EVALUATION OF THE HEALTH ASPECTS OF PROPIONIC ACID,
CALCIUM PROPIONATE, SODIUM PROPIONATE, DILAURYL
THIODIPROPIONATE, AND THIODIPROPIONIC ACID
AS FOOD INGREDIENTS

1979

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health, Education, and Welfare
Washington, D.C.

Contract No. FDA 223-75-2004
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Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014

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NOTICE

This report is one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior sanctioned food substances as food ingredients, being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-75-2004 with the Food and Drug Administration (FDA), U.S. Department of Health, Education, and Welfare. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshalling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances, were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the Office of the Hearing Clerk, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB

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I. INTRODUCTION

This report concerns the health aspects of using propionic acid, calcium propionate, sodium propionate, dilauryl thiodipropionate, and thiodipropionic acid. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (1), which summarizes the world's scientific literature from 1920 through 1973*. To assure completeness and currency as of the date of this report this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, an announcement was made in the Federal Register of January 12, 1979 (44 FR 2687-2690) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using propionic acid, calcium propionate, sodium propionate, dilauryl thiodipropionate, and thiodipropionic acid as food ingredients. The Select Committee received no requests for such a hearing.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (2) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimentation data. FDA (2) recognizes further that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety, the Committee, in accordance with

*The document (PB-228538/5) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.
FDA's guidelines, is relying primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. While the Committee realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited, it recognizes that there can be instances where, in the judgment of the Committee, there are insufficient data upon which to base a conclusion. The Committee is aware that its conclusions will need to be reviewed as new or better information becomes available.

In this context, the LSRO Select Committee on GRAS Substances has reviewed the available information on propionic acid, calcium propionate, sodium propionate, dilauryl thiodipropionate, and thiodipropionic acid and submits its interpretation and assessment in this report, which is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act.
PART A.
PROPIONIC ACID, CALCIUM PROPIONATE AND SODIUM PROPIONATE

II A. BACKGROUND INFORMATION

Propionic acid, CH₃CH₂COOH, is an aliphatic monocarboxylic acid. The Code of Federal Regulations (2) lists propionic acid [21 CFR 182.3081], calcium propionate [21 CFR 182.3221], and sodium propionate [21 CFR 182.3784] as GRAS chemical preservatives. Standards of identity for certain cheeses and related cheese products provide for the addition of not more than 0.3 percent by weight of sodium propionate, calcium propionate or a combination of the two [21 CFR 133]. The propionates serve to inhibit the growth of Bacillus mesentericus which causes "rope" in breads, and they are active against fungi but not against yeasts (3). Optimum antimicrobial activity is exhibited below pH 5 but activity occurs in some foods above pH 6.

Commercial processes produce propionic acid by chemical synthesis and in small quantities by bacterial fermentation (4,5). Food grade calcium propionate, propionic acid and sodium propionate are described in the Food Chemicals Codex (6). Each may contain not more than 3 ppm arsenic and 10 ppm heavy metals as lead. Calcium propionate assays not less than 98.0 percent of C₆H₁₅CaO₄ (anhydrous) and contains not more than 30 ppm fluoride, 0.2 percent insoluble substances, 5 percent water and about 0.4 percent magnesium (as MgO). Propionic acid assays not less than 99.5 percent of C₃H₆O₂ and contains not more than about 0.05 percent aldehydes (as propionaldehyde), 0.01 percent nonvolatile residue, about 0.05 percent readily oxidizable substances (as formic acid) and 0.15 percent water. After drying, sodium propionate assays not less than 99.0 percent of C₅H₁₅NaO₂. Additional limits of impurities in sodium propionate are alkalinity (as Na₂CO₃) of about 0.15 percent and not more than 30 ppm iron and 1 percent water.

Calcium propionate and sodium propionate are free-flowing, readily soluble powders that have a slight, cheese-like flavor (3). These two salts are used to a greater extent and at higher concentrations as antimicrobial agents than propionic acid which is a mildly corrosive liquid having a strong odor characterized as slightly pungent, disagreeable and rancid (4,7).

Propionic acid as propionyl CoA is a metabolic intermediate in the metabolism of odd-carbon fatty acids and of methionine and isoleucine. It is metabolized via methylmalonyl CoA to succinyl CoA (8).

