement decision. The absence of positive effects in the mutagenicity data together with the negative oncogenic potential demonstrated in a second species (B6C3F1 mouse) as well as a second strain of rat (Sprague-Dawley) provide a reasonable basis for reducing our concern. Nonetheless, the increased incidence of malignant kidney transitional cell tumors is unusual and therefore, a Q* estimate is indicated for this Group C carcinogen.

The CAG Guidelines clarify the use of the Group C category in noting that Group C carcinogens "includes a wide variety of evidence: (a) definitive malignant tumor response in a single well-conducted experiment". Although TB believes this guidance is somewhat contradictory to the criteria for assessing "sufficient evidence" of carcinogenicity, it emphasizes the apparent ambiguities of classifying and categorizing animal oncogens in terms of the "carcinogenic risk to humans". Therefore, the classification of Trifluralin as a Group C carcinogen is not inconsistent with the Proposed CAG Guidelines.

Finally, it was mentioned that TB was inconsistent in its application or interpretation of hyperplastic changes with regard to the oncogenic potential of a chemical, e.g. Vinclozolin vs Trifluralin. TB wishes to note that, in the case of Vinclozolin, no hyperplasia was observed at any dose level, the lung adenomas were a frequent finding in the strain of mouse evaluated, and therefore, the chemical was not classified as an oncogen. TB noted then and reiterates again that "hyperplastic changes are a strong indicator of oncogenic effects". TB confirms that the hyperplastic changes and tumor incidence observed in the 1980 Eli Lilly rat study are definitely compound related. The kidneys and urinary tract are target organs for trifluralin. Effects on these organs have been further elucidated through short-term evaluations in the Fischer 344 male rat provided by Eli Lilly. Hyaline droplet incorporation in the kidney architecture and triphosphate crystal formation demonstrated a definite species sensitivity in the rat and preliminary or initial events in the progression of changes involving hyperplasia and eventually neoplasia, if time and dose are sufficient. Hyperplasia, per se, should not be used in a weight-of-evidence evaluation as a reason for "downgrading" the classification and it was not intended as such in the 4/11/86 Peer Review. Nonetheless equally important, in the absence of positive mutagenicity data and oncogenic response in other species or strains, hyperplasia per se should not be sufficient justification, by itself, for "upgrading" the classification. It was utilized, along with the increased incidence of malignancies, for classifying Trifluralin a Group C carcinogen requiring a Q^* calculation. The Q^* for Trifluralin was calculated in the PD 4 to be 7.7×10^{-3} (mg/kg b. wt./day)⁻¹. The omission of this fact in the 4/11/86 Peer Review was an oversight.

cc: Ed Gray (OGC)
John Tice (SIS)
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carcinogenicity, which indicates that there is a causal relationship between the agent and human cancer.

2. Limited evidence of carcinogenicity, which indicates that a causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding, could not adequately be excluded.

3. Inadequate evidence, which indicates that one of two conditions prevailed: (a) There were few pertinent data, or (b) the available studies, while showing evidence of association, did not exclude chance, bias, or confounding.

4. No evidence, which indicates that no association was found between exposure and an increased risk of cancer in well-designed and well-conducted independent analytical epidemiologic studies.

5. No data, which indicates that data are not available.

B. Assessment of Evidence for Carcinogenicity From Studies in Experimental Animals

These assessments are classified into five groups:

1. Sufficient evidence* of carcinogenicity, which indicates that there is an increased incidence of malignant tumors or combined malignant and benign tumors: (a) in multiple species or strains; or (b) in multiple experiments (preferably with different routes of administration or using different dose levels); or (c) to an unusual degree with regard to incidence, site or type of tumor, or age at onset. Additional evidence may be provided by data on dose-response effects, as well as information from short-term tests or on chemical structure.

2. Limited evidence of carcinogenicity, which means that the data suggest a carcinogenic effect but are limited because: (a) The studies involve a single species, strain, or experiment; or (b) the experiments are restricted by inadequate dosage levels, inadequate duration of exposure to the agent, inadequate period of follow-up, poor

* Under specific circumstances, such as the production of neoplasms that occur with high spontaneous background incidence, the evidence may be decreased to "limited" if warranted (e.g., there are widely diverging scientific views regarding the validity of the mouse liver tumor as an indicator of potential human carcinogenicity when this is the only response observed, even in replicated experiments in the absence of short-term or other evidence).

§ Benign and malignant tumors will be combined unless the benign tumors are not considered to have the potential to progress to the associated malignancies of the same morphologic type.

survival, too few animals, or inadequate reporting; or (c) an increase in the incidence of benign tumors only.

3. Inadequate evidence, which indicates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect.

4. No evidence, which indicates that there is no increased incidence of neoplasms in at least two well-designed and well-conducted animal studies in different species.

5. No data, which indicates that data are not available.

The categories "sufficient evidence" and "limited evidence" refer only to the strength of the experimental evidence that these agent(s) are carcinogenic and not to the power of their carcinogenic action.

C. Categorization of Overall Evidence

Group A—Human Carcinogen

This category is used only when there is sufficient evidence from epidemiologic studies to support a causal association between exposure to the agent(s) and cancer.

Group B—Probable Human Carcinogen

This category includes agents for which the evidence of human carcinogenicity from epidemiologic studies ranges from almost "sufficient" to "inadequate." To reflect this range, the category is divided into higher (Group B1) and lower (Group B2) degrees of evidence. Usually, category B1 is reserved for agents for which there is at least limited evidence of carcinogenicity to humans from epidemiologic studies. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard agents for which there is sufficient evidence of carcinogenicity in animals as if they presented a carcinogenic risk to humans. Therefore, agents for which there is inadequate evidence from human studies and sufficient evidence from animal studies would usually result in a classification of B2.

In some cases, the known chemical or physical properties of an agent and the results from short-term tests allow its transfer from Group B2 to B1.

Group C—Possible Human Carcinogen

This category is used for agents with limited evidence of carcinogenicity in animals in the absence of human data. It includes a wide variety of evidence: (a) Definitive malignant tumor response in a single well-conducted experiment; (b) marginal tumor response in studies

having inadequate design or reporting; (c) benign but not malignant tumors with an agent showing no response in a variety of short-term tests for mutagenicity; and (d) marginal responses in a tissue known to have a high and variable background rate.

In some cases, the known physical or chemical properties of an agent and results from short-term tests allow a transfer from Group C to B2 or from Group D to C.

Group D—Not Classified

This category is used for agent(s) with inadequate animal evidence of carcinogenicity.

Group E—No Evidence of Carcinogenicity for Humans

This category is used for agent(s) that show no evidence for carcinogenicity in at least two adequate animal tests in different species or in both epidemiologic and animal studies.

V. References

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