UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

DATE: 4/3/80

SUBJECT: Meeting on March 21, 1980 to duscuss requirements for establishment of potable water tolerance of Acrolein.

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TO: Files (EPA Registration No. 10707-9)

Participants

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Magna Corp.
Frederick F. Caserio
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Dr. Caserio gave a long presentation on history, use patterns, nature, and occurance of Acrolein.
Dr. Fielder discussed the toxicology data found in a literature search. Refer to attachment distributed at the meeting.

The purpose of the meeting was that the Bureau of Reclamation and Irrigation Districts want to use Acrolein in canals that can be used for potable water sources, and Magna Corporation needs "human tolerances" established for this use. But they don't have money to conduct long term tests. They presented the arguments that since it is a naturally occurring compound, only a mutagenicity battery was needed for approval.

Discussion occurred about a draft document prepared by Water Criteria Commission which unofficially indicated Acrolein was a weak mutagen and would set maximum permissible levels for potable water at 6.5 ug/L and 1.2 ug/L average 2" of water for fish and wildlife habitats.

After it was indicated that the chemists did not want to listen to the toxicology data requirements, the requirements for residue chemistry were detailed. The actual residue levels from the proposed use, as measured in water, irrigated crops, and fish must be determined. They must determine what effect location and time have on residues. It was indicated use restrictions could ameliorate the problems of high dose levels, and that we did not set tolerances based on misuse, but the restrictions, precautions, use limitations, and directions for use must be practical. The analytical method and all future testing must be specific for acrolein. The method has to be validated.

It was indicated that potable water tolerances are established under Section 409 of the Pure Food, Drug and Cosmetic Act and given in 21 CFR 193. Crop tolerances and exemptions from requirement of tolerances were under Section 408 and given 40 CFR 180.

If there are any detectable residues in fish, crops, or potable water then the chronic toxicity data as specified in the proposed guidelines of August 22, 1978 must be submitted including 2-year rat and 18-month mouse oncogenicity studies, a chronic feeding study, a reproduction study, and a teratology study.

When a petition for temporary tolerance is submitted with an Experimental Use Program, at a minimum, a 90-day rat and a 90-day dog study which should be extended to a 6-month dog study are required. If the theoretical mean residue contribution does not exceed 50% of the average daily intake from the 90-day study, a temporary tolerance may be granted.

It was pointed out that if the nature of the compound is fundamentally different from the factors that were used to write the guidelines, they may request a waiver of the data requirement. One example is if after a naturally occurring compound is applied to a food crop, the residues of the compound in that food crop are not increased, it may be possible to waive the chronic toxicity testing requirement, but valid acute studies, 90-day studies (rats and dogs), teratology study, and mutagenicity studies are necessary to support registration of the product.

cc Cebarly
Quick
Fielder

2

Information Relevant to a Tolerance For Acrolein
In Potential Sources of Potable Water

I. Occurrence of Acrolein

A. Beverages

B. Foods

II. Exposure Parameters

III. Toxicity Data

A. Introduction

B. Acute and Subchronic Toxicity

C. Oncogenicity Studies

D. Teratogenicity Studies

E. Reproduction Studies

F. Mutagenicity Testing

1. Detecting gene mutations

a. Bacteria, with or without metabolic activation
b. Insects (e.g. sex-linked recessive lethal test)

2. Detecting chromosomal aberrations:

Dominant lethal effects in rodents

3. For detecting primary DNA damage

a. Inhibition of DNA synthesis in bacteria
b. Inhibition and activation of DNA synthesis in mammalian cells

G. General metabolism studies

IV. Comments

I. Occurrence of Acrolein

As a product of food processing, acrolein at present may be ingested from several different sources. It has been detected in off-flavored rums and peppery whiskies,[1] as well as "pricked" brandies distilled from ciders.[2] Mirin, a sweet rice wine used in Japan for cooking, also has been shown to contain acrolein.[3] Acrolein has been identified in chocolate liquor from cocoa beans,[4] sugar cane molasses,[5] and Ceylon tea.[6]

Combustion, heating, or heat-processing of certain foods also produces acrolein. Acrolein, along with other carbonyl compounds which are presumably responsible for the aroma of bread, can be steam-distilled from several types of rye bread.[7,8] Heating lard and butter increases the acrolein content of those foods;[9] and boiling or roasting processes increase the acrolein content of turkey meat.[10] Acrolein has also been found in the breast muscles of chickens,[11] and in fruit vinegars.[12]

The concentrations of acrolein found in random samples of produce are listed in Table 1.