Many dairy products contain propionic acid as a naturally occurring component. It is part of the aroma of butter and cheese, and may constitute as much as 1 percent of Swiss cheese (3).
III A. CONSUMER EXPOSURE DATA

A National Research Council (NRC) subcommittee surveyed food processors concerning their use of GRAS substances in 1970 (7). Respondents to the survey provided information on each GRAS substance they used concerning its level of addition to foods, the total quantity added to foods annually and the 10-year trend in this quantity, its importance in providing certain technical effects, and other information considered relevant to its food use. The subcommittee's report indicated that propionates are added to one or more food products in the food categories shown in Table I. The tabulated values represent weighted means of the reported "usual" levels of addition. Therefore, products may contain a higher level of the propionate but many products within these categories probably contain no added propionates. This latter situation is expected for the many entries in Table I that represent the reports of three or fewer firms. These levels of addition are less representative of the propionate content of foods in their respective categories than are the addition levels representing the processing practice of more firms. For example, the addition of calcium propionate to baked goods at a level of 0.1 percent represents a level of addition derived from the survey reports of over 30 firms. A weighted mean derived from the reported "maximum" levels of addition for calcium propionate to baked goods was 0.28 percent, about three times the "usual" level.

Estimates of the expected average intake and the distribution of daily intakes throughout the population for calcium propionate and sodium propionate have been made by another NRC Committee (9). Data utilized in their calculation included: the usual level of addition as shown in Table I, an estimate of the probability that a specific food contains added propionates, mean portion sizes obtained from the U.S. Department of Agriculture 1965 survey, and frequency of eating of specific foods as reported to the Market Research Corporation of America in a 1967-1968 menu survey. For persons over the age of 2 years, the daily intake of calcium propionate was estimated as 45 mg for persons consuming the mean amount and 95 mg for persons consuming at the 90th percentile of intake; the daily intake of sodium propionate averaged 33 mg and had a 90th percentile of 66 mg. Estimates of the expected average intake were not made by the NRC Committee for propionic acid.

The NRC subcommittee has suggested comparison of estimates of daily intake of GRAS substances with the amounts used by the food industry on a per capita basis (7). The survey of 1970 usage was about 60 percent complete in its coverage for most GRAS ingredients, and this factor can be applied when other means of estimating an industrial usage figure are not available. About 14,000 kg of propionic acid was used in foods by respondents to the survey. Assuming the survey coverage was 60 percent the resulting per capita daily consumption was 0.3 mg propionic acid.
TABLE I

Level of Addition of Calcium Propionate, Sodium Propionate and Propionic Acid to Foods by Food Category (7)

<table>
<thead>
<tr>
<th>Food category</th>
<th>Calcium propionate Weighted mean ppm</th>
<th>Propionic acid Weighted mean ppm</th>
<th>Sodium propionate Weighted mean ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>1100</td>
<td>75</td>
<td>2000</td>
</tr>
<tr>
<td>Fats and oils</td>
<td></td>
<td>24*</td>
<td></td>
</tr>
<tr>
<td>Cheese</td>
<td>12</td>
<td>2700</td>
<td></td>
</tr>
<tr>
<td>Frozen dairy, desserts, mixes</td>
<td></td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Meat products</td>
<td></td>
<td></td>
<td>50*</td>
</tr>
<tr>
<td>Soft candy</td>
<td>3.3</td>
<td>9*</td>
<td></td>
</tr>
<tr>
<td>Sugar, confections</td>
<td></td>
<td>1*</td>
<td></td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td></td>
<td>1.4</td>
<td>500*</td>
</tr>
<tr>
<td>Snack foods</td>
<td></td>
<td>0.2*</td>
<td></td>
</tr>
<tr>
<td>Beverages, nonalcoholic</td>
<td>2.5</td>
<td>170*</td>
<td></td>
</tr>
<tr>
<td>Beverages, alcoholic</td>
<td></td>
<td>4.3*</td>
<td></td>
</tr>
<tr>
<td>Dairy products analogs</td>
<td></td>
<td>5.0*</td>
<td></td>
</tr>
<tr>
<td>Hard candy</td>
<td></td>
<td>1.0*</td>
<td></td>
</tr>
<tr>
<td>Chewing gum</td>
<td></td>
<td>2.0*</td>
<td></td>
</tr>
</tbody>
</table>

(*) Only one, two, or three firms reported use in any product included in the food category.
The manufacturers of calcium propionate disclosed the amount sold in 1970 was 7.3 million kg (9). The 1.5 million kg of calcium propionate reported in the 1970 survey of food processors was only 21 percent of this amount. The difference may result from nonfood uses and because commercial baking done by small- and medium-sized firms was not covered extensively by the survey. The per capita daily intake estimated on the basis of the amount of calcium propionate sold was 83 mg (15 percent allowed for nonfood uses). An average daily intake of 45 mg of calcium propionate appeared more realistic to the NRC Committee in terms of the increments of intake contributed by individual eatings of baked goods, and the discrepancy was attributed to extensive waste. The intake estimate of 33 mg per day for sodium propionate is about the same as the 38 mg per capita daily consumption figure arrived at by assuming a similar survey coverage and nonfood use as was applied for calcium propionate.

