DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

JCG 9-15

FAP 3B36

Memorandum

Date September 9, 1997

From Division of Health Effects Evaluation (HFS-225)

Subject Acrylamide, new information and re-evaluation of the neurotoxicity potential and tentative ADI of acrylamide as a migrant.

To Division of Product Policy (HFS-205)
Attn: Dr. Rosalie Angeles

Division of Petition Control (HFS-215)
Attn: Dr. Daniel Harrison

Through, Francis Lin, Ph.D. F. Lin 9-9-97
Team Leader, Division of Health Effects Evaluation (HFS-225)

Through David Hattan, Ph.D. David S. Hattan 9/11/97
Director, Division of Health Effects Evaluation (HFS-225)

Food Additive Petition Nos. 9A4175 McKenna, Conner and Cuneo
1575 Eye Street, N.W.
Washington, D.C. 20005

3B3677	Calgon
6B3940	American Cyanamide Co.
3B3696	National Starch and Chemical Co.
9B4131	Lubrizol Co.
9B4132	Lubrizol Co.
9B4133	Lubrizol Co.

Our memorandum of Jan. 22, 1990 (Bleiberg/Harris, HFF158/HFF-334 re FAP 9A4175), evaluated the safety of acrylamide (AA) as a migrant from dimethylamine-epichlorohydrin resin (DEC) and acrylic acid - acrylamide resin (AAR) for immobilization of glucose isomerase. Since the issues relating to the carcinogenicity of AA and the quantitative assessment of upper-bound risk are being resolved, as well as completion of the ongoing review of the reproduction and developmental toxicity data, the issue of the ADI for AA based on the neurotoxicity remains. This memo reviews the previously established ADI of AA

in the context of new information and provides a re-evaluation of the neurotoxicity potential and a tentative ADI of acrylamide as a potential migrant from approval of pending new food additive petitions. The ADI will remain tentative pending completion of the ongoing contract review of the reproduction and developmental toxicity data on acrylamide.

Prior to the Jan. 22, 1990 memo referred to above, the accepted NOEL for AA quoted in the reviews of earlier food additive petitions, 0.3 mg/kg/d, was based on its neurotoxicity, and was derived from data stated to be based on a study in cats, submitted with FAP 0A0388. For example, see the memo of Carson/-McGowan, 12/23/74, re FAP 4H3005. Studies were conducted in cats and monkeys[1] to establish the neurotoxic dose of acrylamide. Appendix 1, attached reviews this study in detail.

The studies may be summarized as follows:

Two cats each (12 cats) received oral doses of acrylamide at levels of 0.03, 0.1, 0.3, 1, 3, or 10 mg/kg, respectively, for 5 days/week. At the highest dose level (10 mg/kg), the treated cats showed definite limb weakness at 26 days, leading to loss of control at 40 days, at which time dosing was stopped. At 85 days, there were definite signs of recovery. "No-effect" was noted at dose levels of 0.03 to 0.1 mg/kg, and one cat at 0.3 mg/kg showed no grossly observable effects during 1 year of acrylamide dosing. A similar study was also conducted in rhesus monkeys. However, close re-examination of the data reveals that in this study cats at the NOEL and lower doses only survived for 3 to 4 months. In addition cats were dosed 5 days/week. For these reasons this was not a good study and in my previous review I gave the no-effect level as 0.03 mg/kg/d. The "no-effect" level in monkeys was determined as 1.0 to 3.0 mg/kg/day.

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[1] The neurotoxic potential of acrylamide as a constituent of polyacrylamides was considered in the publication: "Role of the Food and Drug Administration in Regulation of Neuroeffective Food Additives", by Hattan, D.G. et al, in *Nutrition and the Brain*, Vol. 6., ed by Wurtman, R.J. and Wurtman, JJ, Section 2. By Marvin J. Bleiberg, Indirect: acrylamide and polyacrylamides, pp54 - 59

The issue of a safe exposure level to acrylamide has also been a concern of the Environmental Protection Agency and the Centers for Disease Control (CDC), National Institute for Occupational Safety and Health (NIOSH). CDC issued a report: Acrylamide: a review of the literature[2] setting forth a review of the studies which served as a basis for setting an occupational health standard. Table 6 of this review, "Key animal studies of the neurotoxic effects of subchronic and chronic exposure to acrylamide" was adopted from an EPA report issued in 1988: "Preliminary assessment of health risks from exposure to acrylamide."

EPA Office of Pollution Prevention and Toxics later reevaluated the toxicology of AA, and issued a document: Chemical Summary for Acrylamide, Sept. 1994 (See Attached document).