Table 1

Produce	ppm Acrolein (Average of three methods
Almonds	22
Apples	8
Bananas	45
Grapefruit	10
Lemons	66
Olives	less than 1
Oranges	10
Tangelos	18
Tangerines	10
Tomatoes	10

*The methods of analysis included gas chromatography, thin layer chromatography and colorimetry. All analyses were made using the edible portion of the produce.

II. Exposure Parameters

The Magnacide "H" Herbicide Process Handbook[14] recommends the following concentration for application:

> The concentration of Magnacide "H" in water reaching crops should not exceed 15 ppm. Concentrations in this range are obtained readily by: (1) controlling water off-take downstream from the point of application until natural dissipation has reduced the herbicide concentration to 15 ppm; or (2) extending the application time so that the concentration never exceeds 15 ppm.

Distribution of the chemical on usage varies with water temperature, water flow amount and velocity, and weed density. As explained in the Magnacide "H" handbook,

> The herbicide is "used up" as the blanket of treated water moves downstream because of absorption by the weed tissue and vapor loss. Therefore, in long canals it may be necessary to "reinforce the wave" at points downstream from the first application...[A]t recommended dosages, control distances up to 15 miles have been experienced. Algae may be controlled for even greater distances.

Studies on determining the dilution and decay rate of aquatic herbicides[15] have estimated the decay rate constants of acrolein at 0.14 to 0.21 per hour, under different conditions. Where k is the decay rate constant of the herbicide, the half-life (T) is given by the following formula:

$$T = 0.693/k$$

Therefore the half-life may range from three hours and thirty minutes to five hours.

III. Toxicity Data

A. Introduction

The extremely unpleasant odor and taste of acrolein is a major factor in mammalian oral toxicity and long-term feeding studies. According to a study by

the Ontario Water Resources Commission[16], acrolein can be detected by taste and odor at very low concentrations. At 40°C, a concentration of 0.1 ppm was required for human panelists to note the absence of the taste of acrolein. At 21°C, a dilution of acrolein to 0.02 ppm was required. At 60°C, a concentration of 0.07 ppm was required to obtain no detection by odor. Decreasing the temperature had no effect on odor detection limits.

In spite of this low detection limit, human consumption of water from a treated canal in a field trial in Egypt has been reported.[17] According to the report,

> Water analysis showed that three of the labourers drank water with 16 ppm and the fourth water with 10 ppm of acrolein. The only reaction they experienced was some tickling and a very slight burning sensation in the throat. No other incidents or complaints were noted in connexion [sic] with these trials.

B. Acute and Subchronic Toxicity

The acute and subchronic toxicity studies already available[18] are summarized in Table 2.

Table 2

A. Acute Toxicity

1. Mice, oral LD_{50} = 26-30 mg/kg
2. Rats, oral LD_{50} = 37-49 mg/kg

B. Subchronic Toxicity

1. Range-finding study (Rats)

0.0 ppm 10 ppm 20 ppm 40 ppm	Variable growth response of male rats; female rats grew as well as or better than controls.
80 ppm	Water intake of male rats decreased.

160 ppm	Water intake of male and female rats decreased.

2. 90-day feeding study (Rats)

0 ppm 5 ppm 13 ppm 32 ppm 80 ppm	Growth of male and female rats as good as or better than controls. No difference from controls in hematological results or organ weight differences.
200 ppm	5% poorer growth rate in male rats.
80 ppm 200 ppm	Occasional occurrence of stomach ulcers.

3. 60-day feeding study (rats)

600 ppm	Death in 1 out of 5 rats
1200 ppm 1800 ppm	Death in all animals, apparently due to refusal to drink unpalatable solution.

4. 24-hour feeding study (dairy cows)

30 ppm 60 ppm	No apparent adverse effects.
90 ppm	Decrease in water consumption; transitory drop in body weight.

III. Toxicity Data

C. Oncogenicity Studies

No records have been found of oncogenicity studies comparable to the proposed guidelines.

D. Teratogenicity Studies

No data have been found concerning teratogenicity studies comparable to those of the proposed guidelines. However, in an embryotoxicity study[19] involving acrolein, acrylonitrile and acrylamide in developing chick embryos, no evidence

of induced teratogenicity by acrolein was observed. The LD_{50} values of acrolein were estimated to be in the range of 0.01-0.1 μ mol (approximately 0.05μmol/egg). Eye and beak malformations and edematous cysts were found in acrolein-treated chicks at the dose level of 0.001μmol/egg. As the control eggs injected with normal saline also showed the same malformations the authors concluded that their data showed no clear cut evidence of teratogenicity.

