DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Memorandum

Date July 18, 2002

From Toxicology-Group 1, Division of Food Contact Substance Notification Review (DFCSNR)

Subject Addendum memorandum to Final Toxicology Memorandum (Young to Peiperl, 3/08/02) for FAP No. 8A4610. Penta- and Hexa-esters

To Division of Petition Review
Attn: Martha Peiperl

Through: Garfield N. Biddle, Ph.D. Garfield N. Biddle
Director, Division of Petition Review

REF 4

FAP No. 8A4610

Related FAPs:
SFAE:
1A3564 9A4166
2A3590 0A4183
3A3708 2A4321
5A3839 5A3859
6A3914
Higher esters: 6T3900
Olestra: 7A3997

Keller and Heckman, LLP
100 G. Street, N.W.
Suite 500 West
Washington, D.C. 20001

On behalf of: Mitsubishi Chemical Co.
Tokyo 100, Japan

Toxicology has previously determined, in their memorandum for this petition dated March 8, 2002, that the primary focus of the safety assessment for this Food Additive Petition (FAP) would be the tetra-, penta-, and hexa-ester components of SOE. Additionally, in this memorandum, the conclusion of "no safety concerns regarding the potential intake of sucrose tetra-ester in SOE" was presented.

In continuing our safety assessment, the potential exposure and safety of the penta- and hexa-ester components of SOE have also been considered. Toxicology has carefully reviewed the information submitted by the petitioner and other available relevant information. In particular, we have considered an absorption study of ^{14}C-labeled sucrose polyester consisting of approximately 81% hexa- and lower esters in rats that was submitted with the Olestra petition (FAP 7A3997) and previously reviewed by Dr. Pellicore (see attached memorandum for FAP 7A3997 dated August 4, 1993). This study demonstrated that only a small amount (i.e., not more than 1.5%) of the administered radiolabel was absorbed, and that the majority of the absorbed radioactivity was found in expired air and urine. Additionally, interim necropsies

demonstrated that the amount of radioactivity detected in tissues decreased rapidly over time, and therefore did not bioaccumulate.

CONCLUSION

Based on the totality of information available to us regarding sucrose fatty acid esters and the low increase in exposure to the penta- and hexa-ester components of SOE, Toxicology concludes that no safety concerns are raised for the penta- and hexa-ester components of SOE as described in this FAP. However, if exposure to these components is increased in the future, additional safety studies may need to be conducted.

Carolyn Young, PhD.

Carolyn Young, Ph.D.

Attachment (1): Memorandum dated August 4, 1993: Absorption of Low Chain-Low Ester Olestra Formulation in Rats. Food Additive Petition No. 7A3997 - Olestra

cc: (Lin, Edwards, Biddle, Varner, Zajac)
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DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Memorandum

Date August 4, 1993

From Additives Evaluation Branch #1 (HFS-226)

Subject **Absorption of Low Chain-Low Ester Olestra Formulation in Rats. Food Additive Petition No. 7A3997 - Olestra.** VOL 122-23

To Novel Ingredients Branch (HFS-207)

ATTN: Frederick O. Fields, Ph.D.
THRU: Kirk Biddle, Ph.D.
Kirk Biddle
Chief, Additives Evaluation Branch #1 (HFS-226)

Food Additive Petition No. 7A3997 Procter & Gamble Company
Cincinnati, OH 45224-1703

Test Article:

A high specific activity ^{14}C-labeled-sucrose-low chain-low ester olestra test composition was formulated to assess the absorption potential of olestra components which are usually present at low (≤1%) levels. The low chain-low ester olestra formulation tested in this study contained more than 50% C-14 and C-12 fatty acid chain lengths and more than 80% hexa and lower esters. This test composition is outside the current olestra specifications. This olestra formulation was unheated and by analysis contained less than 0.4 % polymer.