EPA has examined a number of published and unpublished studies on AA. The EPA oral reference Dose (Rfd, equivalent to our ADI) for AA based on nerve damage, is derived from a study by Burek, J.D., R.R. Albee, J.R. Beyer et al., 1980: "Subchronic toxicity of acrylamide administered to rats in drinking water for 92-93 days followed by up to 144 days of recovery, J. Environ. Pathol. Toxicol. 4: 157-182. The criteria for setting the NOEL in the Burek et al. study was light and electron microscopic examination of sciatic nerve tissue. This study is reviewed in Appendix 2 attached to this memorandum.

An ultrastructural study was conducted by Schaumberg, H.H. et al (1974)[3]. Tissue was sampled from the limbs of cats through various stages of intoxication, including two cats which received AA for 252 to 294 days at a level of 3 mg/kg in the drinking water. These cats exhibited clinical signs of gait disorder by 70 days. The authors described lesions of peripheral sense organs as well as peripheral nerve.

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[2] NIOH and NIOSH basis for an occupational health standard, Acrylamide: a review of the literature, 1991, Publication No. PB 92-133222, National Technical Information Service, Springfield, Va., 22161

[3] Schaumberg, H.H., Wiśniewski, H.N., and P.S. Spencer, Ultrastructural Studies of the Dying-Back Process, 1. Peripheral Nerve Terminal and Axon Degeneration in Systemic Acrylamide Intoxication, J. Neuropath. Exp. Neurol. 33, 260-84, 1974.

The NOEL for AA was determined by EPA to be 0.2 mg/kg/d. My review agrees with this assessment. Using an uncertainty factor of 1,000 (equivalent to a safety factor) gives the Rfd (ADI) of 2 x 10^{-4} mg/kg/d or 0.2 ug/kg/d. For a 60 kg adult, this is 12 ug/p/d as a safe exposure. However, the ADI of 12 ug/p/d should be regarded as tentative pending completion of contractor's review of the reproductive and developmental toxicity data on AA.

EPA noted that a chronic study conducted by Dow Chemical Co. (1985) in rats gave similar results. However, it was not selected for use in the Rfd calculation, since the NOEL was found using light microscopy, said to be a fairly insensitive measure of structural integrity of nerve vs electron microscopy. Additionally, the Burek et al. study, selected for the Rfd determination, allowed a significant period for recovery from nerve damage, in a satellite group of rats.

A recent publication, Crofton, K.M. et al.,(1996)[4] describes research conducted by EPA at its laboratory at Research Triangle Park. The authors note that health agencies are often required to predict the effects of long-term, low-level exposures based on animal data involving short-term, low-level exposures. The aim of the study was to evaluate the adequacy of short-term exposures to acrylamide using intra-peritoneal injection for predicting neurotoxicity produced by long-term exposures. This study is not directly applicable to determining the NOEL of AA by oral administration. Both behavioral endpoints and histopathologic examination of sciatic nerve were performed. Internal and target tissue doses of acrylamide were measured. Functional and pathological results demonstrated that effects of acrylamide depended on dose-rate and that the neurotoxicity of acrylamide was less than that predicted by a strict dose x time relationship.

Behavioral endpoints showed both qualitative and quantitative changes as a function of dose-rate. Recovery of behavioral function in these studies was independent of the duration of

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[4] Crofton, K.M. et al.(1996) The Impact of Dose Rate on the Neurotoxicity of Acrylamide: The Interaction of Administered Dose, Target Tissue Concentrations, Tissue Damage and Functional Effects, *Toxicology and Applied Pharmacology*, **139**, 163-176.

dosing. Because duration of dosing had no impact on the kinetics of acrylamide, the authors state: "these data indicate that the toxicity of acrylamide is not due to an accumulation of acrylamide in the target tissue. The less than strict cumulative toxicity of acrylamide may result from an interaction between administered dose, tissue damage, and repair processes." This study gives some assurance that the NOEL for neurotoxicity determined in a 90 day study will not grossly overestimate a safe dose if dosing were continued for a longer interval.

Safety Evaluation

The EDI estimates for Acrylamide, for the pending petitions are as follows:

Petition No.	EDI	
9A4175	41 ng/d =	0.041 ug/p/d
3B3677	110 ng/d =	0.110 ug/p/d
6B3940	Substitutional	
3B3696	110 ng/d =	0.110 ug/p/d
9B4131	0.6 ng/d =	0.0006 ug/p/d
9B4132	0.15 ng/d=	0.00015 ug/p/d
9B4133	0.15 ng/d=	0.00015 ug/p/d

Total EDI for petitioned uses: 0.2573 ug/p/d

The total (cumulative) EDI for all uses of acrylamide under § 176.180 and 175.105 are stated to be less than 0.15 ng/p/d for each use (Chemistry memo of 4/28/94) and are stated to be extremely conservative. As such, these petitions could be safely regulated provided that the tentative ADI of 12 ug/p/day can be affirmed following completion of the ongoing contract review of the reproductive and developmental toxicity data on AA.

We also await the CAC assessment of carcinogenicity and QRAC assessment of upper bound-risks for AA before completing the toxicology evaluation of FAP 9A4175.

Marvin J. Bleiberg, Ph.D., D.A.B.T.

CC: HFS-200, HFS-225(Hattan, Biddle, Lin, Edwards), HFS-247
HFS-225:MJBleiberg:R/D:7/6/97,Rev:8/6/97,F/T:9/9/97:DOC9a4175aa

Attachments
1. Review of Publication: Neurotoxicity of acrylamide; Studies of McCollister, D.D., Oyen, F., and V.K. Rowe; Toxicology and Applied Pharmacology, 6, 172-181, 1964.

2. Review of Publication: Subchronic Toxicity of Acrylamide administered to rats in the Drinking Water Followed by up to 144 Days of Recovery. Burek, J.D. et al., *Jour. Environmental Pathology and Toxicology* 4, 157-182, 1980.

Appendix 1. Review of Publication: Neurotoxicity of acrylamide; Studies of McCollister, D.D., Oyen, F., and V.K. Rowe; Toxicology and Applied Pharmacology, 6, 172-181, 1964.

Test Material

A white powder obtained from American Cyanamide Co. was stated to be 99+ % pure acrylamide. In some studies the monomer was administered with a powdered polymer of molecular weight of 1,000,000.

Chemical formula

$CH_2=CHCONH_2$

Methods

A series of studies were conducted in rats, cats, and monkeys by several routes of administration. An acute oral toxicity study was conducted in rats. The focus of this memo will be on the oral route of administration to establish a NOEL for neurotoxicity.

1. Short term dietary feeding in rats:

Rats - Dow Wistar strain, males and females, approximately 60 days of age at start of study.
No. of Rats /Dose group: 10M + 10 F.

Dietary level (ppm)
0 (control) 3, 9, 30, 70, 90, 110, 300, 400 ppm
Dose level (mg/kg/d)
0, 0.3, 0.9, 3, 7, 9, 11, 30, 40 mg/kg/d
Duration of feeding
Diet level: 3, 9, and 90 ppm: 90 days - at which time some rats were sacrificed and subjected to necropsy and others were placed on control feed and allowed to recover up to 90 days.

Dietary level 71 - 110 ppm: Rats were continued on diets for 189 days.

Observations:

Appearance, behavior, signs of neurotoxicity (not otherwise described).
Food consumption, growth and mortality.

At necropsy the lungs, heart, liver, kidneys, spleen, brain, and testis were removed and weighed. Portions of these organs, as well as pancreas, adrenal, and spinal cord were prepared in fixative for histopathologic examination. Some affected rats were sacrificed in extremis and subjected to necropsy while others were placed on control diets to note possible recovery from neurotoxic signs.

Results
Rats sacrificed at 90 days-No adverse effects were observed in rats on 3, 9, or 30 ppm diets.

Rats receiving 70 or 110 ppm AA for 189 days - no neurotoxic signs in regard to locomotor activity or balance on rear legs.

A male rat receiving 90 ppm diet at 56 days lost use of hindquarters but reacted to pain stimulus.

A male rat receiving 300 ppm AA as a residue in polymer lost use of hindquarters at 21 days. This was seen in 4 males and one female in the 90-day experimental period. Toxic signs became severe by 42 days. At this time some rats were therefore placed on control diets to see if recovery was possible. By 99 days there was no evidence of toxicity.

Microscopic examination of the spinal cords of male rats on 400 ppm diets sacrificed at 42 days showed no adverse effects. A no-effect level for neurotoxic signs could be given as 70 ppm or 7 mg/kg/d.

2. Cat studies
Number of controls - 3 cats started, two died early in study (25 and 30 days).

Treatment - 2 cats/dose level x 6 dose levels and control
Basic ration - one-half can dog food
AA was added to ration 5 days per week. No weekend dosing.

Observations - weighed once/week; hematology and blood cholinesterase levels before necropsy. Necropsy with gross examination of organs and tissues saved for histopathology.

Effects

0.03, 0.1 and 0.3 mg/kg/d. Cats at 0.03 and 0.1mg/kg/d succumbed to spontaneous disease before 108 days with no neurological signs. A cat at 0.3 mg/kg/d survived a year of dosing with no adverse effects noted. The other cat at this dose level was found dead at 106 days.

1 mg/kg/d
One cat sacrificed after 152 days was stated to have suffered no adverse effects from AA. The other cat was carried for a full year experimental period. Observations: 26 days some "twitching" apparent in hindquarters. At 335 days considerable "stretching in hindquarters" present. At 367 days stated to appear normal.

3 mg/kg/d
Two cats receiving this dose 5 days/wk survived the dose. At 26 days twitching motions in hindquarters; 47 days slightly unsteady when walking; 68 days definite weakness in hindquarters. At 240 days still weak, at 367 days still weak.

10 mg/kg Definite neurotoxic symptoms with weakness in hindquarters and inability to walk.

No effect level- Tentatively 0.3 mg/kg/d; however, one of the two cats at this dose level was sacrificed at 105 days. At 1 mg/kg/d, also only one cat survived one year. The investigators claim that the surviving cat appeared normal at 367 d., some indications of neurotoxicity were seen at earlier time intervals. It is also not clear why cats at lower doses succumbed before the end of the dosing period but cats at higher doses survived. Therefore, this study must be considered flawed. Since the study involved only five days per week dosing it has only limited utility in setting NOELS. Histopathologic examination of brain and spinal cord failed to demonstrate evidence of adverse effects at any dose level.

Monkeys

Gross observations included body weights and periodic examinations for signs of neurologic deficits by twelve main nervous responses plus sharp pain, proprioception, Babinski reflex, knee jerk, and grabbing.

Controls - Two monkeys
Dose levels from 0.1 to 10 mg/kg/d

Dose level	No. Of monkeys	days on AA	Adverse effects
0.1 mg/kg/d	One	363	None
0.3 " " "	Two	363	None
1.0 " " "	One	363	None
3.0 " " "	One	363	Occasional/isolated effects on reflexes
10 " " "	One	69	41 d.- questionable 48 d.-very weak hindquarters; recovery by 123 days.

One monkey, received 0.1 mg/kg/d AA for 235 doses in 349 days, the dose was then increased to 5 mg/kg/d for 63 doses in 88 days, then 7.5 mg/kg/d for 19 doses in 28 days, and again increased to 10 mg/kg/d for 48 doses 66 days. At the 10 mg/kg level the hind legs became weak and the animal became moribund. The monkey was restored to the control chow but expired. The no-effect level in monkeys lies between 1.0 and 3.0 mg/kg/d.

Conclusion

Cats were the most susceptible species to the neurotoxic effects of AA. Since these studies are based on five day a week exposure, thus allowing for weekend recovery periods, and since some cats receiving low dose levels died from intercurrent disease, a true no-effect level lies between 0.03 and 0.3 mg/kg/d.

The authors recommend that in considering applications to which the public may be exposed daily to acrylamide the total absorption not exceed 0.0005 mg/kg/d (for a 60 kg adult this would be 30 ug/p/d). They state that this provides a large margin of safety (1,000-fold) but "such a margin seems justified on the basis of the serious consequences of overexposure."

Appendix 2 - Review of Publication: "Subchronic Toxicity of Acrylamide administered to Rats in the Drinking water followed by up to 144 days of recovery." J.D. Burek et al., *Jour. Environmental Pathology and Toxicology* 4, 157-182, 1980

The focus of this review is on the neuro-pathological findings.

Materials and methods

Test material - The acrylamide sample used was obtained from Eastman Organic Chemicals, Rochester, N.Y. The purity was greater than 99%. The contaminants included 0.5 ppm acetone + acetonitrile, 1.3 ppm acrylonitrile, 1 ppm oxazole and 510 ppm ethyl acetate.

Rats - Fischer-344 derived CDF rats from Charles River Breeding labs., Wilmington, MA. Males and females approximately 4 weeks of age, weighing between 50-60 grams.

Experimental Design:

Dose of Acrylamide mg/kg/d	Total No of rats Males Females	No. Sacrificed at 90 days Males Females	Number held for Recovery Males Females	Additional rats for interim kill Males
0	26 10	10 10	10 10	6
0.05	23 10	10 10	10 10	3
0.2	23 10	10 10	10 10	3
1.0	23 10	10 10	10 10	3
5.0	23 10	10 10	10 10	3
20.	29 10	10 10	10 10	9

Observations

Rats were observed during the work week for health status and signs of possible toxicity.