E. Reproduction Studies

No records have been found for reproduction studies comparable to the proposed guidelines.

F. Mutagenicity Testing

1. Detecting gene mutations

a. Bacteria, with or without metabolic activation.

In a test involving the response of histidine-requiring mutants of Salmonella typhimurium[20], acrolein had a negative test result, whereas three known mutagens obtained positive test results.

In mutation-detecting systems in Escherichia coli 343/113 including forward mutations as well as back mutations, acrolein did not exhibit mutagenic activity, with or without metabolic activations through rodent liver homogenates.[21]

Another test system involved Salmonella typhimurium,[22] to detect induction of substitution and of frameshift mutations. Acrolein showed no mutagenicity of either type.

b. Insects (e.g., sex-linked recessive lethal test).

Rapoport[23] found that acrolein, like other unsaturated aldehydes such as crotonaldehyde, can produce sex-linked lethals in Drosophila.

These pilot experiments were discontinued.

2. Detecting chromosomal aberrations

Dominant lethal effects in rodents.

In a test involving male Swiss mice[24], a mutagenic index (M.I.) was calculated by the formula:

$$M.I. = \frac{\text{deciduomata + late deaths}}{\text{total implantations}} \times 100$$

Acrolein rated a mutagenic index of 5, which was greater than that for theophylline (2) and DDT (3); but less than the ethyleneimine alkylating agents TEPA (27), METEPA (38), and THIO TEPA (18).

In contrast, a later study by the same investigator[25] rated acrolein as an agent not meeting any screening criteria for mutagenic effects. These criteria were:

1. One or more weekly means exceeding 1.00 early fetal deaths per pregnancy, with at least 55% of the pregnant females having early deaths; by considering these parameters together, any instance where an elevated mean was due to an atypical individual female would be eliminated.

2. One or more weekly means of less than 8 total implants per pregnancy.

3. One or more weekly mean pregnancy rates of less than 30%.

3. For detecting primary DNA damage

No reports have been found comparable to the tests specified in the proposed guidelines. However, several reports indicate that acrolein has an effect on nucleic acid and protein synthesis.

a. Inhibition of DNA synthesis in bacteria.

Very low levels (0.01-0.02 m M) of acrolein have been found to inhibit

nucleic acid and protein synthesis in E. coli.[26] The investigators reported that "RNA synthesis could be totally inhibited by concentrations of acrolein as low as 0.013 m M. At 0.009 m M, DNA synthesis was totally inhibited for a short period of time, while RNA and protein synthesis continued at reduced rates."

b. Inhibition and activation of DNA synthesis in mammalian cells.

In a study of RNA polymerase activity in isolated nuclei from rat liver tissue[27], addition of acrolein lead to an inhibition of transcription. RNA polymerase enzymes appeared to be inhibited.

In a later study involving rat liver DNA polymerase[28] two effects of acrolein were found. An inhibitory effect was found at higher molarities, possibly because of oxidation of the active thiol groups of the enzyme by acrolein.

At very low molarities, acrolein activated DNA polymerase.

G. General metabolism studies.

No reports comparable with the proposed guidelines have been found. Acrolein is, however, a mammalian metabolite of cyclophosphamide, a drug that is not mutagenic unless it is metabolically activated.

IV. Comments

The EPA "Proposed Guidelines for Pesticide Registration; Hazard Evaluation: Humans and Domestic Animals", Supplementary Information (1979) states that:

> "A compound would be considered a mutagen if it produced positive results in: two different kinds of tests for demonstrating gene mutations; a mouse specific locus test; or any kind of test for demonstrating chromosome aberrations. Positive results from any DNA damage test or from a single gene mutation study (other than the mouse specific locus test) would be considered inconclusive."

A question which can be answered at this time is whether information in the literature is sufficient to establish acrolein as a mutagen according to these criteria. If the information is sufficient, further mutagenicity testing might have no effect on the granting of a tolerance level.

The supplementary information also states that, "data will not be rejected merely because they were not developed in accordance with the guidelines test standards." Therefore, it is important to determine whether the 90-day feeding study (Newell, 1958) can be accepted under the current criteria.

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