In 1974, the Joint FAO/WHO Expert Committee on Food Additives reviewed the available data on propionic acid and its calcium and sodium salts (10). No reports of toxicological studies exceeding 1 year duration were available. It was noted that propionate is a normal intermediary metabolite, and the acceptable daily intake for man was not limited.
IV A. BIOLOGICAL STUDIES

Absorption and metabolism

Absorption and metabolism of short-chain fatty acids were studied in isolated rat jejunum in anesthetized rats (11,12). Propionic acid was transferred from 0.037 percent solutions in bicarbonate saline against a concentration gradient by everted rat jejunum; however, concentrations of propionic acid above about 0.3 percent had an inhibitory effect on transfer. Comparison of jejunal and ileal segments showed preferential absorption of propionic acid in the jejunum. Acetate and butyrate were metabolized by the isolated intestine during a 60-minute incubation period but propionate was not. The in vivo absorption rate of propionate approximated that of acetate from 20 mM solutions placed in the intestine of anesthetized rats.

When vagally denervated gastric pouches in four dogs were irrigated for two 30-minute periods with 0.1 M propionic acid (0.74 percent) in 0.015 mM sodium chloride, propionic acid passed through the mucosa and produced increased fluxes of sodium, potassium and chloride ions from the mucosa (13). These changes and an increased flux of hydrogen ion in pouch fluid in two subsequent 30-minute control irrigations with 0.1 mM hydrochloric acid were interpreted by the author as signs of mucosal damage. No damage, however, was reflected when a neutralized salt of propionic acid was used.

Six female Heidenhain pouch dogs with total antrum resections were used to investigate gastric acid secretion (14). The intravenous administration of 185 to 370 μg sodium propionate per kg body weight per minute had a stimulatory effect on gastric acid secretion similar to that produced by sodium acetate.

Merino sheep, 2- to 3-year-old wethers, were infused via the portal vein with solutions of sodium $^{14}C$ propionate at a rate of 370 mg propionate per minute per kg body weight (15). The animals were not fed on the day of the experiment. Blood acetate concentration in samples drawn from the right jugular vein 60 minutes after infusion began averaged 0.23 μmoles per ml and was described as markedly reduced below the normal lower limit of 0.3 μmoles per ml observed in fasting sheep. The specific radioactivity of liver and muscle glycogen (less than 0.2 percent of that of the infused propionate) was low relative to plasma glucose (30 percent).

Two adult ewes received subcutaneously 1 g of phlorhizin dissolved in 5 ml of ethylene glycol twice daily during a 48-hour fast, followed by oral administration of 1.6 g propionic acid per kg body weight (16). There was a significant increase in blood glucose 1, 3, 6, and 24 hours post-treatment; the greatest concentration was recorded at 3 hours. Blood ketone levels were significantly lower than controls. Urine samples showed
a marked increase in glucose excretion during a 48-hour post-administration period indicating propionic acid is metabolized to glucose.

Propionic acid was administered to female goats by gavage or intravenously, alone or in combination with acetic acid or n-butyric acid (17). The data revealed propionic acid caused a marked increase in blood glucose; and, because of its ability to form oxalacetate, propionic acid counteracted the formation of ketone bodies from acetate and n-butyrate.

The absorption of propionic acid contained in a mixture with other short-chain fatty acids was studied in eight patients (18). A solution (neutralized with NaOH) containing approximately equimolar amounts of acetic, propionic (about 1.3 g per liter), butyric, isobutyric, valeric, isovaleric and caproic acids was introduced at a rate of 20 ml per minute via a double lumen polyvinyl tube into the jejunum of seven patients at a point 5 cm past the duodeno-jejunal flexure. After 30 minutes, samples were siphoned from 30 cm below the point of infusion. The absorption of these fatty acids averaged 0.89 μmoles per minute per 30 cm jejunum; propionic acid was absorbed at about one-half the rate of valeric and caproic and nearly twice the rate of acetic acid. The four occasions of diarrhea were attributed to the relatively poor absorption of the acids. One 55-year-old female patient who had had a colonic exclusion operation permitted the study of the absorption of a mixture of acetic, propionic, butyric, valeric and caproic acids. In general, the pattern of colonic absorption was similar to the low uptake noted for the small bowel, and the investigators suggested it was not necessary to invoke an active transport mechanism.

