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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

February 9, 1983

TO: John W. Melone, Director
Hazard Evaluation Division (TS-769)

SUBJECT: Thiodicarb - Response to Your Memo of 1/26/83

Attached are my brief reviews of the three papers concerning the oncogenic potential of acetamide. As you requested, the papers were reviewed only for protocol acceptability regarding present day standards, dose effect relationships and oncogenic effects. Also attached is a risk assessment done by B. Litt.

None of the studies meet today's standards for oncogenicity testing. For example only a small number of male rats were used in all three studies and only single dietary levels of acetamide were employed in two papers Dessau and Jackson (1955) and Weisburger et al. (1969). This is also true of two experiments out of three described by Jackson and Dessau (1961). These studies were not designed to test for carcinogenicity per se. There purpose was to study the liver alteration produced by a simple compound which also produced liver tumors.

No dose response relationship can be discovered in the Dessau and Jackson (1955) and Weisburger et. al. (1969) studies. In the Jackson and Dessau (1961) paper only one experiment among three used multiple dietary doses. The reported hepatoma incidence for the 1.5, 2.5 and 5.0% levels are: 4/24 (16.7%); 6/02 (27.3%); and 1/18 (5.6%). Litt has shown a time related dose response which may or may not be real from a biological point of view.

The positive hepatotoxic (oncogenic) response of male rat to acetamide fed in the Wayne Laboratory Blox diet versus the purported negative response to acetamide fed in Purina Laboratory Chow casts doubts on the certainty of acetamide's oncogenic potential as well as its potential hazard to humans.

If we are going to take on the Delaney clause, it is my opinion that we should use something more potent than this ping pong ball.

I can only conclude that, under the conditions described in these papers, acetamide appears to have an oncogenic potential for male rat livers. Weisburger et. al. (1969) concluded that is a relatively weak carcinogen when compared with other compounds affecting the liver (p. 171).

OEP 7/9/83

Orville E. Paynter, Ph.D.
Chief
Toxicology Branch
Hazard Evaluation Division (TS-769)

Attachment

cc:
PGray
EJohnson
DCampt
BLitt
CASWELL File

2

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

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

February 7, 1983

TO: John W. Melone, Director
Hazard Evaluation Division (TS-769)

SUBJECT: Review of Acetamide Papers Regarding Oncogenic Potential

FROM: Dr. Orville E. Paynter, Chief
Toxicology Branch, HED (TS-769)

OEP 2/9/83

1. Dessau, F.I. and Jackson, B (1955). Acetamide-induced liver cell alterations in rats. Lab. Invest. 4, 387-397.

Two groups of Rockland male rats (10 control and 15 treated) were administered distilled water (1.0 cc/100 gm body weight) or 4000 mg/kg acetamide (40% aqueous solution) by intubation 5 days/week. Group 1, consisting of 5 control and 8 treated males, was killed after 117 days and Group 2, consisting of 5 control and 5 treated males, was killed after 205 days.

Liver sections were prepared and cell counts and nuclear measurements were performed and hepatocellular changes described. Rats killed after 117 days exhibited no gross liver lesions. Among Group 2 rats, one rat liver showed "several round, whiteish nodules" near the surface and one liver contained a "solid tumor". Two diagnostic labels were given to this tumor: 1) hepatocellular adenoma (p. 389) and 2) hepatoma (Fig. 4 & 5).

The liver lesions were described as follows: "In 5 of 8 rats in Group 1, and 3 of 5 in Group 2, the liver sections showed cytologic irregularities which were absent from the 10 controls. The irregularities consisted of a greater variability of cellular and nuclear size (Fig. 2), giant nuclei, and the presence of numerous mitoses, some of these of unusual appearance. Fewer binucleate liver parenchyma cells were observed in the treated animals. Hyperchromatic nuclei were more numerous in the treated. While some of these suggest cell disintegration, others most probably are indicative of beginning or completed cell division. There was marked vacuolation of the cytoplasm of large groups of liver cells in some of the treated rats (Fig. 3). The cytoplasm in these cells was foamlike in

3

appearance, quite unlike the vacuole of the fatty liver after deparaffinization. The vacuolated cells were absent from many lobules and formed rather large foci in others, with no particular topographic preference and no limitation to one lobule. Whether these vacuoles contained fat could not be determined, as the frozen sections which proved negative for fat could not be taken from the same area of the liver. More conclusive was the evidence that the vacuoles were not due to deposits of glycogen, as the vauolated cells were found to contain less PAS-positive material than the surrounding nonvacuolated cells.

The tumor which was mentioned earlier was found to be a hepatocellular adenoma (Fig. 4 and 5). In general, the tumor was sharply separated from the surrounding liver tissue, but there were areas of transition from the usual trabecular pattern to a tubular structure with complete loss of lobular arrangement within the tumor. The cells within the tumor were rather uniform in size and appearance. No glycogen was stainable in the tumor cells." (pp 389-92)

The authors summarized the study results, on p. 316, as follows:

"A qualitative and quantitative study has been presented of the changes in the livers of rats after prolonged treatment with large doses of acetamide. Liver cell alterations occurred in several rats and formation of a hepatoma in 1 rat. The changes resembel those observed by other investigators after treatment with thioacetamide. The changes are interpreted not as a result of regeneration but as a result of injury affecting cell multiplication directly."

Reviewer's Comments:

The pathologic evaluation in this paper fit with the prevailing descriptions used at the time. However this paper is out of date when compared to todays standard for an oncogenicity study and outdated by more recent views and interpretations of the liver lesions and their significance.

4

2. Jackson, B. and Dessau, F.I. (1961). Liver tumors in rats fed acetamide. Laboratory Investigations. 10, 909-923

The experimental design of this study is presented by the authors in their Table 1. (attached) Male Wistar rats were used.

The compounds ~~were~~ mixed into ground Wayne Blox.

The incidence of gross lesions in livers is presented in the authors Table 2 (p. 913).

ACETAMIDE TUMORS IN RATS

TABLE 2. Incidence of Gross Lesions in Livers

Exp. no.	No. of rats	Liver findings: Grossly normal	Disseminated foci	Hepatomas
1	48	32 (23 + 9)*	12	4 (2 + 2)*
	43	43 (41 + 2)*	0	0
2	18	6	11	1
	22	6	10 (9 + 1)*	6 (3 + 3)*
	24	9	11	4 (3 + 1)*
	25	25	0	0
3	81	39 (35 + 4)*	20	22 (19 + 3)*

*The first number in the parentheses indicates the number of animals with this finding at scheduled sacrifices; the second number, those dying spontaneously that had this finding.

5

TABLE I. Plan of Experiments and Fate of Animals

Exp. No.	Plan of experiments: Treatment	Plan of experiments: Autopsy schedule	No. of rats	Fate of rats: Died spontaneously*: Total	Fate of rats: Died spontaneously*: Autolytic: Discarded	Fate of rats: Died spontaneously*: Autolytic: Used	Fate of rats: Sacrificed as scheduled (used)	Rats used in study
1	Continuous feeding of 5% acetamide diet	One rat weekly until week 26; 1 rat biweekly thereafter	50	13	2	11	37	48
	Control diet		50	9	7	2	41	43
2	Continuous feeding of 5% acetamide diet	One rat monthly, remainder at 1 year	25	7	7	0	18	18
	2.5% acetamide diet	Same as above	25	7	3	4	18	22
	1.25% acetamide diet	Same as above	25	2	1	1	23	24
	Control diet	Same as above	25	0	0	0	25	25
3	All rats fed 5% acetamide diet at start. Each week, treatment of 2 rats was stopped and they were placed on control diet for remainder of year	Survivors sacrificed after a year	99	25	18	7	74	81

* Includes 2 rats sacrificed in moribund condition: 1 rat from the group given the 2.5% acetamide diet in Experiment 2 and 1 rat used in Experiment 3.

The incidence of tumors, by microscopic examination is not given by the authors. They do indicate that some tumors showed signs of malignancy. All are simply classified as "hepatomas". A dose relationship is not apparent in this paper.

Reviewer's Comment:

Because of the vagueness concerning tumor incidence it is difficult to properly evaluate this paper. It appears that at dietary levels of 15,000 to 50,000 ppm, acetamide procedures liver tumors in rats within a one year period of feeding. The use of ground Wayne Laboratory Blox as the feed may be a confounding factor in this study. In the following Weisburger et. al. paper, footnote #5 p. 164 states that "In a personal communication, Dr. Jackson (see footnote 2) indicated that for unexplained reasons acetamide was not carcinogenic in Purina Laboratory Chow." The reviewer wonders just how reproducible the oncogenic effects of acetamide might be.

3. Weisburger, J.H., Yamamoto, R.S., Glass, R.M. and Frankel, H.M. (1969). Prevention by arginine glutamate of the carcinogenicity of acetamide in rats. Tox. Appl. Pharma. 14, 163-175.

The compounds(see Table 1, attached) were administered via diet to male Wistar rats for 12 months. This study was designed to discover the underlying mechanisms of acetamide oncogenicity. The feed used was Wayne Laboratory Blox.

In rats receiving 2.5% (25,000 ppm) acetamide for 12 months 2/8 hematomas were produced. In rats receiving acetamide for 12 months and control feed for an additional 3 months 7/16 rats exhibited hepatomas. Rats receiving acetamide and ~~original~~ arginine glutamate exhibited no hepatomas and only 1/19 had hyperplastic nodules.

The authors concluded "Our data entirely confirm the previous report (Jackson and Dessau, 1961) that acetamide is carcinogenic to rat liver. The dosage required was high and the latent period fairly long. Thus, the compound can be classified as a relatively weak carcinogen, in comparison to other agents affecting the liver (Clayson, 1962)." [p. 171]

Reviewer's Comments:

This study is a mechanistic study of acetamide oncogenicity. No dose relationships were expected to develop from this design. Footnote #5, p. 164 casts doubts on the reproducibility of the oncogenic effects observed in this and the Jackson et. al. (1969) paper. Dietary factors seem to play an important part in the mechanism of actamide oncogenicity. How this relates to human risk is uncertain.

8

TABLE 1

EFFECT OF DIETS CONTAINING ACETAMIDE, ACETAMIDE PLUS ARGININE GLUTAMATE, OR AMMONIUM CITRATE FED TO MALE WISTAR RATS TO 12 OR 15 MONTHS

Diet supplement[a]	Time (months)	Total number of rats	Liver histology[b]				Body weights (g)	Liver weights (g/100 g)
			No Hyperplasia	Focal Hyperplasia	Hyperplastic nodules	Hepatoma		
			Number of rats					
None	12	4	4	0	0	0	—	—
	15	7	7	0	0	0	562 ± 16	2.92 ± 0.07
2.5% Acetamide	12	8	3	3	0	2	—	—
	15	16	2	4	3	7	508 ± 12	3.40 ± 0.35[c]
2.5% Acetamide + 5.6% arginine glutamate	12	11	8	2	0	1	—	—
	15	19	8	10	1	0	534 ± 14	2.99 ± 0.08
5.6% Arginine glutamate	12	3	3	0	0	0	—	—
	15	10	9	1	0	0	533 ± 24	2.84 ± 0.08
4.8% Ammonium citrate	12	10	10	0	0	0	—	—
	15	17	15	2	0	0	498 ± 13	2.89 ± 0.07

[a] Rats were fed the experimental diets for 12 months. A sample of each group was killed, and sections of tissues were studied microscopically after histologic processing. The balance of the animals was continued on control diets for another 13 weeks.

[b] Liver histology was classified by the criteria of Firminger and Reuber (1961), and Reuber (1965). In increasing severity of lesions the liver had (1) normal aspect or no hyperplasia, which may include livers with diffuse increase in size of cells in periportal area, or with basophilia in these cells; (2) focal hyperplasia, as small foci or larger areas distinct from surrounding liver and often displaying different architecture; (3) hyperplastic nodule, a clearly defined nodule, with distinct compression of surrounding parenchyma, and going from well-organized cords to disorganized growth pattern and cellular atypia, but only to such an extent that diagnosis of malignancy was not justified; and (4) hepatoma, from highly differentiated to undifferentiated, anaplastic growths.

[c] The increased liver weight in this group was primarily due to weight of liver tumor. Thus, the average liver weight of the 9 rats without frank hepatoma was 2.86 ± 0.10 g/100 g; that of the 7 rats with hepatoma was 3.96 ± 0.67 g/100g.