Experimental Design:

Twenty-six male and 26 female Fischer rats (3 weeks of age) were given a detailed physical examination and were observed during a 13-day acclimation period for any clinical signs of disease. The rats were individually housed in wire-mesh cages during the acclimation period. Of the 26 male and 26 female rats originally obtained, 16 male rats (12 treated, 4 control) and 16 female rats (12 treated, 4 control) were placed on study. The rats were individually housed in metabolism cages during the

remainder of the in-life phase of the study. The rats were randomized and identified by cage group and individual ear tag. All unassigned rats were killed and discarded. After 28 days of feeding 10 % (by weight) of an unheated low chain-low ester olestra formulation (BPO-7810) in a Purina rodent chow (#5002) diet, 24 Fischer rats (12 males, 12 females) were fasted 12 to 18 hours and given a single oral dose of ^{14}C-labeled-sucrose low chain-low ester olestra test composition (BPO-7810) by gavage. The target level of radioactivity to be administered was 100 uCi per rat. The total volume administered to each rat was adjusted based on the individual body weight obtained on study day 29, just prior to the radiological dose. Immediately after the test article was administered, an additional 5 ml/kg of deionized water was administered by gavage using a second syringe and dosing needle to ensure that all of the test substance was delivered to the stomach of the rat. Clean syringes and dosing needles were used for each animal for both the test article formulations and the distilled water. Eight control animals, 4 of each sex, were included to provide background count rates in different tissues and to provide a check on the procedures instituted to minimize cross-contamination. During the 28-day pre-treatment period and after dosing with the radiolabelled olestra, the animals were inspected daily for signs of toxicity, morbidity or mortality. Animal weight and feed consumption were determined weekly. Expired CO_2 was collected at 24 hour intervals for 7 days after dosing (Day 30 through Day 36). Carbon dioxide absorption solution was drained from the metabolic tower and fresh trapping solvent was immediately poured into the tower after each draining. The expired radiocarbon output was determined by scintillation counting (1 gram aliquots of each absorption solution; 5 minutes). Urine and feces were continuously collected from all animals on study for 21 days after dosing. Samples collected on the first seven days (Day 30 through Day 36) and the 13th (Day 42), 14th (Day 43), 20th (Day 49), and 21st (Day 50) day after dosing were analyzed for radioactivity. Urine and feces samples collected from rats on Days 37 through 41 and Days 44 through 48 were pooled but not analyzed. Urine was lyophilized, reconstituted in 5 ml of deionized water, and the radiolabel content was determined. In addition, urine samples collected one day after dosing were extracted with hexane/ethyl ether and the radiolabel content of the aqueous and organic fractions were determined. Urine samples collected on the first day after dosing (Day 30) were also analyzed for ^{14}C-labeled-sucrose. Fecal samples collected during the first three days after dosing were solubilized by adding 5 ml Soluene for every 350 g of homogenized feces and the radiolabel content was determined by liquid scintillation counting (5

minutes). Other feces samples were homogenized with deionized water, an aliquot combusted with a small amount of CombustAid, and the radiolabel content was determined by liquid scintillation counting (5 minutes).

One control rat and three olestra-treated rats of each sex were killed with carbon dioxide 1 (Day 30), 3 (Day 32), 7 (Day 36), and 21 (Day 50) days after dosing. Blood samples were obtained from the orbital sinus prior to killing for determination of radiolabel in whole blood and plasma. The skin was frozen on dry ice and saved for possible analysis. Prior to skinning, and again after skinning, the carcasses were rinsed with methylene chloride to remove possible contamination and any loose fur. A clean set of instruments was used to skin each animal. Gloves were changed between animals. The animal rinses were pooled and saved for radiolabel counting. Liver, spleen, lung, kidney (pair), perirenal fat pad, mesenteric lymph nodes, heart, brain, gastrointestinal tract and its contents were excised and analyzed for radioactivity. The GI tract from the cardiac sphincter to the anus, and contents were removed last to prevent contamination of the other organs. Each step of the necropsy was conducted in a separate area to prevent cross-contamination. In addition the absorbent mats used to cover the work area were changed between animals. The persons handling the tissues changed to clean gloves for each organ and tissue. The remaining carcass was frozen and later processed for radioassay. The cages were rinsed with methylene chloride and the cage rinses were saved for counting. The lipids were extracted from the tissues and carcass and then analyzed for intact olestra by high-performance liquid chromatography (HPLC). The radiolabel content of the blood, CO_2, cage wash and animal rinse was determined by direct counting. The GI tract and contents were stirred for three days with 100 ml of Soluene (or until not tissue was visible) and the radiolabel content determined by scintillation counting. Lipids from the liver and the carcass were extracted and the radiolabel content of the extracts were determined by direct counting. The liver and carcass residues and portions of all other tissues were combusted and the radiolabel content determined.