Neuropathy was evaluated by the landing foot-spread method (hind limb splaying) of Edwards and Parker, 1977.[5] This procedure was conducted on 4 rats per group and repeated 3 times per rat. Once a clear effect was found on the high-dose level rats (20 mg/kg/d), they were no longer dropped. They were monitored by clinical appearance. Rats at the next dose level (5 mg/kg/d) were then monitored. The authors state that because of a lack of response at this level, the lower levels were not monitored.

In addition to neurological testing, body weights and water consumption were monitored weekly. Hematology was evaluated on blood samples taken from 7 rats/sex from control and high-dose groups at day 76. Blood serum was collected from rats at necropsy by decapitation for BUN, alkaline phosphatase, SGPT and serum cholinesterase. A complete necropsy exam was conducted on rats in the basic 90 day study and those held for the recovery phase.

Electron Microscopy Studies.

The sciatic nerves of male rats were submitted for electron microscopic examination after fixation with paraformaldehyde and glutaraldehyde, post-fixation with osmium tetroxide, dehydration and Epon 812 infiltration. Sections were stained with lead citrate and uranyl acetate. Representative electron micrographs were taken, developed and printed. Ultrastructural alterations were counted with a minimum of 50 fields/block.. A field refers to a section through any Schwann cell with or without its associated myelinated axons.

Statistical Evaluation

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[5] Edwards, P.M. and Parker, V.H., "A simple sensitive and objective method for the early assessment of acrylamide neuropathy in rats" *Toxicology and. Applied Pharmacology*, 1977 **40**:589-591.

Quantitative data on laboratory measurements were statistically evaluated using a one-way Analysis of Variance followed by Dunnett's test with $p<0.05$ as the level of statistical significance.

Results- Part I (90-Day Toxicity phase)

Body weights

No body weight tables are provided. According to the report, there were no statistical differences noted when comparing mean weekly body weights for dose- levels between 0.05 mg/kg/d and 5 mg/kg/d to respective controls for males and female dose-groups. There was a marked decrease in body weight gain at the 20 mg/kg/d level, which was statistically different at the 13th day for males and at the 20th day for females.

Water consumption.

No water consumption tables are provided. Water consumption was marginally decreased in the male high-dose group, but only sporadically to a statistically significant extent. The female high-dose group showed a significant depression of water consumption from the 21st to 90th day of the study.

Landing foot Spread Test, Clinical Signs

For the high-dose level (20 mg/kg/d) statistically significant increases in the landing foot spread measurements were noted on day 22 of the study for both males and females. This became more severe at day 29 and therefore discontinued for this group. No landing food spread effect was noted at 5 mg/kg/d or lower dose-levels. All rats at the high-dose level were dragging their hind legs by the end of the study. No changes in clinical condition were detected at lower dose levels.

Clinical Chemistry, Hematology, Urinalysis

Tabular data are not presented. Cholinesterase levels were said to be depressed in the high level rats. The hematology values in high-dose rats were said to reveal decreases in PCV, rbc and Hbg

levels at days 76 and 93. Urinalysis values are reported to show no abnormalities.

Gross Necropsy and Histopathology

Several observations made at gross necropsy and histopathology were considered dose-related. These were related to poor grooming and loss of body weight and emaciation and loss of control of urination and defecation and were principally seen in high-dose level rats.

Neurologic treatment-related effects were principally observed in peripheral nerves and spinal cord in rats of the 20 mg/kg/d groups and the peripheral nerves in the 5 kg/kg/d groups. Histopathologically, the spectrum of nerve tissue alterations included lesions typical of both axon and myelin degeneration. Special staining techniques (Luxol Fast Blue/PAS, Bodian's Stain) were used in addition to hematoxylin and eosin to better characterize the nerve damage.

Classification of nerve damage

(1) Severe - Approximately 50 % or more of nerve fibers affected
(2) Moderate - Approximately 20 - 50 % of nerve fibers affected
(3) Slight - Less than 20 % affected
(4) Very slight - When focal or multifocal changes were seen in individual nerves.
(5) equivocal - Nerve could not be graded as entirely normal.

A tabulation based on this classification was not furnished

At 90 days, all rats on 20 mg/kg/d exhibited moderate to severe peripheral nerve lesions.. The lesions of all the females and half the males were classified as severe. Peripheral nerve lesions were also seen in most rats (male and female) of the 5 mg/kg dose group. These were said to be much less severe and ranged from equivocal to very slight. No peripheral nerve lesions were observed in rats of the 0.05, 0.2 or 1 mg/kg/d groups.