The role of propionic acid as a precursor of methylmalonic acid in normal and vitamin B_{12}-deficient man was studied by using fasting subjects into whom propionic and/or methylmalonic acids labeled at carbon 2 were injected intravenously (19). In addition, one of the vitamin B_{12}-deficient subjects received a 5 g oral dose of sodium propionate 30 minutes prior to the injection of the labeled propionate and showed a marked augmentation of the methylmalonic aciduria. Results indicated that propionate is a precursor of urinary methylmalonic acid and, therefore, intracellular methylmalonyl CoA in both normal and vitamin B_{12}-deficient subjects. Urinary excretion accounted for 20 to 50 percent of the methylmalonic acid turnover in normal subjects and 20 percent in vitamin B_{12}-deficient patients.

**Acute oral toxicity**

Tests of the acute oral toxicity of propionic acid, calcium propionate, and sodium propionate are summarized in Table II. The oral LD_{50} of propionic acid in rats was reported by FDA to the Joint FAO/WHO Expert Committee on Food Additives as 2.6 g per kg body weight (20). In DD-strain mice, the oral LD_{50} of calcium propionate was reported as 3.34 g per kg (21).
TABLE II

Acute Oral Toxicity of Certain Propionates

<table>
<thead>
<tr>
<th>Substance</th>
<th>Species</th>
<th>LD₅₀</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Propionic acid</td>
<td>Rats</td>
<td>2.6</td>
<td>20</td>
</tr>
<tr>
<td>Sodium propionate</td>
<td>Mice (DD-strain)</td>
<td>5.1</td>
<td>21</td>
</tr>
<tr>
<td>Calcium propionate</td>
<td>Mice (DD-strain)</td>
<td>3.34</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Rats</td>
<td>5.16</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;5.0</td>
<td>23</td>
</tr>
</tbody>
</table>
Calcium propionate administered orally to rats was found to have an LD₅₀ of 5.16 g per kg in one test (22) but to have no adverse effects at 5.0 g per kg when tested in another laboratory (23). Sodium propionate had an oral LD₅₀ in DD-strain mice of 5.1 g per kg (21).

**Short-term feeding studies**

One of four adult chickens fed a diet containing calcium propionate at a level of 2.5 percent propionate (about 2 g calcium propionate per kg body weight per day) for 2 weeks showed a "slightly eroded" crop lining when examined postmortem (24). No propionate was found in the ingesta beyond the first and second quarters of the small intestine, and the investigators concluded that the propionate was completely digested.

Groups of eight 1-month-old rats received a diet containing 1 percent sodium or calcium propionate (about 750 mg per kg per day expressed as propionic acid) for 4 weeks and then at a level of 3 percent for an additional 3 weeks (an average intake of 1200 mg per kg per day) (25). The weight gain of the experimental group did not differ from control groups.

Weanling and adult rats were fed a diet containing 26 percent tripropionin that was substituted for one-half of the glucose and starch of the control diet (26). High mortality and weight loss led to termination of the experiment with weanling rats after 5 days. The adults ingested the equivalent of 30 g propionate per kg per day for 20 days; "rough hair coats, reddened paws, and extreme excitability" were described. Measurements of digestible, metabolizable, and net energy of the tripropionin ration did not differ from the control.

Three of four mixed strain male and female rats, 50 to 80 g body weight, fed rice diets containing 5 percent propionic acid (about 5 g per kg) developed "umbilicate" or "warty" lesions of their forestomachs after 110 days (27). No evidence of infiltrative malignant growth was detected.

The growth depression observed for chicks and young Sprague-Dawley rats fed a vitamin B₁₂-deficient diet was accentuated by including sodium propionate in the diets at a level of 5 percent in the case of the chick and 2 percent for the rat (28). Liver methylmalonyl-CoA mutase activity was also measured for the rat and found to be reduced in the presence and absence of vitamin B₁₂ in the diet; however, the growth depression was not seen when a vitamin B₁₂ supplement was included in the diet. Other investigators had reported no appreciable improvement in growth rates of weanling male rats after vitamin B₁₂ supplementation of 30 and 70 percent soy protein diets containing 2 or 4 percent calcium propionate (about 2 and 4 g per kg body weight) (29).
Male and female lambs, 28 kg average weight, were fed a ration containing 10 percent sodium propionate twice daily *ad libitum* (5.6 g per kg per day) for 50 days (30). Neither body weight gain nor feed required per unit gain was affected. The treated lambs had more fat in their weight gain, indicating better storage of energy.

The effects of high doses of propionates have received limited study in humans. Faintly alkaline urine was the only effect noted after daily oral doses of 6.0 g sodium propionate were administered to an adult male for an unspecified number of days (31).

