CASWELL FILE

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

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AUG 30 1995

6(A)(2)

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA Id No.: 129098 and 050534-EUP-E. Fluazinam: 6(a)2 submission reporting vacuolation of the white matter in the brain and spinal cord.

PC No.: 129098
Barcode No.: D216906
Submission No.: S488873

FROM: John Doherty, PhD 8/7/95
Acting Section Head

and

Guruva Reddy, DVM, Ph.D. 8/7/95
Section IV, Toxicology Branch I
Health Effects Division (7509C)

TO: James Stone/Cynthia Giles-Parker
Product Manager #22
Registration Division 7509C

THROUGH: Marion Copley, DVM 8/19/95
Acting Branch Chief
Toxicology Branch I
Health Effects Division 7509C

I. CONCLUSION

Pathology data for the mouse chronic feeding/ carcinogenicity study with fluazinam indicate that the spinal cord and brain white matter develop vacuolation in response to exposure to in the later months of the study. All dose levels (1000, 3000 and 7000 ppm or approximately 143, 429 and 1000 mg/kg/day) were affected. Females appeared to be more seriously affected and the effects were noted earlier than males. TB-I recommends that no further granting of EUPs for fluazinam be approved until the mouse study is reviewed in its entirety and the rat and dog chronic feeding studies are evaluated for potential neurotoxicity responses to fluazinam.

II. Action Requested and Discussion

The ISK Biosciences Co. (refer to letters from Jerry R. Lucietta, dated May 16, 1995 and the attached letter dated July 26, 1995) has advised the Agency that mice dosed with fluazinam in a chronic feeding/carcinogenicity study develop vacuolation of the white matter in the brain and spinal cord. Refer to copy of the July 26 letter attached. This information indicates that fluazinam is a potentially neurotoxic substance that may result in pathological lesions in the nervous system. No information on the exact identification of this study such as the laboratory, study number and date of initiation were provided at this time.

Toxicology Branch I (TB-I) cannot further evaluate this phenomena based on the information provided. The data raise the question if rats and dogs also develop neuropathological lesions in response to treatment with fluazinam.

Demonstration of neurotoxicity especially pathological changes is a trigger for special review. Thus, TB-I recommends that no new EUPs for products containing fluazinam be granted until the mouse chronic feeding/carcinogenicity study demonstrating these neuropathological lesions is reviewed in its entirety and the rat and dog chronic feeding studies are evaluated for evidence of neuropathology.

ISK BIOSCIENCES™

July 26, 1995

Dr. John Doherty
Toxicology Branch I
Health Effects Division (7509C)
U.S. Environmental Protection Agency
401 M Street SW
Washington D.C. 20460
fax: (703)395-5147

Dear Dr. Doherty:

With regard to your fax dated July 18, 1995 and further to my letter dated July 19 please find attached a draft summary and incidence tables relative to additional information you requested from a mouse study conducted on IKF-1216 (Fluazinam).

I am also sending this information by mail in case the faxed copy is difficult to read/

If you should have any question, please do not hesitate to contact me.

Very Yours Truly,

J. R. Lucietta

Jerry R. Lucietta
Manager Regulatory Affairs
ISK Biosciences Corporation

JRL/hab

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ISK Biosciences Corporation
5966 Heisley Road, P.O. Box 8000, Mentor, Ohio 44061-8000, U.S.A.
216/357-4100 · FAX: 216/354-9506

Females

Dosage level (ppm)		Control			1000		3000		7000		
Kill		I	D	T	D	T	D	T	I	D	T
Mice with altered hepatocyte foci	Total	0	1	2	0	4	1	6	3	5	10**
Mice with hepatocyte enlargement (any zone)	Total	0	1	0	8**	10**	13**	22**	13**	22**	9**
Mice with hepatocytes with pale/vacuolated cytoplasm (any zone)	Total	0	0	0	0	11**	4*	20**	12**	16**	12**
Focal swollen/vacuolated hepatocytes		0	0	2	0	1	0	5	0	0	5*
Brown pigment in centrilobular hepatocytes		0	0	0	0	0	0	0	0	1	0
Parenchymal inflammatory cells (Minimal or moderate)		0	2	0	0	0	1	1	0	4	1
Aggregates of macrophages containing brown pigment (mainly centrilobular)		3	7	3	14*	13**	20**	21**	13**	36**	10**
	Total	2	3	2	5	5	4	6	2	4	3
	Trace	1	3	0	8	8**	10*	9**	8**	14**	6**
	Minimal	0	1	0	1	0	6*	4*	2	16**	1
	Moderate	0	0	1	0	0	0	2	1	2	0
	Marked										
Number of mice examined		12	27	29	24	26	23	27	13	42	13