Spinal cord sections from the cervical, thoracic and lumbosacral regions were examined with H&E and special stains. Degenerative myelopathy was found in the dorsomedial funiculi (fasiculus gracilis) of one or all spinal cord sections in 5 of 10 males and

9 of 10 females at the high-dose level. No treatment related effects were seen in transverse sections through the brain.

Electron Microscopic Studies

The principal findings seen on EM examination of peripheral nerves are related to cellular debris associated with degeneration of Schwann cells and demyelinization. The findings are tabulated as axolemma invaginations, axolemma invaginations with cell organelles and/or cell bodies, Schwann cells without axons and with/or without degenerating myelin, Table 1 of the report (attached to this review) summarizes the electron microscopic findings on the sciatic nerves of rats. The key data appear to be the percentage of fields examined with any alteration in EM findings.

The table shows a dose-related response; however, because the rats of the control group showed 15 % of fields examined with an alteration from normal; statistically significant effects ($p<.05$) requires an alteration in almost 2x (30%) of fields examined. Thus a marginal effect may be defined at the 1 mg/kg/d level, in which 25 % of the fields examined showed an EM alteration. It should be noted that on H&E histopathological examination the peripheral nerves of the female rats were more severely affected than the males. Therefore, the sole selection of sciatic nerve tissues from the males for EM examination may be considered a limitation of this study.

Recovery Studies

Recovery from nerve injury was noted in EM observations made at 92 days on test plus 111 days and 92 days on test plus 144 days.

Conclusion

Based on EM examination, a no-effect level for neuropathology in male rats after 90 day oral administration of acrylamide in drinking water may be set at 0.2 mg/kg/d.

Attachment: Table 1, Electron microscopic observations on sciatic nerves from male rats administered acrylamide in the drinking water for up to 90 days

TABLE 1. Electron Microscopic Observations on Sciatic Nerves from Male Rats Administered Acrylamide in the Drinking Water for up to 90 Days

Number of Days on Test	7 Days		31 Days	90 Days					
Dose in mg/kg/day	0	20	20	0	0.05	0.2	1	5	20
Number of Rats in Group	3	3	3	3	3	3	3	3	3
OBSERVATIONS									
Total fields examined	450	450	430	450	450	350	453	443	435
Axolemma invaginations	37	37	30	16	24	27	30	33	8
Axolemma invaginations with cell organelles and/or dense bodies	13	18	20	12	15	17	78	109	48
Schwann cells without axons and/or with degenerating myelin	0	0	20	0	0	0	0	7	183
Percentage of fields examined with any alteration	11%	12%	21%	15%	9%	12%	25%	34%	55%

DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Memorandum

AD

Date January 24, 2000

From Division of Health Effects Evaluation (HFS-225)

Subject Final Safety Evaluation of Acrylamide acrylic acid resin (AAR) and dimethylamine-epichlorohydrin resin (DEC) as fixing agents for immobilized glucose isomerase used in foods. Memo of Div. of Product Manufacture and Use, Chemistry and Environmental Review Team (CERT) 4/28/99, received 5/5/99. QRAC concurrence of estimation of the upper bound lifetime risk from residual epichlorohydrin and acrylamide (S. Henry memo dated Dec. 20, 1999).

To Division of Product Policy (HFS-205)
Attn: Dr. Rosalie Angeles
Through Francis S. Lin, Ph.D.
Team Leader, Division of Health Effects Evaluation (HFS-225)

Food Additive Petition No. 9A4175

Enzyme Bio-systems Ltd.
Represented by
McKenna, Conner and Cuneo
1575 Eye Street, N.W.
Washington, D.C. 20005

This memo addresses the final Safety Evaluations of acrylamide-acrylic acid resin (AAR) and dimethylamine-epichlorohydrin resin (DEC) as fixing agents for immobilized glucose isomerase for the production of high fructose corn syrup (HFCS). The toxicology of these resins was initially reviewed in a memorandum dated Jan. 22, 1990.

The estimated intake for AAR resin is 83 ug/p/d based on the amount of high fructose corn syrup produced over lifetime use of an enyzyme preparation (HFF-415, memo of DiNovi, Nov. 22, 1989). Chemistry states this estimate may be highly exaggerated since the petitioner estimates that 90 % of the leachate will be removed with further processing.

Acrylamide

The EDI of acrylamide from the proposed use of the AAR resin is given by FCARS (HFF-415, memo of DiNovi, Nov. 22, 1989) as 41 ng/p/d. However, this EDI also includes acrylic acid, a reaction product of acrylamide degradation. The neurotoxicity of acrylamide is addressed in our memorandum of Sept. 9, 1997 (Bleiberg/Harrison(HFS-215) and Bleiberg/Angeles(HFS-205). The acceptable daily intake (ADI) based on neurotoxicity evaluation is 12 ug/p/d.