**Long-term studies**

Male and female Wistar rats were fed a baked, dried, and ground bread containing 5 percent sodium propionate as three-fourths of their diet for 1 year without adverse effects (about 4 g per kg per day) (32). Another group of rats was fed the unbaked bread ingredients as three-fourths of their diet and a similar concentration of sodium propionate (about 4 g per kg per day) for 32 weeks (33). Depression of growth occurred in the first few weeks, in the latter test, but there were no adverse effects on mortality or on hemoglobin levels, organ weights, and histology of the tissues in either feeding study.

**Special studies**

Feeding calcium propionate to pregnant mice and rats for 10 days at levels up to 300 mg per kg daily, to pregnant hamsters for 5 days at levels up to 400 mg per kg daily, and to pregnant rabbits for 13 days at levels up to 400 mg per kg daily showed no significant effects on nidation or on maternal or fetal survival (34). The number of abnormalities seen in fetal or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls.

Calcium propionate was not teratogenic to the developing chicken embryo when administered at levels up to 100 mg per kg of egg preincubation or at 96 h via the yolk and air cell (35). A dose of 10 mg per kg of egg produced high mortality rates compared to solvent controls, and a dose of 5 mg per kg administered preincubation via the yolk caused a high mortality rate.

Calcium propionate was not mutagenic in microbial assays using *Salmonella typhimurium* strains TA-1535, TA-1537, and TA-1538 or *Saccharomyces cerevisiae*, strain D-4 (36). Mammalian tissue activation preparations were from lung, liver, and testis of mouse, rat, and monkey. In a host-mediated assay in mice, data for *S. typhimurium* strain G-46 indicate that this compound increased reversion frequency under the conditions of the test; the increases were unrelated to dose (23). Calcium
propionate was not mutagenic in cytogenetic studies with bone marrow meta-
phase chromosomes of rats or with the anaphase chromosomes of human
tissue culture cells; the results of a dominant lethal assay in rats were also
interpreted as showing calcium propionate as nonmutagenic under conditions
of the test.

Microbial assays for mutagenicity of propionic acid (37) and sodium
propionate (38) were negative with and without the addition of mammalian
metabolic activation preparations. The assays employed \textit{S. typhimurium}
strains TA-1535, TA-1537, TA-1539, TA-98, and TA-100 and \textit{S. cerevisiae}
strain D-4.

Sodium propionate was administered to developing chicken embryos
at preincubation (0 h) and at 96 h via air cell and yolk; the incidences of
abnormalities were significantly higher (p<0.05) than those in the solvent
controls only at the highest test levels after air cell administration (39).
Yolk treatment did not produce significant increases in abnormalities. The
highest levels tested by air cell at preincubation, 10 mg per egg, and at
96 h, 5 mg per egg produced mortalities of 92 and 74 percent, respectively.
Propionic acid occurs naturally in various foods including butter and cheese. Its absorption and metabolism are demonstrated in experimental animals and humans where it is a normal intermediary metabolite. As incorporated in foods as its sodium or calcium salt or as the free acid, propionic acid does not occur at the concentrations or under the conditions that are necessary to produce signs of mucosal damage in experimental animals.

Propionic acid, sodium propionate, and calcium propionate have demonstrated low acute toxicity after oral administration to mice or rats. The adverse effects observed in chicken embryos occurred only after injection of large amounts of calcium propionate or sodium propionate into the yolk sac, and the reversions observed in a host-mediated assay of calcium propionate were unrelated to dose. These results in chicken embryos and the host-mediated assay must be viewed in the light of other microbial assays and animal studies that demonstrate no adverse effects and the fact that propionate is a normal intermediary metabolite. Microbial assays for mutagenicity of propionic acid and calcium and sodium propionate were negative. Investigations of the teratogenicity of calcium propionate in four mammalian systems also were negative. Short-term feeding tests show the most sensitive animals tested, young and vitamin B₁₂-deficient animals, experience adverse effects on weight gain only when propionate intakes are many orders of magnitude greater than the estimate of human dietary intake of propionate used as a food ingredient, about 1 mg per kg per day. Long-term feeding studies of propionic acid and calcium propionate have not been reported. However, a long-term feeding study of sodium propionate showed no adverse effects in rats.

The Select Committee has weighed the foregoing and concludes that:

There is no evidence in the available information on propionic acid, calcium propionate, and sodium propionate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.
PART B.