Tissues for which there were sufficient amounts remaining after the initial radioassay were extracted with chloroform:methanol (2:1 v/v) and the lipid extracts fractionated by size exclusion chromatography to determine if radiolabel was present in the olestra-containing fraction. The radiolabel contents of all lipid fractions were determined by scintillation counting.

Results:

There were no mortalities during the study. No visible abnormalities were observed for any animal during the study period. There were no remarkable changes or significant differences observed in mean body weights among control and olestra-fed animals of the same sex during the four week measurement interval. Mean food and compound consumption values were comparable between groups of animals throughout the study. Body weight gains were comparable between groups. Hematocrit values were unremarkable.

The ^{14}C-labeled-sucrose low chain-low ester olestra test composition administered to male rats ranged from 30.5 uCi (67,760,000 dpm) to 105.5 (234,119,000 dpm) uCi. The female rats recieved calculated levels of radioactivity ranging from 78.2 (173,592,000 dpm) uCi to 103.8 (230,391,000 dpm) uCi. There were no biologically significant sex-related differences in routes or rates of radiocarbon excretion.

The mean recovery of radiolabel from the females was 99.5 $\pm$ 12.2 % of the administered dose. The recovery from the males was 140.4 $\pm$ 27.7 % of the administered dose. The mean recovery of radiolabel from all animals was 119.9 $\pm$ 29.6 % of the administered dose. Excretion of radiolabel in the feces was rapid with 111.6 % (males) and 87.6 % (females) of the administered dose detected in the feces samples collected within 24 hours of dosing (Day 30). Cumulative excretion of administered radiolabel in the feces was 137.5 % and 103.8 % (Day 32), 138.2 % and 102.9 % (Day 36) and 154.6 % and 93.6 % (Day 50) for male rats and female rats, respectively. The radiolabel detected in the G.I. tract and contents was 12.0 % (males) and 3.7 % (females) 24 hours after dosing (Day 30). However, the radiolabel detected in G.I. tract and contents rapidly decreased after dosing to 1.1052 % and 0.2569 % on Day 32, 0.0355 % and 0.0211 % on Day 36, and 0.0734 % and 0.0124 % on Day 50 for males and females, respectively. [Results are reported here and elsewhere to four or more decimal places to make the point.] Radiolabel in these samples represents unabsorbed test material. The absorbed radiolabel, found in the CO_2, urine, blood, tissues, carcass and extracts, amounted to 1.0605 % and 0.7539 % on Day 30, 1.4708 % and 1.7794 % on Day 32, 2.1745 % and 1.5104 % on Day 36, and 1.6366 % and 1.6173 % on Day 50 for males and females respectively. On the average, for all animals on all days, the absorbed radiolabel amounted to 1.5 % of the administered dose.

Cumulatively 0.01 % - 1.09 % of the administered dose was expired as CO_2 over the first seven days (Day 30) after dosing. By Day 32, 0.69 % - 2.27 % of the administered dose

was expired as CO_2, while 0.67 % - 2.29 % and 1.02 % - 1.59 % was expired as CO_2 by Day 36 and by Day 50, respectively. There were no significant differences in the expiration of radiolabel by males and females.

Cumulatively 0.15 % - 0.21 % of the administered dose was excreted in the urine over the first seven days (Day 30) after dosing. By Day 32, 0.18 % - 0.28 % of the administered dose was excreted in the urine, while 0.20 % - 0.35 % was excreted in the urine by Day 36. Cumulatively 0.21 % - 0.39 % of the administered dose was excreted in the urine within 21 days (Day 50). There were no biologically significant differences between sexes. About 97 % of the urinary radiolabel was found in the aqueous fraction following hexane:ethyl ether extraction. [The original submission failed to account for a five-fold dilution factor in the calculation of the amount of radiolabel in the aqueous fraction of the urine following solvent extraction. At this Reviewer's request, the petitioner revised their submission to reflect this recalculation. Correction of this error increased the percent of urinary radiolabel in the aqueous phase and decreased the percent of the urinary radiolabel in the lipid phase.] The urine from animal #72844, had about 34% of the detected radiolabel in the organic extract, suggesting a contamination problem. Radiolabel profiles of the HPLC fractionated urine samples were compared to control urine samples "spiked" with sucrose. A few urine samples showed an indication of a peak in the sucrose region of the chromatogram. The average radiolabel detected in the sucrose-containing fraction was 0.027 % of the total recovered radiolabel for all 24 animals.