A review of a combined two-generation Reproductive and Developmental Toxicology study of acrylamide was prepared for OPA by Sciences International, Inc. The report, dated June 1998, concludes that a NOAEL for prenatal toxicity is 2.0 mg/kg/d. Applying a 1,000 fold safety factor yields 2 ug/kg/d or 120 ug/p/d as a safe dose.

The cumulative EDI for acrylamide in leachate and as a migrant in 7 pending petitions including 9A4175, 3B3677, 6B3940, 3B3696, 9B4131, 9B4132, and 9B4133 is 0.2573 ug/p/d (Chemistry memo of 4/28/94) and are stated to be extremely consrvative. This exposure is safe based on neurotoxicology and reproduction toxicity studies.

A risk assessment for acrylamide was conducted (memo of Quantitative Risk Assessment Committee, QRAC, April 10, 1998). The upper bound risk for exposure to acrylamide was determined to be 2.2 x 10-8 based on an EDI of $3x10^{-8}$ mg/kg-bw/d (memo of CERT, Bailey/Angeles, April 28, 1999) and a unit risk estimated from total significant male rat tumors. The CERT estimate, as submitted by you, memo of May 4, 1999, was concurred to by QRAC, of S. Henry memo dated Dec. 20, 1999. As noted above this estimate of migration is conservative assuming all leachate remains in HFCS although petitioner states 90% is removed in further processing. Moreover, Chemistry states that this figure actually represents the combined exposure to acrylamide and acrylic acid.

As discussed below the executive secretary of the CAC in an opinion concurred with by the Risk Assessment Manager, states that acrylic acid has not been demonstrated to be an oral carcinogen. Therefore this risk assessment is very conservative, and can be considered acceptable.

Acrylic acid

The toxicology of acrylic acid was reviewed in a memorandum (Bleiberg/Ekelman, Jan. 14, 1999) prepared for the Executive

Secretary of the Cancer Assessment Committee. Based on an expedited review of the relevant studies, the Executive Secretary (memo of Feb. 9, 1999) with the concurrence of the Risk Assessment Manager, concluded that there was no credible study to indicate that acrylic acid is an oral carcinogen. The no-effect level for acrylic acid in a chronic drinking water study conducted by Hellwig, et al.1993[1] can be taken as 27 mg/kg/d for the male mid-dose group since there was some early mortality of rats on the high-dose level. Using a 200 fold safety factor (based on the use of one species) an ADI of 0.135 mg/kg/d x 60 kg, or 8.2 mg/p/d (Memo of Bleiberg/Ekelman, 1/14/99) was determined for acrylic acid. There is therefore an adequate margin of safety based on an exposure of 41 ng/p/d for acrylic acid plus acrylamide and a risk assessment of less than 1 x 10-6.

The safety data for AAR includes 2 year rat and dog studies at dietary levels of 1, 2, 5, 10 and 15 %., and metabolic fate data, were initially reviewed for evaluation of FAP 1019 by Blumenthal, Feb 15, 1963. The no-effect level of AAR was determined to be 1% (10,000 ppm) in the rat and 2% in the dog. By applying a safety factor of 100 to the lower no-effect level, an ADI of 100 ppm or 300 mg/p/d is obtained as compared with the estimated intake (EDI) for AAR resin, 83 ug/p/d. Since the EDI of AAR resin is much less than the ADI, it is safe for the proposed use.

Dimethylamine - epichlorohydrin Resin

Chemistry (memo of Bailey, Aug. 7, 1997) states that the estimated daily intake (EDI) for DEC resin will be 210 ug/p/d. Chemistry states this estimate is conservative since the petitioner estimates that 90 % of the leachate will be removed with further processing (Di Novi/Harris memo of Nov. 22, 1980). The ADI for the polymer is 1 mg/kg/d or 60 mg/p/d based on a NOEL of 4 %(memo of Siu/Pauli, Oct. 20,1980 re FAP No.0A3500). There is an adequate margin of safety Since the EDI is much lower than the ADI.

Epichlorohydrin

Chemistry (Bailey/Harrison, Aug. 7, 1997) states that residual epichlorohydrin (EC)in DEC resin, at 10 ppm, may give a level of exposure in HFCS of 2.1 ng/p/d. The upper bound lifetime carcinogenic risk from exposure to EC for the requested use, based on unit risks provided in the QRAC memorandum for FAP 6A3905, Feb.

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[1] Subchronic and Chronic Studies of the Effects of Oral Administration of acrylic acid to rats. Fd. Chem. Toxic 31 (1), 1-18, 1993.

Secretary of the Cancer Assessment Committee. Based on an expedited review of the relevant studies, the Executive Secretary (memo of Feb. 9, 1999) with the concurrence of the Risk Assessment Manager, concluded that there was no credible study to indicate that acrylic acid is an oral carcinogen. The no-effect level for acrylic acid in a chronic drinking water study conducted by Hellwig, et al.1993[1] can be taken as 27 mg/kg/d for the male mid-dose group since there was some early mortality of rats on the high-dose level. Using a 200 fold safety factor (based on the use of one species) an ADI of 0.135 mg/kg/d x 60 kg, or 8.2 mg/p/d (Memo of Bleiberg/Ekelman, 1/14/99) was determined for acrylic acid. There is therefore an adequate margin of safety based on an exposure of 41 ng/p/d for acrylic acid plus acrylamide and a risk assessment of less than 1 x 10-6.

The safety data for AAR includes 2 year rat and dog studies at dietary levels of 1, 2, 5, 10 and 15 %., and metabolic fate data, were initially reviewed for evaluation of FAP 1019 by Blumenthal, Feb 15, 1963. The no-effect level of AAR was determined to be 1% (10,000 ppm) in the rat and 2% in the dog. By applying a safety factor of 100 to the lower no-effect level, an ADI of 100 ppm or 300 mg/p/d is obtained as compared with the estimated intake (EDI) for AAR resin, 83 ug/p/d. Since the EDI of AAR resin is much less than the ADI, it is safe for the proposed use.

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[1] Subchronic and Chronic Studies of the Effects of Oral Administration of acrylic acid to rats. Fd. Chem. Toxic 31 (1), 1-18, 1993.

24, 1989) was estimated as 9.5×10^{-11} as forwarded by you to QRAC (your memo May 4,1999) and concurred (memo of Henry/Angeles, Dec. 20, 1999). This risk may be considered to be negligible and acceptable.

1,3-dichloropropanol

The degradation product of EC in water is 1,3-dichloropropanol (DCP), which may be at residual levels of up to 1,000 ppm in the resin (21CFR§173.60), may remain in HFCS at levels of 2.5 ppb (Chemistry memo of Aug. 7, 1997). Since this is a carcinogenic contaminant, an expedited risk assessment was performed (memo of Executive Secretary of Cancer Assessment Committee, Aug. 24, 1998, re 1,3-dichloropropanol). The risk estimate, a worst case upper bound, lifetime cancer risk was calculated as 1.2 x10-7 and was confirmed by K. Ekelman, who initialed the memo of M.Bleiberg/K. Ekelman, re FAP 9A4175, March 25, 1999. Since the estimated residual levels are very conservative and do not account for further HFCS processing, in which 90% of the leachate containing DCP will be removed, this upper bound lifetime cancer risk can be considered below 10-8 and acceptable for regulating this petition (memo of M. Bleiberg/K. Ekelman, re FAP 9A4175, March 25, 1999).

Conclusion

All of our safety questions have been answered and the petition is ready for regulation.

Marvin J. Bleiberg, Ph.D., DABT

CC: HFS-200, HFS-225 (Hattan, Biddle, Lin), HFS-247
MJBleiberg:HFS-225:R/D:1/06/00:F/T:1/24/00:DOC:h:/9A4175c.wpd

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The degradation product of EC in water is 1,3-dichloropropanol (DCP), which may be at residual levels of up to 1,000 ppm in the resin (21CFR§173.60), may remain in HFCS at levels of 2.5 ppb (Chemistry memo of Aug. 7, 1997). Since this is a carcinogenic contaminant, an expedited risk assessment was performed (memo of Executive Secretary of Cancer Assessment Committee, Aug. 24, 1998, re 1,3-dichloropropanol). The risk estimate, a worst case upper bound, lifetime cancer risk was calculated as 1.2 x10-7 and was confirmed by K. Ekelman, who initialed the memo of M.Bleiberg/K. Ekelman, re FAP 9A4175, March 25, 1999. Since the estimated residual levels are very conservative and do not account for further HFCS processing, in which 90% of the leachate containing DCP will be removed, this upper bound lifetime cancer risk can be considered below 10-8 and acceptable for regulating this petition (memo of M. Bleiberg/K. Ekelman, re FAP 9A4175, March 25, 1999).

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