DILAURYL THIODIPROPIONATE AND THIODIPROPIONIC ACID

II B. BACKGROUND INFORMATION

Dilauryl thiodipropionate [21 CFR 182.3280] and thiodipropionic acid [21 CFR 182.3109] are GRAS for addition to food as antioxidants when the total content of antioxidants is not over 0.02 percent of fat or oil content, including essential (volatile) oil content of the food, provided they are used in accordance with good manufacturing practice (2). Thiodipropionic acid [(CH₃CH₂COOH)₂S] and its dilauryl ester [C₁₂H₂₅OOCCH₂CH₃S] also are prior sanctioned antioxidants when employed in the manufacture of food packaging material at a level that results in less than 0.005 percent addition to the packaged food [21 CFR 181.24].

The Chemical Abstracts Service Registry number for thiodipropionic acid is 111-17-1 and for dilauryl thiodipropionate it is 123-28-4. The following lists indicate some of the synonyms used in the scientific and trade literature as contained in the CHEMFILE system of the National Library of Medicine's computerized literature retrieval system. The generic, trivial, and trade names may not represent a unique compound.

Thiodipropionic acid

- propionic acid, 3, 3'-thiodipropionic acid, 3, 3'-thiobis-diethyl sulfide, 2, 2'-dicarboxylic acid
- TDPA
- 4-thiaheptanedioic acid
- thiodihydracrylic acid
- beta, beta'-thiodipropionic acid
- 3, 3'-thiodipropionic acid
- Tyox A
- thiodipropionic acid

Dilauryl thiodipropionate

- propionic acid, 3, 3'-thiodi-, didodecyl ester
- propanoic acid, 3, 3'-thiobis-, didodecyl ester
- bis(dodecyloxy carbonyl)ethyl sulfide
- dilauryl beta, beta'-thiodipropionate
- dilauryl 3, 3'-thiodipropionate
- didodecyl 3, 3'-thiodipropionate
- lauryl 3, 3'-thiodipropionate
- Plastanox LTDP Antioxidant
- thiobis(dodecyl propionate)
- Antioxidant LTDP
- Neganox DLTP
- Antioxidant AS

- DLTP
- Ipognox 89
- DMPTP
- Advastab 800
- Plastanox LTDP
- DLT
- DLTP
- Stabilizer DLT
- Tyox B
Stuckey (40) suggests these sulfur-containing antioxidants are relatively ineffective and often produce problems with odor and flavor. They are useful in combination with phenolic-type antioxidants in the stabilization of food packaging film such as polyolefins.

Food grade dilauryl thiodipropionate is described in the Food Chemicals Codex (6) as white crystalline, water insoluble, flakes having a characteristic sweetish, ester-like odor. It should assay as not less than 99.0 percent \( C_{30}H_{58}O_4S \) and have an acidity (as thiodipropionic acid) of not more than 0.2 percent of \( C_6H_{16}O_4S \). Arsenic content is limited to 3 ppm and heavy metals (as Pb) may not exceed 20 ppm with not more than 10 ppm of lead. Food grade specifications are not given in Food Chemicals Codex for thiodipropionic acid.
III B. CONSUMER EXPOSURE DATA

A survey of the food industry performed by NRC indicated that dilauryl thiodipropionate and thiodipropionic acid were not added to food in the United States in 1970 by those firms that responded to the survey. It is possible that the distribution of these compounds in premixes and under different names may have been unreported in the survey.

The Joint FAO/WHO Expert Committee on Food Additives evaluation of the available data on thiodipropionic acid and its dilauryl ester resulted in an estimate of an acceptable daily intake for man of 3 mg per kg body weight calculated as thiodipropionic acid (41).
IV B. BIOLOGICAL STUDIES

Absorption and metabolism

Rats consuming 3.0 percent thiodipropionate (741 mg per rat per day) or 3.0 percent dilauryl thiodipropionate (715 mg per rat per day) for a "prolonged period" excreted less than one-third of the ingested antioxidant as the acid in their urine (42). No thiodipropionic acid was detected in the feces collected over a 48-hour period. Dogs receiving 3.0 percent of their diet as dilauryl thiodipropionate excreted in urine an amount of thiodipropionic acid equivalent to about one-third of that ingested. None was excreted when the level in the diet was 0.1 percent, the only other level fed. More recent studies using tracer techniques indicated a slightly greater rate of excretion.

