HAZARD REVIEW

OF

2-ACETYLAMINOFLUORENE (2-AAF)

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Wilson, DeEds, and Cox, [1] studying the toxicity of a promising new pesticide, 2-acetylaminofluorene (2-AAF), discovered that it was a potent carcinogen. As a consequence this substance could not be used for pesticidal effects although it became a unique tool for the study of carcinogenic mechanisms. Morris et al [2] stated in 1961 that more than 500 reports dealing with 2-AAF, often called N-2-fluorenyl acetamide (2-FAA), or related compounds, had appeared in the literature since 1941. Many of these studies and some reported since that time have confirmed the carcinogenicity of 2-AAF. Although the majority of these studies has concerned rats, the carcinogenic effects of 2-AAF have been demonstrated in other laboratory animals as well. Workers in cancer research have been exploiting the consistent responses of the rat and of other animals to 2-AAF as experimental models for the study of carcinogenesis. The results of the most pertinent of these studies relating to the carcinogenicity of 2-AAF are summarized below.

The original work of Wilson et al [1] demonstrated a definite relationship between dosage level in the diet and the time required for tumor induction. Dosage levels of 0.25 to 1.0 percent 2-AAF were too toxic and all rats died within 93 days. At a level of 0.125 percent 9 of 20 female rats developed tumors within 273 days while 9 rats died early without tumors. A similar group of 5 female rats fed 0.31 percent 2-AAF developed tumors within 280 days. Although tumors

occurred at various sites, carcinomas of the liver and bladder predominated. Other authors have shown a sex variation in the type of tumors produced by 2-AAF. Sidransky et al [3] reported that male rats had a higher incidence of liver tumors than female rats. In one experiment, male and female rats fed a diet containing 0.03 percent 2-AAF had an incidence of liver tumors of 100 percent (12/12) in the males and 60 percent (5/12) in the females. In a second experiment in which female rats were fed a diet containing 0.06 percent 2-AAF, the incidence of liver tumors was 40 percent (6/15). In a third experiment, in which the average duration of survival of female rats fed a diet containing 0.03 percent 2-AAF was prolonged by surgical removal of breast tumors, a higher incidence of liver tumors (7/9) was found. Ross et al [4] reported an incidence of 17 percent mammary tumors in rats fed a diet containing 0.04 percent 2-AAF for 11 months in contrast to 5 percent in controls. Among other organs of rats which have developed tumors following treatment with 2-AAF are salivary glands, [5,6] auditory sebaceous glands, [6,7] the eye, [8] and the ear duct. [8]

The effect of diet on the carcinogenicity of 2-AAF has been studied by several investigators. Engel and Copeland (8) studied the influence of diet on the relative incidence of eye, mammary, ear duct, and liver tumors in rats fed 2-AAF. Eye tumors, presumably originating in the harderian glands, were induced in 10/31 weanling rats fed 0.03 percent 2-AAF in a low fat diet (no added fat) for 7-8

months. Growth was very poor in the rats on the low fat diet and some developed dermatitis, indicating essential fatty acid deficiency. No eye tumors appeared in any rats receiving diets containing 15 to 29 percent lard. The incidence of mammary tumors was low (2/31) in animals receiving the low fat diet. In contrast, mammary tumors occurred in 55/71 of animals that received the diet containing 15-30 percent of lard. The incidence of ear duct tumors was in contrast to that of mammary tumors. Only 4/18 rats on the 15 percent fat diet developed ear duct tumors in contrast to 24/44 in the more slowly growing rats on the low fat diets.

Engel and Copeland, [9] in a similar study, fed weanling rats synthetic diets containing from 9-60 percent casein plus 2-AAF for 16 to 40 weeks. The rats on the diets containing 9-27 percent casein plus 2-AAF had an 86 percent (57/66) mammary tumor incidence compared to an incidence of 12 percent (3/25) in controls fed casein alone. The daily intake of 2-AAF in these groups averaged from 1.8 to 2.1 mg per rat. When the 2-AAF exceeded 2.1 mg daily the protective effect of high protein was largely overcome. There was also suggestive evidence that the high protein diet protected against ear duct and liver tumors. Engel and Copeland [10] likewise showed that stock diets consisting of natural foodstuffs offered considerable protection against 2-AAF. Rats fed 2-AAF in stock diets lived longer and developed fewer mammary tumors than did rats fed 2-AAF in semi-purified diets.

The action of the essential amino acid, tryptophane, and of its metabolite, indole, has been studied in the hope of shedding some light on the mechanism of 2-AAF induced bladder tumors. Dunning et al [11] fed 344 female rats of the Fisher line (3-4 months of age) on four diets differing in protein content to which was added 0.06 percent 2-AAF. The four diets contained, respectively: 45 percent casein; 26 percent casein; 25 percent tryptophane-free casein hydrolysate plus 1.4 percent DL-tryptophane; and 22 percent tryptophane-free casein hydrolysate plus 4-3 percent DL-tryptophane. Hepatomas occurred in a majority of the rats in each group. Bladder tumors occurred in 100 percent of rats on the 25 percent tryptophane-free casein hydroloysate plus 1.4 percent tryptophane diet and in 92 percent of the rats on the 22 percent tryptophane-free casein hydrolysate plus 4.3 percent tryptophane diet. However, no bladder tumors occurred in animals fed the 45 or the 26 percent casein diets. In a similar study, Oyasu et al [12] confirmed the studies of Dunning et al [11] that indole prolonged the life, modified damage to the liver, and increased the incidence of bladder tumors in 2-AAF fed rats. They expressed the belief that the primary factor in the increased incidence of bladder tumors was increased survival time and, secondarily, degree of liver damage. This was confirmed by a later study by McDonald et al [13] in a study of the action of indole as a co-carcinogen.

Melicow et al [14] studied the effect of 2-AAF on the incidence of bladder tumors in male rats maintained on a pyridoxine (Vitamin B6) deficient diet. Bladder tumors were observed in 2/18 rats fed a diet containing 4 mg vitamin B6 and 600 mg 2-AAF per kg of food and none in a group fed vitamin B6 and 2-AAF for 1 month, at which time vitamin B6 was continued and 2-AAF discontinued. In both groups average survival time was less than five months. In the group fed the vitamin B6 deficient diet plus 2-AAF one sub-group, surviving an average of 175 days, had an incidence of 50 percent (6/12) bladder tumors whereas in the other sub-group, surviving an average of 272 days, 11 of 12 rats (92 percent) exhibited bladder tumors. Thus, the primary factors appeared to be the survival time of the animal and the accompanying exposure to the carcinogen during this time.

Mori [15] studied the effect of liver feeding on the production of liver tumors by 2-AAF. A polished rice diet supplemented with vegetables was used. 2-AAF was supplemented at the level of 0.3 g per kg of dry food to experimental diet containing 10 percent liver powder and to a control diet without liver, for a 6 month period. Groups of 20 female rats were placed on each diet. The experiment was discontinued after 300 days. Approximately one-half of each group died early and were not counted. Although there were no striking differences in liver damage in the two groups, there was slight inhibition of liver cancer by the liver feeding. Two of 10 animals

(20%) were diagnosed with definite liver cancers in the experimental group versus 7 liver cancers of 16 (44%) in the controls.

Numerous authors have studied changes in the liver and in other organs in an attempt to explain the mechanisms of 2-AAF carcinogenesis. Among these, Epstein et al [16] made a cellular analysis of the liver of rats fed diets containing 0.05 percent 2-AAF for 2 or 3 weeks. All animals used for histological and biochemical studies showed both nodular and non-nodular foci in the liver. The nodules were composed predominantly of hepatocytes which showed morphological biochemical differences from the surrounding liver. The glycogen, or its metabolic control, was different in the nodule in that a significant amount of glycogen was present even after a 48 hour period of fasting. The authors presented evidence to implicate the hyperplastic nodule as a step in the carcinogenic process. Trams et al [17] studied the effects on the liver enzymes of feeding a diet containing 250 mg per kg of 2-AFF for up to 24 weeks. The enzymes which N-demethylate morphine and hydroxylate acetanilide decreased progressively toward the time when hepatoma formation occurred. The glucuronyl transferase and glutathione reductase enzymes demonstrated significant increases in activity. Likewise there was a marked increase in activity of uridinediphosphoglucose (UDPG) dehydrogenase. King and Guttman [18] demonstrated that 2-AAF fed to male rats at a dietary level of 0.03 percent for 6 weeks depressed the activity of liver catalase to the extent of 20-25 percent, but had no effect on