* $p < 0.05$, ** $p < 0.01$ with Fisher's Exact test

Altered hepatocyte foci includes basophilic/(and vacuolated), eosinophilic/(and vacuolated), clear cell, and altered (on ORO section) hepatocytes

Central nervous system - Treatment-related vacuolation of white matter, sometimes widespread, in brain and cervical spinal cord was seen in a large number of mice receiving 3000 or 7000 ppm, and in a few mice receiving 1000 ppm. The incidences and degrees of the lesions were dose-related. In the high dose group, with the exception of four male mice dying in the first year of the study (Weeks 26 to 47), moderate vacuolation of white matter was first seen in female mice at Week 59 and in male mice at Week 79; the marked grade was first seen in female mice at Week 76 and in male mice at Week 87; and the severe grade was first seen in females at Week 81 and in males at Week 91. The vacuolation seems therefore to increase in degree with time, and affect females earlier than males. Electron microscopic examination of tissue from four affected mice, showed the vacuolation seen by light microscopy to be marked myelin oedema. The severity of the CNS lesions did not correlate well with the severity of the liver pathology.

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"Vacuolation of cerebellar white matter" has been reported separately, because it was seen in untreated control mice. This finding is different from the treatment-related vacuolation; in the treatment-related lesion, vacuolation was particularly prominent in white matter of the cerebrum (especially in the corpus callosum and optic tract), as well as being present elsewhere in the brain, including the cerebellum. The treatment-related vacuolation masked the background vacuole in the cerebellum, hence the apparently decreased incidence of vacuolation of cerebellar white matter in mice receiving 3000 or 7000 ppm compared with controls. Male mice receiving 1000 ppm showed a statistically significant increase in this finding compared with controls, but no such effect was seen in female mice receiving 1000 ppm. The increased incidence in males at this dosage was mainly of the 'trace' grade. This may be fortuitous, or may possibly represent a treatment-related exacerbation of a spontaneous change.

Males

Dosage level (ppm)		Control			1000		3000			7000	
Kill		I	D	T	D	T	D	T	I	D	T
Brain											
Widespread vacuolation of white matter	Total	0	0	0	0	0	1	0	0	7**	10**
	Marked	0	0	0	0	0	1	0	0	5*	9**
	Severe	0	0	0	0	0	0	0	0	2	1
Vacuolation of white matter	Total	0	0	0	0	1	17**	20**	16**	16**	5*
	Trace	0	0	0	0	1	8**	3	4	4	0
	Minimal	0	0	0	0	0	9**	10**	10**	16**	0
	Moderate	0	0	0	0	0	0	7**	2	6*	5*
Vacuolation of cerebellar white matter	Total	0	4	8	8*	23**	5	1	0	0	0
	Trace	0	3	7	4	19*	3	0	0	0	0
	Minimal	0	1	1	4	4	2	1	0	0	0
Spinal cord											
Widespread vacuolation of white matter	Total	0	0	0	0	0	1	0	0	5*	7**
	Marked	0	0	0	0	0	1	0	0	3	6**
	Severe	0	0	0	0	0	0	0	0	2	1
Vacuolation of white matter	Total	0	0	0	0	0	1	12**	8**	12**	8**
	Trace	0	0	0	0	0	1	9**	7**	5*	1
	Minimal	0	0	0	0	0	0	3	1	4	4*
	Moderate	0	0	0	0	0	0	0	0	3	3
Number of mice examined		14	38	18	24	26	29	21	16	39	15

* $p < 0.05$, ** $p < 0.01$ with Fisher's Exact test

Females

Dosage level (ppm)		Control			1000		3000		7000		
Kill		I	D	T	D	T	D	T	I	D	T
Brain											
Widespread vacuolation of white matter	Total	0	0	0	0	0	1	2	0	10**	11**
	Marked	0	0	0	0	0	0	2	1	4	10**
	Severe	0	0	0	0	0	1	0	0	6*	1
Vacuolation of white matter	Total	0	0	0	0	5*	12**	23**	12**	27**	2
	Trace	0	0	0	0	2	5*	4*	1	5	0
	Minimal	0	0	0	0	2	4*	14**	6**	11**	0
	Moderate	0	0	0	0	1	3	5*	5*	11**	2
Vacuolation of cerebellar white matter	Total	9	3	24	7	20	0	2	0	1	0
	Trace	8	2	17	6	15	0	1	0	1	0
	Minimal	1	1	7	1	5	0	1	0	0	0
Spinal cord											
Widespread vacuolation of white matter	Total	0	0	0	0	0	1	0	0	9**	5**
	Marked	0	0	0	0	0	1	0	0	4	5**
	Severe	0	0	0	0	0	0	0	0	5	0
Vacuolation of white matter	Total	0	0	0	0	1	3	14**	10**	10**	8**
	Trace	0	0	0	0	1	2	5*	5*	3	1
	Minimal	0	0	0	0	0	1	6**	4	5	0
	Moderate	0	0	0	0	0	0	3	1	2	7**
Number of mice examined		12	27	29	24	26	23	27	13	42	13

* $p < 0.05$, ** $p < 0.01$ with Fisher's Exact test

Other findings

Heart - Statistically significant increased incidences of atrial thrombi were recorded in decedent treated mice of both sexes compared with controls. Atrial thrombi were also seen in a few male mice receiving 7000 ppm killed after 78 weeks. In most cases, the thrombi were visible macroscopically. They were generally associated with moderate or marked amyloidosis in the heart. At termination, only a single male mouse receiving 7000 ppm showed an atrial thrombus, therefore microscopic examination was not extended to mice receiving 1000 or 3000 ppm. The toxicological importance of the increased incidence of atrial thrombi in decedent mice is uncertain.

TABLE 9

(Microscopic pathology - incidence summary of non-neoplastic findings - continued)

Males on study	Group 1		Group 2		Group 3		Group 4	
	70		50		50		70	
Animals completed	Decedent 38	Terminal 18	Decedent 24	Terminal 26	Decedent 29	Terminal 21	Decedent 39	Terminal 15
Spinal Cord								
Examined	38	18	24	26	29	21	39	15
No abnormalities detected	33	9	21	25	26	9	21	0
Widespread vacuolation of white matter (Total)	0	0	0	0	1	0	5	7
Marked	0	0	0	0	1	0	3	6
Severe	0	0	0	0	0	0	2	1
Vacuolation of white matter (Total)	0	0	0	0	1	12	12	8
Trace	0	0	0	0	1	9	5	1
Minimal	0	0	0	0	0	3	4	4
Moderate	0	0	0	0	0	0	3	3
Nerve fibre degeneration (Total)	5	9	3	1	1	2	0	1
Trace	2	2	1	1	1	0	0	0
Minimal	3	6	2	0	0	2	0	0
Moderate	0	1	0	0	0	0	0	1
Necrosis and inflammation (Total)	0	0	0	0	0	0	1	0
Minimal	0	0	0	0	0	0	1	0
Brain								
Examined	38	18	24	26	29	21	39	15
No abnormalities detected	24	7	14	2	6	0	5	0
Widespread vacuolation of white matter (Total)	0	0	0	0	1	0	7	10
Marked	0	0	0	0	1	0	5	9
Severe	0	0	0	0	0	0	2	1
Vacuolation of white matter (Total)	0	0	0	1	17	20	26	5
Trace	0	0	0	1	8	3	4	0
Minimal	0	0	0	0	9	10	16	0
Moderate	0	0	0	0	0	7	6	5
Vacuolation of cerebellar white matter (Total)	4	8	8	23	5	1	0	0
Trace	3	7	4	19	3	0	0	0
Minimal	1	1	4	4	2	1	0	0
Vacuolation in diencephalon (Total)	1	0	0	0	0	0	0	0
Minimal	1	0	0	0	0	0	0	0
Encephalomalacia (Total)	1	0	0	0	1	0	0	0
Minimal	1	0	0	0	0	0	0	0
Moderate	0	0	0	0	1	0	0	0
Mineralisation in diencephalon (Total)	7	7	4	7	3	1	0	2
Trace	2	3	3	5	3	1	0	2
Minimal	5	4	1	2	0	0	0	0
Necrosis and inflammation (Total)	1	0	0	0	0	0	1	0
Minimal	1	0	0	0	0	0	1	0
Infarction in cerebrum (Total)	1	0	0	0	0	0	0	0
Marked	1	0	0	0	0	0	0	0
Thrombus	1	0	0	0	0	0	0	0
Mononuclear cells in choroid plexus (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Dorsal compression	0	0	0	1	0	0	0	0

TABLE 9

(Microscopic pathology - incidence summary of non-neoplastic findings - continued)

Females on study	Group 1		Group 2		Group 3		Group 4	
	70		50		50		70	
Animals completed	Decedent 27	Terminal 29	Decedent 24	Terminal 26	Decedent 23	Terminal 27	Decedent 42	Terminal 13
Spinal Cord								
Examined	27	29	24	26	23	27	42	13
No abnormalities detected	25	14	21	24	16	8	22	0
Widespread vacuolation of white matter (Total)	0	0	0	0	1	0	9	5
Marked	0	0	0	0	1	0	4	5
Severe	0	0	0	0	0	0	5	0
Vacuolation of white matter (Total)	0	0	0	1	3	14	10	3
Trace	0	0	0	1	2	5	3	1
Minimal	0	0	0	0	1	6	5	0
Moderate	0	0	0	0	0	3	2	7
Nerve fibre degeneration (Total)	1	15	2	1	2	7	0	2
Trace	0	7	0	0	2	1	0	0
Minimal	1	7	2	1	0	6	0	2
Moderate	0	1	0	0	0	0	0	0
Gliosis (Total)	0	0	0	0	1	0	0	0
Minimal	0	0	0	0	1	0	0	0
Perivascular mononuclear cells (Total)	0	0	1	1	0	0	0	1
Trace	0	0	0	1	0	0	0	0
Minimal	0	0	1	0	0	0	0	1
Monouclear cells in meninges (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1
Brain								
Examined	27	29	24	26	23	27	42	13
No abnormalities detected	19	5	16	0	9	0	4	0
Widespread vacuolation of white matter (Total)	0	0	0	0	1	2	10	11
Marked	0	0	0	0	0	2	4	10
Severe	0	0	0	0	1	0	6	1
Vacuolation of white matter (Total)	0	0	0	5	12	23	27	2
Trace	0	0	0	2	5	4	5	0
Minimal	0	0	0	2	4	14	11	0
Moderate	0	0	0	1	3	5	11	2
Vacuolation of cerebellar white matter (Total)	3	24	7	20	0	2	1	0
Trace	2	17	6	15	0	1	1	0
Minimal	1	7	1	5	0	1	0	0
Encephalomalacia (Total)	0	0	0	0	1	0	2	0
Minimal	0	0	0	0	1	0	1	0
Moderate	0	0	0	0	0	0	1	0
Mineralisation in diencephalon (Total)	2	0	0	8	1	3	1	4
Trace	2	0	0	5	1	3	0	4
Minimal	0	0	0	3	0	0	1	0
Necrosis and inflammation (Total)	0	0	0	0	1	0	0	0
Minimal	0	0	0	0	1	0	0	0
Periarteritis	0	0	1	0	0	0	0	0
Necrosis in hippocampus (Total)	0	0	0	0	1	0	1	0
Moderate	0	0	0	0	1	0	1	0
Dilated ventricles (Total)	0	0	0	0	0	0	1	0
Minimal	0	0	0	0	0	0	1	0
Perivascular mononuclear cells (Total)	0	0	0	1	0	1	0	1
Minimal	0	0	0	1	0	1	0	1
Mononuclear cells in meninges (Total)	0	0	0	0	0	0	0	1
Minimal	0	0	0	0	0	0	0	1

IV. Studies Reviewed

Study Identification	Material	MRID No.:	Results	Classification
85-2. Carcinogenicity - mice [Exact identification of study not yet identified]	Technical fluazinam	Not assigned	6(a)2 submission as of May 16, 1995 indicates that mice have vacuolation of the white matter in the brain and spinal cord.	~~Reserved~~ Supplemental