Single, oral doses of [1-\textsuperscript{14}C]thiodipropionic acid administered to rats were rapidly eliminated, 87 to 95 percent of 241 to 650 mg per kg doses being recovered in 4 days: 78 to 88 percent in urine, 0.1 to 0.9 percent in feces, and 3 to 8 percent as carbon dioxide (43). Radioactivity in tissues and organs was less than 1.5 times background. A 3 mg per kg dose of thiodipropionic acid was handled similarly. Urinary radioactivity at the higher dose was due almost entirely to unchanged thiodipropionic acid, while the lower dose resulted in an unidentified acid-labile conjugate. Similar excretion patterns occurred after single, oral doses of dilauryl [1-\textsuperscript{14}C]thiodipropionate (107 and 208 mg per kg) and after 1-day feeding of 166 mg per kg body weight. However, radioactivity levels of fat were elevated 34 days after dosing. Evidence suggests that dilauryl thiodipropionate may be hydrolyzed in the organism to the parent acid, which is then eliminated similarly to thiodipropionic acid given orally.

Acute toxicity

Tullar (42), summarizing unpublished data, estimated the oral LD\textsubscript{50} of thiodipropionic acid in the mouse as approximately 2.0 g per kg body weight. Deaths occurred over a period of 2 days. Two of 15 rats died, one on day 4 and the second on day 7, after receiving an oral dose of 2.0 g per kg. Dilauryl thiodipropionate (in olive oil) had an oral LD\textsubscript{50} greater than the highest dosages given: 2.0 g per kg in the mouse and 2.5 g per kg in the rat. Lehman et al. (44) reported an oral LD\textsubscript{50} for thiodipropionic acid in the rat of 3.0 g per kg and in the mouse of 2.0 g per kg.

Administration of dilauryl thiodipropionate suspended in 0.85 percent saline as an acute dose of 5.0 g per kg to each of 12 male rats by gastric intubation produced no adverse effects (45). Necropsies on day 6 revealed no gross morphological changes in the organs examined.
Short- and long-term studies

Groups of 20 young male rats were fed thiodipropionic acid or dilauryl thiodipropionate at levels of 0.5, 1.0, and 3.0 percent in the diet (the latter equivalent to about 1.5 g per kg body weight) for a period of 2 years and no adverse effects were indicated by growth rate (42). The investigator noted "a serious number of spontaneous deaths at two of the three feeding levels" of dilauryl thiodipropionate as compared to groups receiving thiodipropionic acid or to controls. However, Lehman et al. (44) reviewed these same data and concluded "none of the treated groups showed significant differences from the controls in the number of animals living at any time up to the end of the experiment." Inclusion of 0.5 percent thiodipropionic acid in the drinking water of guinea pigs for 119 days had no significant effect on weight gain or mortality (42).

A mixture of 10 to 1 by weight of dilauryl thiodipropionate and thiodipropionic acid (heated with lard at 190°C for 30 minutes) was fed to groups of 20 rats for 52 weeks at levels of 0.11 and 1.1 percent of the diet (46). No detectable toxic effects occurred during a 52-week period in the rats fed the mixture at a level of 0.11 percent of the diet. However, a very high incidence of mortality during the last 26 weeks of the experiment occurred in the group fed the mixture at a level of 1.1 percent in the diet. The consensus of the investigators was that deaths were caused by respiratory infection.

Lehman et al. (44) reviewed an experiment in which a mixture of 10 to 1 by weight of dilauryl thiodipropionate and thiodipropionic acid (heated at 190°C for 30 minutes) was fed to two adult male mongrel dogs as 0.1 or 3.0 percent of their diet for 100 days. The Select Committee examined the final report on this study prepared by Tullar (42) and found the data too limited to draw conclusions.

Special studies

No significant effects on nidation or on maternal or fetal survival were produced by dilauryl thiodipropionate fed after the 5th day of gestation to pregnant mice and rats for 10 days at levels up to 1.6 g per kg, to pregnant hamsters for 5 days at levels up to 1.6 g per kg, or to pregnant rabbits for 13 days at levels up to 1.0 g per kg body weight (47). The number of abnormalities occurring in fetal or skeletal tissues of the test groups did not differ from controls.

Dilauryl thiodipropionate was tested for mutagenic effects by the host-mediated assay in mice (50 to 5000 mg per kg), by cytogenetic studies in rats (50 to 5000 mg per kg), in vitro with human embryonic lung culture cells (50 to 600 μg per ml), and by the dominant lethal assay in rats (50 to 5000 mg per kg) (45). The results of the host-mediated assay using S. typhimurium
strain G-46 indicated a slight dose response of induced reversion in this system; results with strain TA-1530 and S. cerevisiae strain D-3 were negative. Results of the cytogenetic and dominant lethal assays indicated this compound was nonmutagenic under the conditions employed.