The sum total of radiolabel found in tissues including spleen, lung, kidney, fat pad, lymph nodes and liver of male rats was 0.0557 % and 0.0398 % (Day 30), 0.0386 % and 0.0369 % (Day 32), 0.0358 % and 0.0170 % (Day 36), and, 0.0119 % and 0.0067 % (Day 50) of the administered dose for males and females, respectively.

Of the tissues, the liver with extract contained the highest levels of radiolabel. Cumulatively, 0.0364 % and 0.0310 % (Day 30), 0.0176 % and 0.0211 % (Day 32), 0.0142 % and 0.0078 % (Day 36), and, 0.0025 % and 0.0019 % (Day 50) of the dose was found in liver and liver extract samples in males and females, respectively.

Radiolabel was found in the olestra-containing fraction of the liver extract from 21 out of 24 rats. The radiolabel levels detected ranged from 0.0032 % to 0.0003 % of the administered dose.

A low level of radiolabel, 0.00013 % - 0.00004 % of the dose, was detected in the olestra-containing fraction of kidney lipids from 15 out of 24 rats. [In the original submission the weight of the lipid aliquot injected into the HPLC rather than the weight of the total lipid extracted from the tissue was used in the calculation of the radiolabel present in the olestra-containing fraction of the tissue lipids and in the calculation of the analytical detection limits. At this Reviewer's request, the petitioner revised their submission to correct their error. The amount of radiolabel present in the olestra-containing fraction of tissue lipids are correctly reported in this review.] In addition, radiolabel was found in the olestra-containing fraction of the brain extracts from 23 out of 24 rats. The radiolabel levels detected in the olestra-containing fraction of the brain extracts ranged from 0.00006 % to 0.00063 % of the administered dose. However, the data (dpm/aliquot) contained in Exhibit 25 of the Final Report indicates that the raw counts were only 2-5 times the 15 dpm/aliquot detection limit. In addition, based on the low level of radiolabel detected and reported in Table 17 (dpm/g tissue) and Exhibit 17 (% of administered dose) of the Final Report, the radiolabel detected in the olestra-containing fraction of the brain extracts may have been increased due to a mathematical noise scale-up.

One day after dosing (Day 30), 0.0973 % (both sexes) of the administered dose of radiolabel was found in the carcass plus extract. However, by 21 days after dosing (Day 50), the level of radiolabel in the carcass plus extract was detected at 0.0923 % and 0.0542 % of the dose in male rats and female rats, respectively.

Radiolabel levels found in the blood from the male rats ranged from 0.0118 % of the administered dose one day after dosing (Day 30) to 0.0047 % on Day 50. Blood radiolabel levels for the female rats tended to be lower than those measured in the male rats, however, the differences are not biologically significant.

Summary:

The mean recovery of unabsorbed radiolabel from the feces, G.I. tract and contents, and cage rinse solutions totaled 118.4 % of the administered dose. The absorbed radiolabel amounted to 1.5 % of the administered dose. The liver with extract contained the highest levels of absorbed radiolabel (0.0337% (Day 30), 0.0194% (Day 32), 0.0110% (Day 36), and, 0.0022% (Day 50)) with radiolabel detected in the olestra-containing fraction of the liver extract from 21 out of 24 rats (0.0032 % to 0.0003 % of the dose). In addition, low

level radiolabel was detected in the olestra-containing fraction of brain extracts in 23 out of 24 rats (0.00063% to 0.00006% of the dose), and in the olestra-containing fraction of kidney lipid extracts in 15 out of 24 rats (0.00013% to 0.00004% of the dose).

Conclusion:

Most of the detected radiolabel (118.4 %) was unabsorbed and excreted in feces, GI contents and cage washes, primarily during the first 24 hours after administration of the radiolabel. However, 1.5 % of the administered radiolabel was absorbed. The majority of the absorbed radioactivity was found in the expired air and urine. The results of interim necropsies demonstrated that the amount of radioactivity detected in tissues decreased rapidly over time. Absorption of radiolabel was expected in this study because of the high content of penta and lower sucrose esters contained in the test article formulation. The total amount of radiolabel absorbed in the present study is more than ten times that absorbed from the heated mid-range olestra composition (1.5 % vs. 0.14%). We have no further questions regarding this rat absorption study.

Linda S. Pellicore

Linda S. Pellicore, Ph.D.

cc:
HFS-207 (Fields, Harris)
HFS-225 (Hattan)
HFS-226 (Biddle, Pellicore)
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