liver arginase. Among other enzyme studies, Roth [19] demonstrated that 0.06 percent 2-AAF fed to rats depressed alkaline RNase activity of the liver mitochondria an average of 50 percent. Serum alkaline RNase in the 2-AAF-fed animals was depressed to a lesser extent and there was no change in the acid RNase activity. Poirier and Pitot [20] found that rats consuming a diet containing 0.03 percent 2-AAF for 4 to 5 weeks had no metabolic increase in ornithine transaminase and histidase, as observed in protein depleted rats following forced feeding of casein hydrolysate. At the same time the induction of tryptophane pyrrolase and serine dehydratase was greatly diminished in animals receiving 2-AAF. No change in tyrosine transaminase was observed.

Among the many investigators who have been interested in the hormonal relationship of 2-AAF-induced carcinogenesis, Reuber [21] showed that both the thyroid hormone and testosterone are necessary for the production of carcinoma and cirrhosis of the liver in male and female rats fed 0.025 percent 2-AAF. Likewise, Toh [22] found that the subcutaneous implantation of testosterone pellets greatly increased the incidence of both the non-neoplastic and neoplastic lesions in the liver of female and of castrated male rats. Perry [23] reported that adrenalectomy protected male rats against the carcinogenic effects of 2-AAF, whereas desoxycorticosterone trimethylacetate did not modify the response to the carcinogen in either the adrenalectomized or the intact rat. Hall [24] produced

tumors of the ovaries of female rats joined in parabiosis to gonadectomized litter mates.

In order to explain the carcinogenic effect of 2-AAF, several authors [25-27] have demonstrated the importance of the binding effect on various tissues, cells, or cellular constituents.

Compounds which have been examined for synergistic or antagonistic effects with the carcinogenic action of 2-AAF are: indole, [11] dietary tryptophane, [11] orotic acid, [28] carbon tetrachloride, [29] methyl cholanthrene, [30,46] cupric oxyacetate, [31] tannic acid, [32] Tween 60, [33] 1-naphthyl-isothiocyanate, [34] m-acetoluidide, [35] m-aminobenzoic acid [35] and phenobarbital. [36]

Several studies have concerned the metabolic fate of 2-AAF as a means of determining the true nature of the carcinogen. Miller et al [37] discovered that the major metabolite of 2-AAF in the rat was N-hydroxy-2-AAF. Subsequently, Miller and his associates [37,39] proved that this metabolite was more potent in producing tumors of the liver, mammary glands, small intestine, and ear duct of the rat than the parent compound. It also produced more types of tumors than the parent compound. These authors surmised that the N-hydroxy 2-AAF was the proximate carcinogenic agent. Miller et al [40] further reported that N-hydroxy-2-AAF is found in vivo in mice and hamsters treated with 2-AAF, but not in the guinea pig. After giving a single dose of radioactive 2-AAF to the steppe lemming, Weisburger et al [41] found the major, almost exclusive, urinary metabolite to be the 7-hydroxy

derivative. However, treatment for a period of two weeks with 2-AAF gave rise to increased 5-hydroxy and some N-hydroxy derivatives. The lemming eliminated much less of the N-hydroxy metabolite than did the rat. Dyer and Kelly [42] believed from their study in the rhesus monkey that the animal metabolized 2-AAF more like the rat than the guinea pig. Cooper [43] administered 0.05 percent 2-AAF in the diet to 34 female and 20 male mice for 64 weeks. Liver and bladder tumors were found in both sexes; however, there were more liver tumors in the surviving females in contrast to more bladder tumors in the surviving male mice. Wood [44] fed a diet containing 0.05 percent 2-AAF to two strains of mice for periods up to 48 weeks and found tumors of the bladder epithelium and liver. Other authors [45,46] have found tumors after oral administration of 2-AAF to mice.

Enomoto et al [47] fed diets containing 0.03 percent 2-AAF to golden hamsters and diets containing 0.03 percent 2-AAF plus 0.02 percent 3-methylcholanthrene to mice for 10 month periods. Benign tumors of the liver occurred in the hamsters fed 2-AAF whereas malignant tumors occurred in the livers, pancreas, and mesentery of the mice given the combination of 2-AAF plus 3-methylcholanthrene. Oyasu et al [48] gave neonatal hamsters intraperitoneal injections of 2-AAF, 5 mg per 100 g of body weight, three times weekly until weaning. They then fed the animals a diet containing 0.06 percent 2-AAF and 1.6 percent indole. Twenty four (92%) of 26 hamsters surviving for 10 to 12 months developed sarcoma of the bladder. No

hepatomas were observed in the liver. Oyasu and associates [49] also produced bladder cancer in hamsters by intratracheal administration of 2-AAF.

Several investigators [50-52] have produced bladder tumors in rabbits by oral administration of 2-AAF. According to Irving et al [51] only the urinary tract of the rabbit is affected by 2-AAF feeding.

Morris and Eyestone [53] fed five dogs diets containing 2-AAF for periods up to 91 months. Four of the 5 dogs developed liver and urinary bladder tumors in 68 to 91 months on the diet. These 4 dogs received a total of 90 to 198 grams of the carcinogen. The one animal not developing tumors received only 45 grams of 2-AAF. Jabara [54] also reported urinary bladder carcinomas and hepatomas in dogs fed 2-AAF for 40 months.

Peacock and Peacock [55] injected 2-AAF into the crop of Leghorn cockerels twice weekly for 4 months. Seven birds out of 12 (58%) repeatedly injected with 25 mg of 2-AAF in aqueous alcoholic suspension (total dose 1.2 to 3.1 g) developed epithelial tumors in the kidneys and lungs. Campbell [56] also produced epithelial tumors of the oviduct and ovaries of hens given oral doses of 25 mg daily, 6 days a week, for 77 doses and then placed on normal diet until death, or about 4 years.

Dyer et al [57] administered relatively large doses of 2-AAF orally to monkeys for a maximum time of 3 1/2 years without observing

tumors. They found that 2-AAF was rapidly excreted by the monkey and that a large percentage of the administered material was metabolized to the questionably carcinogenic compound, 7-hydroxy-2-AAF, and very little to the potent carcinogen, the N-hydroxy derivative. These facts may explain the apparent resistance of monkeys to 2-AAF. The steppe lemming and guinea pig are also resistant to the action of 2-AAF. Weisburger and associates [41,58] explain this resistance by the difference in the metabolism of 2-AAF in these animals from that of the rat. The potent carcinogenic metabolite, N-hydroxy- 2-AAF, is formed in very small amounts in both the steppe lemming and the guinea pig.

In summary, 2-AAF has been shown to be carcinogenic in rats, mice, rabbits, dogs, hamsters, and fowl. [53] Only two species, the guinea pig and the steppe lemming, of the many studied, are definitely resistant to the carcinogenic action of 2-AAF. This is explained on the basis that these species either do not form, or form only in minor amount, the active carcinogenic metabolite. Recently, Weisburger et al [59] noted from 5 patients treated with a single oral tracer dose of radioactive 2-AAF that man may N-hydroxylate 2-AAF in varying degrees to the potent carcinogenic metabolite, N-hydroxy-2-AAF. From this observation it seems reasonable to conclude that 2-AAF, which has been shown to be carcinogenic in many animal species, is probably carcinogenic in man.

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16. Abstract (Limit: 200 words)

The toxic hazards of exposure to 2-acetylaminofluorene (2-AAF) are discussed. Dietary dose response studies in rats are described in which induction times were determined for tumor development, especially liver and kidney tumors. Other studies are summarized on the incidence of eye, mammary, ear duct, and liver tumors in rats fed 2-AAF, and on the effects of other dietary components on 2-AAF carcinogenicity including casein, tryptophane, indole, vitamin-B6, and liver powder. Cellular and biochemical changes caused by 2-AAF are described, such as development of nodular and non-nodular foci in the liver; increases in glycogen, glucuronyl-transferase, glutathione-reductase, and uridinediphosphoglucose; and decreases in liver catalase, alkaline RNase, and serum alkaline RNase. The effect of hormones on 2-AAF carcinogenicity is discussed, and research findings are reviewed on 2-AAF carcinogenic antagonists, metabolic fate, and species differences in carcinogenic response. The author concludes that 2-AAF has been proven to be carcinogenic in rats, mice, rabbits, dogs, hamsters, and fowl, and should probably be considered as a human carcinogen.

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