Acute Oral Toxicity of *N*-2-Fluorenylacetamide (2-FAA) in Several Strains of Mice[1] (37481)

THOMAS J. HALEY, K. L. DOOLEY, AND J. R. HARMON

Department of Health, Education, and Welfare, Public Health Service, Food And Drug Administration, National Center for Toxicological Research, Jefferson, Arkansas 72079

Weisburger and Weisburger (1) have reviewed the chemistry, carcinogenicity and metabolism of *N*-2-fluorenylacetamide and pointed out the differences in response elicited from different species and strains of animals. Wilson, DeEds and Cox (2) in their original studies on 2-FAA toxicity reported that the compound had little or no acute toxicity but they used only 7 C57 strain mice and did not cover a wide enough dosage range. In order to determine the subacute and chronic toxicities of 2-FAA, it was necessary to obtain the acute oral toxicity of this compound in several strains of mice using both males and females to ascertain any differences in response due to strain and sex.

Materials and Methods. Oral LD_{50}/7 days of 2-FAA was determined in the following strains of mice: CD-1, BALB/cj, C57BL/6j, and C57BL/6Cr. Three hundred mice of each strain and sex were used in each experiment. The weight range of the mice was males 20–38 g and females 18–28 g. They were maintained on laboratory chow in a room temperature controlled to 72 ± 5°. 2-FAA was administered by gavage. Except for the first CD-1 experiment where the 2-FAA was suspended in 10% aqueous polysorbate 80, all other experiments used the same vehicle and agitation with a sonic dismembrator set at 90 for 30 min to achieve a better flowing and more stable suspension. Animals dying acutely during the first 7 days were autopsied at time of death and all survivors were necropsied and examined histopathologically 30 days after dosing. The observed pathological changes were compared with normal controls. The results were analyzed statistically by the

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TABLE I. Oral LD_{50}/7 Days of 2-FAA in Several Mouse Strains.

Expt no.	Strain	No. mice	Sex	LD_{50} and range[a] (g/kg)	Slope and range[a]
1[b]	CD-1	100	M	2.02 (1.89–2.16)	1.19 (1.07–1.32)
	CD-1	100	F	1.87 (1.75–2.00)	1.20 (1.06–1.36)
2[c]	CD-1	100	M	1.08 (1.02–1.14)	1.17 (1.06–1.29)
	CD-1	100	F	1.08 (1.02–1.14)	1.17 (1.06–1.29)
3[c]	C57BL/6Cr	100	M	1.22 (1.16–1.28)[d]	1.11 (0.98–1.25)
	C57BL/6Cr	100	F	1.02 (0.96–1.08)	1.13 (1.0 –1.28)
4[c]	C57BL/6j	100	M	1.23 (1.17–1.29)[d]	1.11 (0.98–1.25)
	C57BL/6j	100	F	0.81 (0.76–0.86)	1.18 (1.07–1.3)
5[c]	BALB/cj	100	M	1.20 (0.98–1.46)	1.17 (0.84–1.64)
	BALB/cj	100	F	1.17 (1.08–1.27)	1.26 (1.09–1.46)

[a] All values at $p = 0.05$.

[b] 10% Aqueous polysorbate 80.

[c] 10% Aqueous polysorbate 80 plus sonication.

[d] Significantly more toxic in females.

TABLE II. Comparison of LD_{50}/7 Day Between Males of Different Strains.[a]

Strains	No. 1 CD-1	No. 2 CD-1	C57BL/6Cr	C57BL/6j	BALB/cj
No. 1 CD-1	—	S	S	S	S
No. 2 CD-1	S	—	S	S	NS
C57BL/6Cr	S	S	—	NS	NS
C57BL/6j	S	S	NS	—	NS
BALB/cj	S	NS	NS	NS	—

[a] S = significantly different at $p = 0.05$; NS = not significantly different.

Litchfield–Wilcoxon method (3).

Results. The symptoms of acute toxicity were lethargy, sedation, loss of righting reflex, circling, orange-red urine, bladder concretions and paralysis with all four limbs rigidly extended. One difference between the two CD-1 experiments was the presence of orange-red urine in the second one: this was attributed to better absorption of the smaller particles produced by the sonic dismembrator. The LD_{50} for 2-FAA in the various mouse strains and sexes are given in Table I. In general the females tended to be more susceptible to the toxic effects of 2-FAA than the males. Moreover, both groups of C57BL/6 females showed significant differences from the males (Table I). Experiment No. 1 CD-1 showed a significantly lesser toxicity in males than the other experiments (Table II). Comparisons of responses between No. 2 CD-1 *vs* BALB/cj; C57BL/6Cr *vs* C57BL/6j or BALB/cj and C57BL/6j *vs* BALB/cj showed no significant differences while all other comparisons between groups were significantly different. Comparison between all female groups and No. 1 CD-1 showed significant differences in response (Table III). No significant difference in response was observed when comparison was made between No. 2 CD-1 *vs* C57BL/6Cr or BALB/cj females.

The histopathological examination of the tissues of all strains indicated that the tissues were essentially normal except for the presence of bladder concretions in 7.5% of the animals. Autopsy of the animals dying acutely showed that the concretions were present 48 hr after administration of 2-FAA.

Discussion. The observation of the orange-red urine confirms the previous report by Wilson, DeEds and Cox (2). Using the Hodge–Sterner (4) toxicity classification system as a basis, 2-FAA falls in the moderately toxic class. Since large doses of 2-FAA are required to produce acute lethality in mice it should not lead to complacency regarding low doses of the chemical. Armstrong and Bonser (5) showed that continuous administration thrice weekly to a total dosage of 3 g in 65 wk resulted in tumors of the urinary bladder, liver and uterus. Wilson, DeEds and Cox (6), Foulds (7), Miller, Miller and Enomoto (8), Wood (9), and Levi *et al.* (10) also observed bladder tumors when 2-FAA was fed at levels varying from 0.03 to 0.5% of the diet for periods of 35 to 379 days. There is also the problem of cumulative toxicity from feeding the chemical for prolonged periods. Wood (9) observed animals dying from 2-FAA prior to the development of liver or bladder tumors. Armstrong and Bonsor (11) also obtained

TABLE III. Comparison of LD_{50}/7 Day Between Females of Different Strains.[a]

Strain	No. 1 CD-1	No. 2 CD-1	C57BL/6Cr	C57BL/6j	BALB/cj
No. 1 CD-1	—	S	S	S	S
No. 2 CD-1	S	—	NS	S	NS
C57BL/6Cr	S	NS	—	S	S
C57BL/6j	S	S	S	—	S
BALB/cj	S	NS	S	S	—

[a] S = significantly different at $p = 0.05$; NS = not significantly different.

similar results and felt that more tumors would have developed if their animals had survived long enough.

Summary. The oral LD_{50}/7 day of 2-FAA has been determined in both sexes of the following strains of mice: CD-1, C57BL/6Cr, C57BL/6j, and BALB/cj. Symptoms of toxicity included: lethargy, sedation, loss of righting reflex, circling, orange-red urine, bladder concretions and paralysis with all limbs rigidly extended. Deaths peaked between 48 and 72 hr. Particle size was shown to affect the LD_{50}/7 day in CD-1 mice. Histopathological examination of the tissues indicated they were essentially normal.

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