Walter Reed-Carworth Farms rats in their first pregnancies were fed a total dose of 0.5 g thiodipropionic acid over 22 days (48). On day 22 after positive mating, caesarian delivery of young and counting of resorption sites were done; 7 percent of 128 implantations were resorbed, a slightly lower percentage than the controls.

Thiodipropionic acid was not mutagenic in in vitro assays utilizing S. cerevisiae strain D-4 and S. typhimurium strains TA-1535, TA-1537, and TA-1538 with and without addition of mammalian metabolic activation preparations from the livers and lungs of adult male mice, rats and monkeys (49).

Tests of the teratogenic effects of thiodipropionic acid and dilauryl thiodipropionate to the developing chicken embryo were negative (50, 51).
V B. OPINION

A survey of industry indicated thiodipropionic acid and dilauryl thiodipropionate were not added to processed foods in 1970. There might be some quantity of these compounds in antioxidant formulations in use today that the Select Committee has not been able to identify. If so, it is most likely to be small. In any case, it may be advisable that this value be ascertained. These substances are of limited value as antioxidants in food systems but may be of greater importance in food packaging films. If thiodipropionic acid and dilauryl thiodipropionate are added to food, present limitations require that the total content of antioxidants may not exceed 0.02 percent of fat or oil content, including essential (volatile) oil content of the food. When used in food packaging, the resulting level of addition to the packaged food must be less than 0.005 percent.

Thiodipropionic acid and dilauryl thiodipropionate are of low acute toxicity when orally administered to experimental animals. Doses of 100 mg per kg administered to rats were absorbed and largely excreted in the urine within a few days as thiodipropionic acid or an acid-labile conjugate. Apparently less than 10 percent is otherwise metabolized, and in the case of the ester, evidence of some incorporation into fat depots was detected. No studies of the disposition of these compounds in humans or subhuman primates are available for review. Tests of the teratogenic and mutagenic effects of both compounds did not suggest cause for concern. The only reports of feeding studies are unpublished and of limited thoroughness. The unpublished feeding studies on thiodipropionic acid in rats and guinea pigs presented no adverse effects as measured by growth rate or mortality. However, in unpublished long-term studies, the investigators noted increased mortality in groups of rats fed dilauryl thiodipropionate as 0.5 and 3 percent of the diet and as a mixture with thiodipropionic acid as 1.1 percent of the diet. It would seem advisable to conduct adequate long-term feeding studies on thiodipropionic acid and dilauryl thiodipropionate should it be ascertained that significant amounts are currently being used.

In light of these considerations, the Select Committee concludes that:

There is no evidence in the available information on thiodipropionic acid and dilauryl thiodipropionate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current and in the manner now practiced. However, it is not possible to determine, without additional data, whether a significant increase in consumption would constitute a dietary hazard.
There is no evidence in the available information on thiodipropionic acid and dilauryl thiodipropionate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used as ingredients in food packaging materials at levels that are now current or that might reasonably be expected in the future.
VI. REFERENCES CITED


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VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

Joseph F. Borzelleca, Ph.D., Professor of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Va.

Harry G. Day, Sc.D., Professor Emeritus of Chemistry, Indiana University, Bloomington, Ind.

Samuel J. Fomon, M.D., Professor of Pediatrics, College of Medicine, University of Iowa, Iowa City, Iowa.

Bert N. LaDu, Jr., M.D., Ph.D., Professor and Chairman, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Mich.

John R. McCoy, V.M.D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, N.J.

*Sanford A. Miller, Ph.D., Professor of Nutritional Biochemistry, Massachusetts Institute of Technology, Cambridge, Mass.

Gabriel L. Plaa, Ph.D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

Michael B. Shimkin, M.D., Professor of Community Medicine and Oncology, School of Medicine, University of California, San Diego, La Jolla, Calif.

Ralph G.H. Siu, Ph.D., Consultant, Washington, D.C.

John L. Wood, Ph.D., Distinguished Service Professor, Department of Biochemistry, University of Tennessee Medical Units, Memphis, Tenn.

George W. Irving, Jr., Ph.D. (Chairman), Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Md.

*Did not participate in the final opinion reached in this report.
2. LSRO staff:

Kenneth D. Fisher, Ph.D., Director
Frederic R. Senti, Ph.D., Associate Director
C. Jelleff Carr, Ph.D., Director Emeritus
Richard G. Allison, Ph.D., Staff Scientist
Herman I. Chinn, Ph.D., Senior Staff Scientist
Andrew F. Freeman, Senior Staff Scientist
John M. Talbot, M.D., Senior Medical Consultant
Michael J. Wade, Ph.D., Staff Scientist

Report submitted by:

[Signature]
September 20, 1979
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances