EVALUATION OF THE HEALTH ASPECTS OF STARCH AND MODIFIED STARCHES 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
EVALUATION OF THE HEALTH ASPECTS OF STARCH
AND MODIFIED STARCHES 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

Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20014
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
CONTENTS

I. Introduction .............................................. 1
II. Background information .............................. 3
III. Consumer exposure data ............................ 12
IV. Biological studies .................................... 18
V. Opinion .................................................. 61
VI. References cited ...................................... 80
VII. Scientists contributing to this report .......... 98
I. INTRODUCTION

This report concerns the health aspects of using starch and modified starches as food ingredients. 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 Life Sciences Research Office staff. In addition, announcement was made in the Federal Register on June 16, 1978 (43 FR 26132) 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 using starch and modified starches as food ingredients or, in lieu of an oral hearing presentation, to submit a written statement. Two organizations submitted written statements in lieu of oral hearing presentations: Corn Refiners Association, Inc., 1001 Connecticut Avenue, N.W., Washington, D.C., and CPC International, Inc., International Plaza, Englewood Cliffs, N.J. No other requests were received and a hearing was not held.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premrmarketing 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 [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

*The document (PB-241 956/2) is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield, Virginia 22161.

- 1 -
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 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 starch and modified starches 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.
II. BACKGROUND INFORMATION

This report evaluates the health aspects of the cereal starches [specifically cornstarch, waxy maize starch, high amylose cornstarch, wheat starch, milo starch (also called grain sorghum starch), and rice starch], potato starch, tapioca starch and arrowroot starch as direct food ingredients and as substances migrating to food from paper and paperboard products and cotton and cotton fabrics used in food packaging. The chemical modifications of these starches given in the Food Chemicals Codex (3) also are evaluated as food ingredients.

Starch is a polymer of glucose and is the carbohydrate reserve of plants. Native starch in tubers, roots, seeds, fruits and in the pith of some plants, occurs as minute granules varying in size (about 3 to 100 microns diameter in commercial starches) and shape depending on the plant source. The principal sources of commercial starch are corn, wheat, rice, grain sorghum, potato, cassava, arrowroot and sago palm (4, 5). Starch is produced commercially by extraction from seeds and tubers of plants by wet-milling processes in which the starch is liberated by grinding aqueous slurries of the raw material. Sulfur dioxide is used to aid in the separation of starch from the protein matrix of corn and grain sorghum; inhibit the action of oxidative enzymes that discolor potato starch and, in case of tapioca starch, to aid in starch settling, improve color and inhibit bacterial action (6-8).

Nonstarch constituents in cornstarch, the major starch produced in the United States, are moisture, 10 to 11 percent; fat (total, by acid hydrolysis) 0.65 percent; protein, 0.25 to 0.30 percent; ash, 0.08 percent; and oil (CCl₄ extraction) 0.02 percent (6). Good quality potato starch has the approximate composition: ash, 0.35 percent; nitrogen, trace; fat, practically nil; and cold water solubles, 0.1 percent. Potato starch is unusual in that it contains 0.06 to 0.1 percent phosphorus. Phosphorus is present as dihydrogen orthophosphate groups esterified to the amylopectin fraction (7).

The common starches contain two polysaccharide components, amylose and amyllopectin. Amylose is a linear polymer containing about 200 to 2,000 D-anhydroglucose units (32,000 to 320,000 mol. wt.) joined by α-1,4 bonds. Amylopectin is a highly branched molecule that consists of linear α-1,4 linked segments branched through α-1,6 bonds at intervals of 15 to 25 anhydroglucose units. Molecular weights of the amylopectins of the common starches are in the multimillions (4, 5). The relative proportions of amylose and amylopectin are characteristic of any particular plant species. Cornstarch contains about 27 percent amylose; potato, 22 percent and tapioca, 17 percent. Genetic variants of corn, grain sorghum and rice, the waxy cereal grains, are composed almost entirely of amylopectin (9). Another genetic variant of corn, amylomaize, contains starch having 50 to 80 percent amylose (10).
Starch molecules in the granule are held together by hydrogen bonding. A preferred orientation of the amylose molecules and linear segments of the amyllopectin molecules is manifest in birefringence properties of the granule. When a slurry of starch in water is heated beyond a critical temperature (62°C for cornstarch) the granules swell to many times their original size. This process, referred to as gelatinization, is accompanied by a loss of granule birefringence, increase in optical clarity and abrupt rise in viscosity of the solution. Some molecules leach out of the swollen granules which eventually reach maximum hydration, rupture and collapse as heating is continued to 95°C, yielding a dispersion of granule fragments, molecular aggregates and free molecules. As this occurs, the viscosity decreases and tends to stabilize. On cooling, the viscosity increases and the system may set to an opaque gel depending on the starch source and concentration. Starch gels show syneresis on standing. If the starch gel is frozen and thawed, the effect is magnified. Upon slow cooling of starch dispersions, the amylose molecules tend to associate and become insoluble, a process called retrogradation (5,11).

Unmodified starches are primarily used in foods as thickening or gelling agents and processing aids. Corn or wheat starch may be used to thicken foods where the opacity of the starch dispersions and their gelling and syneresis characteristics are not a serious disadvantage. Ordinary cornstarch, for example, is used where a gel structure is required in custard and cream fillings. Waxy corn and tapioca starches may be used in canned foods to provide temporary viscosity and keep ingredients uniformly suspended during filling operations. However, for a large number of food applications, modified starches have been introduced because of their superior properties (5,11).

Both unmodified and modified starches are widely used in the manufacture of paper and paperboard products. Unmodified starches, generally in the form of gelatinized starch pastes prepared at the paper mill, are added to the wood pulp slurry in the beater to serve as an internal binder and strengthen the sheet formed on the paper machine. In some mills, raw starch is added dry to the pulp furnish. However, to be effective the wet paper sheet must reach temperatures sufficient to gelatinize the starch on the dryer. Pregelatinized starches are used in mills where a dry additive is needed because of lack of starch cooking facilities (12).

Starch pastes are applied as a surface sizing to the formed paper sheet to improve appearance and erasibility, inhibit ink penetration and to form a hard surface for writing or printing. Native starches are enzyme-converted to a low viscosity for this application. Chemically modified starches such as oxidized starch also are used for this purpose. Starch also serves as an adhesive in paper coatings to bond pigment particles to each other and to the paper. Most commonly used pigments are clay, calcium carbonate and titanium dioxide. Coating improves the appearance and printability of the paper. Oxidized and enzyme-converted starches are commonly used as coating adhesives (12).
In the textile industry, pastes of unmodified cornstarch are used in sizing of warp yarns to improve strength and abrasion resistance and reduce fuzz on the yarn prior to the weaving operation. Starch is used primarily in the sizing of cotton yarns and is removed from most fabrics subsequent to weaving. The high biochemical oxygen demand (BOD) of starch wastes from cotton mills has encouraged the use of chemically modified starches and other sizing materials having lower BOD's in recent years (13).

Cornstarch is generally recognized as safe (GRAS) by FDA as a food ingredient (14). Starch, modified, appears on the list of substances presumed GRAS by FDA that was included in the NAS/NRC survey of industry on the use of food chemicals generally recognized as safe (15). Use of specified modified starches in food is regulated under CFR 172.892 (2).

Unmodified starch, cornstarch, pregelatinized starch and acid-modified starch are considered GRAS (2) as substances migrating to food from paper and paperboard products used in food packaging [21 CFR 182.90]. Cornstarch, potato starch, tapioca starch and wheat starch are cited among the GRAS substances that may migrate to food from cotton and cotton fabrics used in dry food packaging [21 CFR 182.70]. Modified starches used as food ingredients that are also used in the manufacture of paper and paperboard are hypochlorite-oxidized, acid-modified, and enzyme-modified starches (2,12,16,17). Starch modified by amylolytic enzymes was considered in another report of the Select Committee (18) and will not be evaluated in this report. Regulations applicable to additional chemically modified starches that are used in paper and paperboard intended for food packaging and for such products that will contact food, are given in 21 CFR 178.3520.

Specifications for modified food starch are given in the Food Chemicals Codex (3). Upper limits of impurities specified are arsenic, 3 ppm; heavy metals (as Pb), 40 ppm; lead, 5 ppm and sulfur dioxide, 80 ppm. Additional specifications for specific starch modifications are given in Table I. These specifications are essentially identical with those given for food starch-modified in 21 CFR 172.892. No specifications are given in the Food Chemicals Codex for food grade unmodified starches.

The following types of chemical treatments are used in making modified starches for use as food ingredients:

1. Degradation of the starch molecule by acid hydrolysis or moderate oxidation in order to reduce the viscosity of starch pastes and permit them to be used at higher concentrations than would be possible with native starches. Acid-modified starches (Table I, product 1) are made by acid-catalyzed (hydrochloric or sulfuric acid) hydrolysis of water suspensions of granular starches at nongelatinizing temperatures. When the desired reduction in
TABLE I
Specifications for Modified Food Starches (3)

<table>
<thead>
<tr>
<th>Product</th>
<th>Starch treatment</th>
<th>Residuals limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Acid-modified starch</td>
<td>Hydrochloric and/or sulfuric acid</td>
<td></td>
</tr>
<tr>
<td>2. Bleached starch</td>
<td>Active oxygen obtained from hydrogen peroxide, and/or peracetic acid, not to exceed 0.45 percent of active oxygen Ammonium persulfate, not to exceed 0.075 percent, and sulfur dioxide, not to exceed 0.05 percent Chlorine, as sodium hypochlorite, not to exceed 0.0082 pounds (3.72 g) of chlorine per pound (454 g) of dry starch Potassium permanganate, not to exceed 0.2 percent Sodium chlorite, not to exceed 0.5 percent</td>
<td>Not more than 50 ppm of residual manganese (as Mn)</td>
</tr>
<tr>
<td>3. Oxidized starch</td>
<td>Chlorine as sodium hypochlorite, not to exceed 0.055 pounds (25 g) of chlorine per pound (454 g) of dry starch</td>
<td></td>
</tr>
<tr>
<td>4. Starch acetate</td>
<td>Acetic anhydride or vinyl acetate</td>
<td>Not more than 2.5 percent of acetyl groups introduced into finished product</td>
</tr>
<tr>
<td>5. Starch sodium succinate</td>
<td>Succinic anhydride, not to exceed 4 percent</td>
<td></td>
</tr>
<tr>
<td>6. Starch sodium octenyl succinate</td>
<td>1-octenyl succinic anhydride, not to exceed 3 percent</td>
<td></td>
</tr>
<tr>
<td>7. Starch aluminum octenyl succinate</td>
<td>1-octenyl succinic anhydride, not to exceed 2 percent, and aluminum sulfate not to exceed 2 percent</td>
<td></td>
</tr>
<tr>
<td>8. Starch phosphate</td>
<td>Monosodium orthophosphate</td>
<td>Not more than 0.4 percent of residual phosphate (calculated as P)</td>
</tr>
<tr>
<td>Product</td>
<td>Starch treatment</td>
<td>Residuals limitation</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>9.  Distarch phosphate</td>
<td>Phosphorus oxychloride, not to exceed 0.1 percent</td>
<td>Not more than 0.04 percent residual phosphate (calculated as P)</td>
</tr>
<tr>
<td></td>
<td>Sodium trimetaphosphate</td>
<td></td>
</tr>
<tr>
<td>10. Phosphated distarch</td>
<td>Sodium tripolyphosphate and sodium trimetaphosphate</td>
<td>Not more than 0.4 percent residual phosphate (calculated as P)</td>
</tr>
<tr>
<td>distarch phosphate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11. Acetylated distarch</td>
<td>Phosphorus oxychloride, not to exceed 0.1 percent, followed by either acetic</td>
<td>Not more than 2.5 percent of acetyl groups introduced into finished product</td>
</tr>
<tr>
<td>distarch phosphate</td>
<td>anhydride, not to exceed 8 percent, or vinyl acetate, not to exceed 7.5 percent</td>
<td></td>
</tr>
<tr>
<td>12. Hydroxypropyl distarch</td>
<td>Phosphorus oxychloride, not to exceed 0.1 percent, and propylene oxide, not to</td>
<td>Not more than 5 ppm of residual propylene chlorohydrin</td>
</tr>
<tr>
<td>phosphate</td>
<td>exceed 10 percent</td>
<td></td>
</tr>
<tr>
<td>13. Hydroxypropyl starch</td>
<td>Propylene oxide, not to exceed 25 percent</td>
<td>Not more than 5 ppm of residual propylene chlorohydrin</td>
</tr>
<tr>
<td>14. Oxidized hydroxypropyl</td>
<td>Chlorine, as sodium hypochlorite, not to exceed 0.055 pounds (25 g) of chlorine</td>
<td>Not more than 5 ppm of residual propylene chlorohydrin</td>
</tr>
<tr>
<td>starch</td>
<td>per pound (454 g) of dry starch; active oxygen obtained from hydrogen peroxide,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>not to exceed 0.45 percent; and propylene oxide not to exceed 25 percent</td>
<td></td>
</tr>
<tr>
<td>15. Acetylated distarch</td>
<td>Adipic anhydride, not to exceed 0.12 percent, and acetic anhydride</td>
<td>Not more than 2.5 percent of acetyl groups introduced into finished product</td>
</tr>
<tr>
<td>adipate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Distarchoxy propanol</td>
<td>Acrolein, not to exceed 0.6 percent</td>
<td></td>
</tr>
<tr>
<td>17. Distarch glycerol</td>
<td>Epichlorohydrin, not to exceed 0.3 percent</td>
<td></td>
</tr>
<tr>
<td>Product</td>
<td>Starch treatment</td>
<td>Residual limitation</td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>18. Acetylated distarchoxy propanol</td>
<td>Acrolein, not to exceed 0.6 percent; vinyl acetate not to exceed 7.5 percent</td>
<td>Not more than 2.5 percent of acetyl groups introduced into finished product</td>
</tr>
<tr>
<td>19. Hydroxypropyl distarch glycerol</td>
<td>Epichlorohydrin, not to exceed 0.1 percent, combined with propylene oxide, not to exceed 10 percent</td>
<td>Not more than 5 ppm of residual propylene chlorohydrin</td>
</tr>
<tr>
<td></td>
<td>Epichlorohydrin, not to exceed 0.1 percent, followed by propylene oxide, not to exceed 25 percent*</td>
<td>Not more than 5 ppm of residual propylene chlorohydrin</td>
</tr>
<tr>
<td>20. Acetylated distarch glycerol</td>
<td>Epichlorohydrin, not to exceed 0.3 percent, and acetic anhydride</td>
<td>Not more than 2.5 percent of acetyl groups introduced into finished product</td>
</tr>
<tr>
<td>21. Succinyl distarch glycerol</td>
<td>Epichlorohydrin, not to exceed 0.3 percent and succinic anhydride, not to exceed 4 percent</td>
<td></td>
</tr>
<tr>
<td>22. Gelatinized starch</td>
<td>Sodium hydroxide, not to exceed 1 percent</td>
<td></td>
</tr>
</tbody>
</table>

*This specification is given in 21 CFR 172.892.*
viscosity has been achieved, the acid is neutralized and the granular starch is filtered, washed, and dried. The basic reaction is hydrolysis of glucosidic bonds of starch molecules and reduction in molecular size. Acid-modified starches are characterized by the fluidity of their pastes as measured under standard conditions. Commercial products range in fluidity from less than 10 to about 90. Molecular weights of amylose and amylpectin fractions of a commercial 90-fluidity starch were about 30,000 as determined by osmotic pressure measurements (17, 19).

Dextrins are modified starches produced by dry heating or roasting starch with or without an acid or alkaline catalyst. Hydrolysis of glucosidic bonds and/or transglucosidation reactions occur, their relative extent depending on reaction conditions. The dextrins were considered in another report of the Select Committee (20) and will not be evaluated in this report.

2. Treatment with minimal quantities of oxidizing agents to produce bleached starches (Table I, product 2). Reagents used are hydrogen peroxide, peracetic acid, ammonium persulfate, sodium hypochlorite, potassium permanganate and sodium chlorite. Oxidation removes color due to traces of pigments such as xanthophyll and carotene. Color is objectionable in applications where starch is added as a moisture sorbing and fluidifying agent for dry powders such as confectioners sugar. Agents used for bleaching also help reduce the microbiological count to levels necessary for certain applications. Treatments are made on aqueous suspensions of starch so that color bodies solubilized by bleaching may be removed by filtering and washing (21). Excess oxidant is neutralized by reduction with sodium bisulfite or sulfur dioxide prior to final filtration, washing and drying (19).

Oxidized starches are made by treatment of aqueous suspensions of starch granules with chlorine, as sodium hypochlorite, at levels up to 5.5 percent of the dry starch weight (Table I, product 3). On completion of the oxidation the reaction mixture is neutralized with acid; bisulfite solution or sulfur dioxide is added to destroy any unspent oxidant, and the reaction mixture is diluted with water. The product is recovered on a filter, washed with water and dried. A limited number of starch hydroxyl groups are converted to carboxyl and carbonyl groups with concomitant scission of the starch chain. Introduction of carboxyl and carbonyl groups reduces the tendency of starch solutions to retrograde and their pastes to gel. The number of carboxyl and carbonyl groups introduced may be about 1 per 30 to 200 anhydroglucose units, the values within this range depending on the particular starch and the reaction conditions (16, 19).

3. Reacting starch with monofunctional reagents to introduce substituents on the hydroxyls by esterification or etherification. Introduction of substituent groups such as acetate, succinate, octenyl succinate, phosphate, and hydroxypropyl into the molecule (Table I, products 4-8, 13) reduces the
tendency of starch to associate in solution, lose clarity and form gels (5,22). Extent of substitution is low in all derivatives (Table I) except hydroxypropyl starch which may contain up to 15 percent or possibly more hydroxypropyl groups. Polyelectrolyte properties result from the introduction of the phosphate and the succinate ester groups.

4. **Cross-linking modification** by treating starch in the granule form with very small amounts of difunctional agents capable of reacting with hydroxyl groups of two different molecules within the granule (Table I, products 9, 15-17). Cross-linking stabilizes the viscosity of starch pastes by maintaining the integrity of gelatinized, swollen granules at high temperatures, in acid solutions and under shear conditions. Flow properties and clarity of pastes also are improved for certain applications. Cross-linking reactions are normally run on aqueous suspensions of granular starch at temperatures below gelatinization. A cross-linking reagent that does not react with starch is hydrolyzed under conditions of the reaction and largely removed by washing (5,23). The residuals limitation of 0.04 percent phosphorus for distarch phosphate (Table I, product 9) corresponds to one phosphate cross-link for about every 500 anhydroglucose units.

5. **Reaction of cross-linked starches** with monofunctional reagents to introduce substituent groups by esterification or etherification (Table I, products 10-12, 18-21). These starches are used as thickeners in canned pie fillings, retorted puddings and similar applications where stability at high processing temperatures and prolonged low temperature storage are needed (5). A survey of infant foods in 1970 indicated that about 80 percent of strained and junior dinners, high meat dinners, desserts and fruits contained cross-linked starches or their monofunctional esters and ether derivatives (24). However, a 1977 survey indicated that only 65 percent of infant foods contained a modified starch at that time (25).

6. **Gelatinization of starch** by physical or chemical treatment. Pregelatinized (i.e., gelatinized prior to incorporation into a food product) starches are commonly prepared in the starch industry by feeding moist starch cake, a starch slurry, or a cooked starch paste to the rolls of a drum dryer which operates at temperatures above the gelatinization temperature of the starch. The dry gelatinized product is classified by screening to give particle sizes best suited to various applications. Chemically modified starches also may be pregelatinized by this process (26).

Specifications for pregelatinized starch prepared by physical treatment are not included in the Food Chemicals Codex (3) but gelatinized starch prepared by treatment with alkali is included as a modified food starch (Table I, product 22). Depending on the conditions of treatment, hydrolytic scission of glucosidic bonds and/or oxidation of hydroxyl groups may occur in addition to gelatinization of the granule (16). It appears that alkali
gelatinized starch is not a commercial product in the United States. However, sodium hydroxide is used by the industry as a catalyst in chemical modification of starch and for neutralization and washing of starch and modified starches (27).

The Food Chemicals Codex (3) specifications in Table I place upper limits on the content of acetyl and phosphate groups that are permitted in the corresponding food starch derivatives. Similar limitations on the content of introduced chemical groups are not specified for the other derivatized food starches. It appears that the conditions specified for starch treatment are insufficient to define the extent of reaction with the various reagents. Levels of unreacted phosphate reagent are limited for starch phosphate derivatives by the residuals limitation on total phosphorus. To the extent that bleached and oxidized starches are treated with sulfur dioxide or sulfite to neutralize excess oxidant, the limitation of 80 ppm on residual sulfur dioxide for specified modified starches is a residuals limitation for these products. A further limitation on residuals for permanganate bleached starches is given by the specification of no more than 50 ppm residual manganese. The specification that modified starches shall have a pH range between 3.0 and 9.0 places a residuals limitation on acids in acid-modified starches and base in sodium hydroxide-gelatinized starches. Residual levels are not specified for vinyl acetate nor the reaction byproduct, acetaldehyde, in starch acetate or acetylated distarch phosphate, derivatives for which vinyl acetate is permitted as an acetylation reagent. Neither are residual reactant limitations specified for the starch succinate derivatives or for distarchoxy propanols. However, manufacture of the latter starches was discontinued many years ago; residual acrolein levels were less than 1 ppm (27a). Propylene oxide will react with chloride ion present in the reaction mixture to form propylene chlorohydrins. Specifications for hydroxypropyl starch derivatives place a limitation of 5 ppm on residual propylene chlorohydrin; however, present commercial starches modified with propylene oxide contain less than 1 ppm total chlorohydrins using a test sensitive to about 0.1 ppm (28). Epichlorohydrin, the cross-linking agent used in producing distarch glycerols will react with water in the presence of alkali used to catalyze the reaction with starch to produce 3-chloro-1,2-propanediol and glycerol; in the presence of trace amounts of chloride ion, it will react to produce 1,2-dichloro, 3-propanol and the isomeric compound, 1,3-dichloro, 2-propanol (29). No residual limitations are specified for these compounds. Present evidence indicates that epichlorohydrin cross-linked starches contain less than 0.05 ppm residual epichlorohydrin, the sensitivity limit of present analytical methods, and less than 3 ppm total of the chlorinated propanols. Industry has, however, discontinued manufacture of epichlorohydrin cross-linked starches (28).
Starch, mainly as a component of cereal products and vegetables, supplies about 20 percent of the energy content of the average American diet. In the years from 1971 through 1974, the available food supply provided about 180 g of starch per capita per day (30).

According to Agricultural Statistics (31), the annual per capita consumption of wet-milled cornstarch as food, 1968-1974, as estimated from disappearance data was 1.9 pounds (0.86 kg) or about 400 million pounds (182 million kg) total. Both unmodified and modified cornstarch are included in these figures. Breakdown of sales of cornstarch, modified and unmodified, to the food industry in 1971 shows 160 million pounds (73 million kg) sold to grocers as packaged starch and 95 million pounds (43 million kg) sold to brewers (4). This leaves 145 (66 million kg) of the 400 million pounds of cornstarch for use as a food ingredient by food processors. Total food usage of potato, wheat, tapioca (cassava), sago and arrowroot starches in 1971 was estimated to be 100 million pounds (45 million kg) (4) which added to the figure for cornstarch gives a total of 245 million pounds (111 million kg) for all starches used by the food industry in 1971. A survey of the food industry by a National Research Council (NRC) subcommittee (15) indicated 1970 usage of modified starch by food processors was 120 million pounds (54 million kg), based on poundage data adjusted to take into account the subcommittee's estimate that about 60 percent of the total poundage used was included in their survey. Assuming modified starch usage also was 120 million pounds in 1971, subtraction of modified starch usage from total starch usage leaves 125 million pounds (57 million kg) as the quantity of unmodified starches used in 1971 by food manufacturers. On a per capita daily basis, this amounts to 0.7 g, a quantity small in comparison to the 180 g of unmodified starch available per capita as a component of foods in the average diet. A recent NRC subcommittee survey indicated that 1975 usage of modified starch by the food industry was 170 million pounds (77 million kg) corresponding to a per capita daily usage of 1 g (32).

The NRC subcommittee survey (15) provided information on the level of addition of modified starch to foods in several food categories as given in Table II. Information on specific modified starches and unmodified starch usage was not requested. However, data were volunteered by three or fewer companies on levels of addition of several unmodified starches to some of the food categories. Because of the limited information base, these data are not included in this report.

The NRC subcommittee estimated the possible average daily intake of modified starches from Market Research Corporation of America data on the mean frequency of eating foods by food category, U.S. Department of
<table>
<thead>
<tr>
<th>Food Category</th>
<th>Starch, modified Weighted mean percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baked goods, baking mixes</td>
<td>1.62</td>
</tr>
<tr>
<td>Grain products, such as pastas or rice dishes</td>
<td>7.47</td>
</tr>
<tr>
<td>Fats and oils</td>
<td>1.77</td>
</tr>
<tr>
<td>Milk products</td>
<td>0.01</td>
</tr>
<tr>
<td>Cheese</td>
<td>1.00</td>
</tr>
<tr>
<td>Processed fruits, juices and drinks</td>
<td>2.20</td>
</tr>
<tr>
<td>Meat products</td>
<td>1.50</td>
</tr>
<tr>
<td>Poultry products</td>
<td>1.46</td>
</tr>
<tr>
<td>Egg products</td>
<td>3.49</td>
</tr>
<tr>
<td>Fish products</td>
<td>2.73</td>
</tr>
<tr>
<td>Processed vegetables, juices</td>
<td>1.09</td>
</tr>
<tr>
<td>Condiments, relishes, salt substitutes</td>
<td>0.94</td>
</tr>
<tr>
<td>Soft candy</td>
<td>8.29</td>
</tr>
<tr>
<td>Sugar, confections</td>
<td>2.38</td>
</tr>
<tr>
<td>Gelatins, puddings, fillings</td>
<td>3.02</td>
</tr>
<tr>
<td>Soups, soup mixes</td>
<td>1.22</td>
</tr>
<tr>
<td>Snack foods</td>
<td>1.81</td>
</tr>
<tr>
<td>Nuts, nut products</td>
<td>1.20</td>
</tr>
<tr>
<td>Reconstituted vegetable proteins</td>
<td>1.36</td>
</tr>
<tr>
<td>Gravies, sauces</td>
<td>1.70</td>
</tr>
<tr>
<td>Dairy products analogs</td>
<td>3.91</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>0.42</td>
</tr>
<tr>
<td>Baby food processed fruit</td>
<td>4.10</td>
</tr>
<tr>
<td>Baby food processed vegetables</td>
<td>4.32</td>
</tr>
<tr>
<td>Baby food puddings</td>
<td>4.53</td>
</tr>
<tr>
<td>Baby food soups, mixes</td>
<td>3.25</td>
</tr>
<tr>
<td>Baby food meat dinners</td>
<td>3.16</td>
</tr>
<tr>
<td>Baby food combination dinners</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Level of addition of modified starches is the weighted mean of the levels reported by manufacturers as their usual addition to one or more products in a food category. For discussion of weighted mean see text, also Section X and Exhibit 50 of Reference (15).
Agriculture data on mean portion size in these categories, and the assumption that all food products within a category contained modified starch at the level shown in Table II. Such an assumption is likely to lead to overestimates of intake. The NRC subcommittee has recognized that in most cases its calculations of possible intakes are overstated, often by considerable margins. This is probably the case with respect to modified starches. For the 2 to 65+ year-old age group the estimated possible average daily intake was 11.8 g as contrasted with the per capita daily usage of 0.7 g of modified starch by the food industry estimated from poundage data. The possible daily intakes given for the 0 to 5 and 6 to 11 month-old age groups are in good agreement with 1970 survey data obtained from one-day dietary histories of 430 infants between one and 14 months of age which indicated that the average daily intake of modified starch for this age group was about 5.7 g or about 2.3 percent (about 0.7 g per kg body weight) of the total caloric intake (24). The maximum intake of modified food starch by any infant in the survey was 35 g per day, about 16 percent of that infant's caloric intake. Modified food starches supplied more than 10 percent of the caloric intake for only four of the 430 infants. Cross-linked starches, esterified cross-linked starches and etherified cross-linked starches were consumed in the proportions of 10:5:1.

From a food consumption survey conducted in 1974, high and low values for the intake of modified food starches were estimated from the average food consumption of infants and the range in content of modified starches in the categories of foods eaten (33). Low values ranged from 2.2 percent (about 0.7 g per kg body weight) of the caloric intake for one-month-old infants to 3.7 percent (about 1 g per kg) for those 12 months old; high values ranged from 4.0 percent (about 1.3 g per kg) at one month to 6.2 percent (1.6 g per kg) at 12 months. Highest value was 7.1 percent (about 1.7 g per kg) of caloric intake for infants 6 months old.

A 1977 survey of infant food and food starch manufacturers disclosed that only three modified starches were being used in infant foods: distarch phosphate (level, >0.1 but <5.5 percent), acetylated distarch phosphate (level, >1 but <5.5 percent), and acetylated distarch adipate (level, >1 but <7 percent) (25). Infant formulas were reported to contain >0.5 but <3.0 percent distarch phosphate. However, only one infant formula listed in the Physicians' Desk Reference (34) is reported to contain modified starch in this concentration range; the level in this formula is given as 1 percent. Some special dietary formulas for feeding infants with particular medical problems contain more than 3 percent of a modified starch (34). During the second month of life, the 50th percentile for energy intake is estimated to be 565 kcal per day and the 90th percentile is estimated to be 680 kcal per day (35). Assuming the entire energy were provided by a formula containing 1 percent distarch phosphate and supplying 67 kcal per 100 ml, intakes of the modified food starch would be 8 and 10 g per day, respectively (approximately 1.8 and 2.1 g per kg per day).
From a food consumption survey conducted in April, 1977, intake of
modified food starches was estimated from the average food consumption of
151 infants and the content of modified starches in the infant foods eaten (25).
About 35 percent of the infant foods reported used contained no modified
starch and about one-fifth of the infants had diets containing no modified
starch. Overall average daily intake was 2.9 g (S.D. 6.1 g). Average daily
intake ranged from 0.55 g (S.D. 0.789) for 0 to 3-month-old infants to 5.1 g
(S.D. 5.1 g) for infants 8 months old. Maximum intakes for individuals in
these age groups were 3.1 and 15.8 g (0.6 and 1.7 g per kg), respectively.
The latter was the highest daily intake of any infant in the survey and repre-
sented 7.8 percent of that infant's caloric intake. About 50 percent of the
infants surveyed had modified starch intakes (g per kg body weight) in the
range 0 to 0.19; 17 percent, 0.20 to 0.39; 10 percent, 0.40 to 0.59; 9 per-
cent 0.60 to 0.79; 13 percent, 0.80 to 1.39; and 2 percent, 1.40 to 1.79.

The NRC subcommittee survey provided no data on the use of starches
or modified starches in paper and paperboard used in food packaging or in
cotton and cotton fabrics used in dry food packaging. Russell (36), however,
has estimated that approximately 3 billion pounds of cornstarch products,
including unmodified and modified starches, were used in industrial or non-
food applications in 1972. About 90 percent of this quantity was starch prod-
ucts used in the paper and paperboard industry. The fraction of these products
that was used for packaging foods was not estimated. Breakdown of corn-
starch sales for industrial applications by type of product is given in Table
III. It should be noted that unmodified starch sold to the paper industry is
largely processed before use by enzymatic hydrolysis or steam-jet cooking
to reduce its molecular size and viscosity. The quantities of the principal
starches sold to the textile industry in 1972 were estimated to be (in million
pounds): unmodified starch, 83; acid-modified, 170; and high-amylose, 22
for a total of 275 million pounds.
### TABLE III

Sales of Cornstarch Products for Industrial Applications in 1972 (36)

<table>
<thead>
<tr>
<th>Starch product</th>
<th>Millions of pounds</th>
<th>g/capita/day*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unmodified starch</td>
<td>1,817</td>
<td>10.8</td>
</tr>
<tr>
<td>Pregelatinized starches</td>
<td>100</td>
<td>0.6</td>
</tr>
<tr>
<td>Acid-modified starch</td>
<td>340</td>
<td>1.9</td>
</tr>
<tr>
<td>Oxidized starch</td>
<td>166</td>
<td>1.0</td>
</tr>
<tr>
<td>Dextrins</td>
<td>150</td>
<td>0.9</td>
</tr>
<tr>
<td>Cationic starches</td>
<td>100</td>
<td>0.6</td>
</tr>
<tr>
<td>All others, including hydroxy-ethyl starch</td>
<td>420</td>
<td>2.6</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>3,093</strong></td>
<td></td>
</tr>
</tbody>
</table>

*Calculated on basis of the U.S. population of 208 million in 1972.
Total per capita sales on a per day basis (Table III), and thus the maximum possible consumption of nonchemically derivatized starches (unmodified, pregelatinized and acid-modified) in paper and paper products was 13 g in 1972. This quantity is small compared to the quantity available (180 g) as a natural component of the foods in the average American diet. Per capita daily sales of the chemically modified starches was 2.6 g or less. As in the cases of the unmodified starches, only a very small fraction of this quantity could be expected to migrate into foods from paper or paper products used in food packaging.

Per capita sales on a per day basis of unmodified and acid-modified starches to the textile industry was 1.2 g. Because these starches are principally used as a sizing that is removed after the looming operation, only a small residual would likely remain in cotton or cotton fabrics used in dry food packaging (36).
IV. BIOLOGICAL STUDIES

Unmodified Starches and Pregelatinized Starches

Digestion and absorption

Cooked (gelatinized) native starch is digested within the intestinal lumen by \( \alpha \)-amylase secreted by the pancreas. The products are maltose, maltotriose and \( \alpha \)-limit dextrins containing five to nine glucose residues and one or more \( \alpha-1,6 \) branching links. The brush border of the intestinal mucosa contains an \( \alpha \)-dextrinase, glucoamylase and maltase that convert the products of intraluminal digestion into glucose. Only the monosaccharide glucose is transported through the intestinal wall into the bloodstream (37). Comprehensive discussions of the metabolism of glucose are given in standard biochemistry textbooks (38, 39).

Raw corn, waxy maize, wheat, rice and tapioca starches in diets containing 63.7 percent starch were 98 percent digested by weanling rats in 28-day feeding experiments whereas digestibilities of raw potato, arrowroot and sago starches in the same concentrations were 51, 80, and 65 percent, respectively. Utilization of starches as evidenced by weight gains at 28 days paralleled digestibility coefficients. Destruction of granule structure of potato starch by gelatinization increased digestibility to 96 percent (40). Digestibility of high amylose cornstarches containing from 35 to 67 percent amylose as determined in vitro by treatment with pancreatin was not directly associated with amylose content but was related to the genetic background of the corn variety (41). Digestibilities in rats of unmodified high amylose cornstarches having 50, 63, and 77 percent amylose were 71, 77, and 66 percent, respectively (42). Digestibility of high-amylose starch ingested as a component of corn meal muffins made with high amylose corn meal was 88 percent in a human subject (43). Microscopic examination showed that the starch was not gelatinized in baking the muffins. Although starch in the meal analyzed 70 percent amylose, starch granules recovered from the feces contained only 47 percent amylose. It has been suggested that susceptibility of granular starches to enzymatic attack is related to the size and number of pores in their granule structure which permit entry of enzyme molecules (44).

Pancreatic amylase activity in human infants is relatively low during the first few months of life and digestion of starch is slower than in older infants. Auricchio et al. (45) reported an \( \alpha \)-amylase activity of 3.9 to 7.7 units per ml in duodenal fluids of four infants 2 \( \frac{1}{2} \) to 5 months old whereas that of six infants 8 to 13 months of age was 48 to 73 units per ml. Average degree of polymerization of the saccharides in duodenal fluid of the younger groups of infants collected 2 to 4 hours after ingestion of amylpectin was 7 as compared to 3.3 for the older group. During balance periods of 3 days, De Vizia et al. (46) found that wheat, tapioca, corn, rice, and potato starch in amounts of 45 and 85 g per \( m^2 \) of body surface per day were almost completely absorbed.
(98 percent) in 1- and 3-month-old infants when fed as cooked flours. Known quantities of starch were fed and the fecal content of lactic acid, glucose, dextrins, and starch was measured. Only minimal rises in glucose concentration in the blood were observed after feeding a thin-boiling (acid-modified) waxy maize starch to 3-day-old infants by nipple which suggests slow hydrolysis and/or absorption of the starch (47). Mean blood glucose levels did not rise above the base line by more than 10 mg per 100 ml in infants 4 to 60 days of age after ingesting a 10 percent solution of a thin-boiling waxy sorghum starch (48). However, Hlavon and Klušaček (49) reported peak increases in blood glucose of 24, 8, 22, 18 and 34 mg per 100 ml after intragastric administration of 2 g rice starch (in 20 percent suspensions) per kg body weight to groups of 15 to 28 infants aged 1, 3, 5, 30 and 180 days, respectively. They state that the differences among age groups were not statistically significant. Similar studies with potato and cornstarch showed no significant differences in digestibilities among starches.

Acute toxicity

No reports were available on the acute toxicity of raw or cooked unmodified starches.

Short-term studies

Raw, unmodified corn, wheat, rice, tapioca and waxy maize starch gave normal weight gains, protein efficiency ratios (PER) and cecal weights when fed for 28 days to Wistar rats, 50 g initial body weight, in diets containing either 6 or 15 percent casein and 77 or 66 percent starch. Feed consumption of animals receiving the 6 percent protein diet containing raw potato starch was relatively high compared to that of animals fed the other starches; cecal weights also were relatively high but feed efficiency and PER were not significantly different. Feed efficiency and PER were relatively low in animals fed raw potato starch in the 15 percent protein diet but were normal when fed pregelatinized (dried, gelatinized) potato starch in the same basal diet. Unmodified arrowroot starch resulted in inferior weight gain and PER on both low and high protein diets; PER was normal for diets containing pregelatinized arrowroot starch but weight gain remained relatively low (50).

Raw cornstarch fed to 61 mice for 4 weeks at a 71 percent level produced fewer lesions of the duodenal mucosa than did glucose or sucrose fed to a similar number of animals (51).

In a study by Harper et al. (52), groups of five or six male weanling Wistar rats were given diets containing 18 percent casein and 73.6 percent of either unmodified potato starch, autoclaved potato starch, glucose, sucrose or dextrin for 4 to 6 weeks. Food intake was limited to 10 g per day. Weight gains were similar for all groups except the one fed unmodified potato starch.
Bulky white feces of animals in this group were composed largely of undigested starch granules. There also was loss of dietary protein in the feces. In another study, rats grew as well on raw wheat starch or cornstarch as on sucrose but raw potato starch was poorly utilized. All diets contained 74 percent carbohydrate. Grinding, heating in an oven at 145°C or autoclaving moistened starch at 120°C improved the utilization of potato starch (53).

Weanling pigs (breed not stated) fed diets containing high levels of pearl cornstarch and low levels of protein (raw cornstarch containing 7 to 10 percent gelatinized starch) developed a high incidence of esophagogastric ulcers within 80 days (54). Frequency of occurrence of ulcers in groups of seven or eight pigs fed 89, 77, 64, or 48 to 58 percent starch in their diets was 6/7, 8/8, 6/8, and 1/8, respectively. Protein contents of these diets were 0, 5, 10, and 12 to 16 percent in the order listed. In view of previous studies which indicated that diets containing gelatinized whole corn or gelatinized corn endosperm were associated with ulcers in swine, the investigators compared cornstarch flour (no heat treatment) with pearl cornstarch in a second feeding experiment with 8-week-old Duroc and Duroc x Yorkshire pigs. Incidence of ulcers in the two groups was similar. Other dietary and stress factors also have been associated with esophagogastric ulcers in swine (55-58).

Compulsive ingestion of raw starch as well as clay is not uncommon among certain segments of the population (59-62). As much as 2 pounds, generally laundry starch, may be ingested per day. On the basis of 14 percent moisture content, this provides 3160 calories. The chief symptoms are obesity and iron-deficiency anemia. In some cases enlargement of the parotid glands has been observed and one patient who consumed 3 to 4 pounds of starch per day developed a starch gastrolith (59).

Seven adult male subjects were given low fat diets containing 500 g of raw cornstarch or sucrose daily for a 25-day period (63). The only adverse effects noted by the subjects ingesting the starch diet were flatus and borborygmi. Serum lipids fell and serum transaminase rose. The authors suggested the latter might reflect a degree of liver damage.

Fifteen hyperlipoproteinemic patients were fed formula diets containing 50 percent of the calories as carbohydrate, 35 percent as fat and 15 percent as protein for two 28-day periods. Substitution of 40 percent of the calories as sucrose for wheat starch resulted in significant increases in levels of serum cholesterol, phospholipid and triglyceride in all patients (64).

Special studies

Animal feeding experiments indicate that diets containing starch as the principal carbohydrate component are less cariogenic than similar diets in which starch is replaced by sucrose, glucose or fructose (65-70).
Orland et al. (71) reported that a dextrin (uncharacterized) was less cariogenic than sucrose when fed to rats as the carbohydrate component in semisynthetic purified diets. Although less cariogenic than sucrose, roll-dried (gelatinized) cornstarch and roll-dried wheat starch (67) were found to be more cariogenic to rats than the corresponding uncooked starches. However, others have reported no difference in the incidence of carious lesions in rats fed cooked and raw wheat starches (70).

No studies designed to test the carcinogenicity, teratogenicity, or mutagenicity of unmodified starches were available to the Select Committee.

Acid-modified Starch

Digestion and metabolism

Acid-modified wheat starch in diets containing 63.7 percent starch was 97.8 percent digested by weanling rats in 28-day feeding experiments. Digestibility and utilization of acid-modified wheat starch as measured by weight gains did not differ significantly from the values found for unmodified wheat starch (40).

Short-term studies

An acid-modified waxy cornstarch (80 fluidity) contributed about 25 percent of the calories in the control diets fed to Pitman-Moore miniature pigs in two studies of chemically modified food starches (72, 73). The pigs were weaned at 3 days of age and fed the starch-containing diets for 25 days. Serum concentrations of cholesterol and triglyceride were markedly lower in the starch-fed pigs than in sow-reared pigs (74), reflecting the lower cholesterol content and highly unsaturated character of the fat in the starch diets. Urea levels in the serum of pigs fed the starch diets (26 mg per 100 ml) were higher than those in sow-reared pigs (18 mg per 100 ml) although protein levels in the diets were similar. All organs, including ceca, appeared to be grossly normal when inspected at autopsy. The liver weight, expressed as a percentage of body weight, was slightly less than that found at 28 days in sow-reared pigs but the weights of other organs expressed as a percentage of body weight were comparable.

Acute toxicity

Attempts to demonstrate an acute toxicity level for starch in rats resulted in gastric rupture. No deaths resulted from the intragastric administration of a 60 percent paste of a soluble acid-hydrolyzed potato starch in volumes of 50 to 100 ml per kg body weight but larger doses produced gastric rupture. Larger total daily doses could be tolerated if 50 to 100 ml volumes were administered every $2\frac{1}{2}$ hours (75).
Special studies

Orland et al. (71) reported that a dextrin (uncharacterized) was less cariogenic than sucrose when fed to rats as the carbohydrate component in semipurified diets.

Bleached Starches

No toxicity data on the bleached starches were available to the Select Committee. All agents listed in Table I for the production of bleached starches are oxidizing agents and, although the mechanism of reaction may differ, at sufficiently high concentrations all react with starch to introduce carboxyl and/or carbonyl groups (76). However, few such groups are introduced because of the low level of oxidant required to bleach and/or reduce the microbial count of starch.

A second consideration concerns the possible toxicity of the reduced form of the oxidants that may remain in the bleached starch. For hydrogen peroxide, peracetic acid, ammonium persulfate and sulfur dioxide, permanganate, sodium hypochlorite and sodium chlorite, the reduced forms include water, acetic acid, ammonium sulfate, manganous sulfate and sodium chloride. Residual sulfite and sodium sulfate may also be present in some of these starches. Because the bleached starches are washed, residual levels of reduction products will be much less than that predicted by the stoichiometry of the reaction. Health aspects of acetates, sulfates, manganese, sodium chloride and sulfites are evaluated in other reports of the Select Committee (77-81).

Hypochlorite Oxidized Starches

Digestion, metabolism and short-term feeding studies

In vitro digestibilities of two commercial hypochlorite-oxidized cornstarch samples were compared with that of unmodified cornstarch in experiments in which 1 percent solutions of the cooked starches were treated with saliva or U.S.P. pancreatin. A "lightly" oxidized starch containing 0.42 percent carboxyl groups was 84.8 percent digested by pancreatin and 98.8 percent digested by saliva as compared to the unmodified starch control. The moderately oxidized sample (0.84 percent carboxyl groups) was 88.4 and 97.6 percent digested by pancreatin and saliva, respectively (82,83).

Digestibility of a commercial oxidized wheat starch in 45 to 60 g rats as determined by analyses of the food ingesta and fecal excretions over a 28-day feeding experiment was equal to that of unmodified wheat starch fed at the same levels (63.7 percent) in a similar diet (40). Extent of oxidation of the commercial oxidized starch was not stated.
Digestibility and caloric values of three starches of different degrees of oxidation were similar to those of regular (unmodified) cornstarch when fed to adult rats at a level of 62 percent in the diet (Table IV). No gross pathological changes were observed on autopsy after 10 days on the test diets (83). Caloric values equal to that of unmodified cornstarch also were found for two commercial oxidized cornstarches in 21-day feeding experiments in which weaning rats were fed 5 g of a basal diet plus 1 or 2 g of the oxidized starches or regular cornstarch. One commercial oxidized starch was oxidized by 6 percent (wt/wt) chlorine as hypochlorite and contained 0.9 percent carboxyl groups; the other was treated with 2.5 percent (wt/wt) chlorine as hypochlorite and contained 0.3 percent carboxyl groups. Caloric value of laboratory-prepared oxidized starch treated with two equivalents of hypochlorite (43.2 percent chlorine) was much lower than the control starch as shown by a weight gain of 19 g as compared to 34 g for rats fed the control starch. Autopsies showed that rats fed the heavily oxidized starch had a marked dilation of the colon. Animals fed the commercially oxidized starches appeared normal (84).

In a 90-day feeding study an oxidized starch, 0.9 percent carboxyl, prepared by treating cornstarch with 5.5 percent chlorine as hypochlorite, was fed at dietary levels of 5, 10, or 25 percent (about 4, 8, or 20 g per kg body weight) to groups of 15 male and 15 female weanling albino rats (85). The oxidized starch was substituted for an equal amount of cornstarch in the control diet. Growth and food intake showed no significant differences between test groups and controls nor were there significant differences in hematologic indices, biochemical blood values and urine composition which could be attributed to starch treatment. Organ-to-body weight ratios of control and test groups were not significantly different for the heart, kidneys, liver, spleen, brain, gonads, thymus and thyroid. Relative weights of the adrenals were significantly higher in females fed 5 and 10 percent oxidized starch but not in the group fed the diet containing 25 percent oxidized starch. The relative cecum weight was slightly increased at the 25 percent level in females only. Histopathological examination of the lungs, salivary glands, prostate, epididymus, uterus, urinary bladder, thoracic aorta, esophagus, stomach, duodenum, ileum, cecum, colon, pancreas and mesenteric lymph nodes revealed no pathological changes that could be attributed to the ingestion of the oxidized starch. Nephrocalcinosis in the corticomedullary region was seen in 7/15 female controls and in 10/15 females fed the oxidized starch at the 25 percent level. No nephrocalcinosis was observed in 15 males receiving the high level diet nor in 15 control males. However, one male in the high dose group showed a "bud-like structure consisting of loose connective tissue covered with transitional connective tissue extending from the outer medulla into the renal calyx."
TABLE IV

Digestibility and Caloric Values of Hypochlorite Oxidized Starches (83)

<table>
<thead>
<tr>
<th>Starch</th>
<th>Chlorine Percent *</th>
<th>Carboxyl Percent</th>
<th>Digestibility Percent</th>
<th>Caloric Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular (unmodified)</td>
<td>0.</td>
<td>0.</td>
<td>100.</td>
<td>100.</td>
</tr>
<tr>
<td>Lightly oxidized</td>
<td>3.9</td>
<td>0.57</td>
<td>97.0</td>
<td>114.7</td>
</tr>
<tr>
<td>Moderately oxidized</td>
<td>4.5</td>
<td>0.80</td>
<td>96.9</td>
<td>99.6</td>
</tr>
<tr>
<td>Moderately oxidized</td>
<td>5.5</td>
<td>0.90</td>
<td>96.5</td>
<td>110.9</td>
</tr>
</tbody>
</table>

* Percent chlorine, in form of sodium hypochlorite, in reaction mixture based on dry starch weight.
Starch Acetate

Digestion and absorption

In vitro digestibilities of two acetylated cornstarches, 1.50 and 2.41 percent acetyl contents, as determined by digestion with amyloglucosidase were 84 and 69 percent of that of unmodified cornstarch. In contrast, in vivo digestibility in rats of an acetylated cornstarch, 1.8 percent acetyl content, was 106 percent that of pregelatinized cornstarch (86). The digestibility of starch acetate containing 1.98 percent acetyl groups by pancreatin and porcine mucosal enzymes was 90 percent of that of the unmodified starch (87).

Groups of 10 male and 10 female weanling rats were fed semipurified diets containing 25 or 50 percent (about 50 or 100 g per kg) starch acetate (1.98 percent acetyl) as replacement for an equal amount of pregelatinized potato starch in the basal diet (88). After 7 days, 4 percent cellulose was added and the diets were fed for 3 more days. Weight gains were slightly lower and fecal dry matter higher in rats fed starch acetate at the 50 percent level as compared to the controls. A slight diarrhea was observed in the test animals fed the 50 percent level; the severity was not decreased by the addition of cellulose to the diet.

Short-term studies

Groups of 10 male Sprague-Dawley rats were fed for 28 days diets containing 60 percent (about 50 g per kg) acetylated starches (plant source not stated) having 1.24, 2, 2.56, or 3.25 percent acetyl groups. Vinyl acetate was the acetyling agent used. Weight gain was reduced in groups receiving starch acetates with more than 2 percent acetylation as compared to control animals but feed efficiency was unaffected. Diarrhea occurred in animals fed acetylated starches containing 2 percent or higher acetyl content and there was noticeable cecal enlargement in these animals. No tissue damage or inflammation was observed in association with the diarrhea (89).

Acetylated potato starch (1.36 percent acetyl groups) was fed for 13 weeks to groups of 10 male and 10 female rats (strain not stated) at levels 15 and 45 percent (about 10 and 30 g per kg) of the diet (90). Growth and hematological parameters were not significantly affected. Relative weights of the liver, kidney, adrenal and pituitary were generally lower than those of the control animals. Cecal weights of males were higher than those of controls and ceca were distended in animals fed acetylated starch at the 15 and 45 percent levels. No histopathological changes were observed that were attributed to starch acetate in the diets.
A potato starch acetate containing 1.98 percent acetyl groups was fed for 8 weeks to groups of 10 male and 10 female weanling rats (Wistar derived) at 25 and 50 percent levels (about 35 and 70 g per kg) in a practical diet (88). Growth rates did not differ significantly from those of controls. No diarrhea was observed. Cecal weights were higher in animals fed the modified starch and were most pronounced at the 50 percent feeding level. However, histological examination showed no abnormalities of the ceca.

Twelve human volunteers consumed on each of 4 consecutive days 60 g (about 1 g per kg) of starch acetate (starch source not stated) containing 1.98 percent acetyl groups. No effect was noted on the frequency of defecation and on the amount of feces, fecal water or its lactic acid content. No adverse effects were observed (91).

Long-term studies

In a 2-year study, a precooked acetylated potato starch, acetyl content 1.98 percent, was fed to groups of 30 male and 30 female rats at 5, 10, and 30 percent levels (about 2.5, 5, and 15 g per kg) in the diet (92, 93). The modified starch replaced an equal quantity of precooked potato starch in the basal diet. Body weights of male rats fed the 30 percent level were significantly lower than those of the controls at 76 weeks but not at 104 weeks. There was no diarrhea. Survival, hematological parameters, urine composition and organ weights, were unaffected in the test animals with the exception of cecal weights which were greater in animals fed 10 and 30 percent modified starch than those of the controls. It was concluded that cecal enlargement had little if any toxicological significance since it was present throughout the 2-year study but did not result in any relevant microscopic changes in the tissues. Histological examination revealed that suburothelial deposits of calcium accompanied by hyperplasia of the epithelium lining the renal pelvis occurred slightly more frequently in the highest dose group than in controls (Table V). Increased incidence did not occur in females and it was suggested that incidence in males might be related to the occurrence of the parasite Trichosomoides crassicauda in the urinary tract. Similar renal changes have been observed in rats fed diets containing 60 percent lactose (97), 15 percent sodium alginate (98), 0.8-0.9 percent magnesium oxide (99), a nonsteroid anti-inflammatory agent (100), or excess sodium chloride after uninephrectomy (101). A similar pathological process of unknown etiology also has been described in Sprague-Dawley rats (102).
### TABLE V

Incidence of Pelvic Nephrocalcinosis in Rats Fed Seven Chemically Modified Starches for Two Years According to Severity of Lesions and Level in Diet

<table>
<thead>
<tr>
<th>Modified starch</th>
<th>Severity of lesion</th>
<th>Incidence of lesion. *( )=No. animals examined Percent of modified starch in diet *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>CIVO/TNO Studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylated distarch phosphate (ref. 92, 94)</td>
<td>slight</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>same control</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>as above</td>
<td>0</td>
</tr>
<tr>
<td>Acetylated diamylopectin phosphate (ref. 92, 94)</td>
<td>slight</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>same control</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>as above</td>
<td>1</td>
</tr>
<tr>
<td>Starch acetate (ref. 92, 93)</td>
<td>slight</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>same control</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>as above</td>
<td>1</td>
</tr>
<tr>
<td>Hydroxypropyl distarch glycerol (ref. 92, 93)</td>
<td>slight</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>same control</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>as above</td>
<td>0</td>
</tr>
<tr>
<td>Phosphated distarch phosphate (ref. 92, 122)</td>
<td>slight</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>moderate</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>same control</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>as above</td>
<td>0</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>19/284</td>
<td></td>
</tr>
<tr>
<td></td>
<td>35/284</td>
<td></td>
</tr>
<tr>
<td><strong>IFFA/CREDO Studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylated distarch glycerol (ref. 96)</td>
<td>discrete</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>marked</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td>4</td>
</tr>
<tr>
<td>Acetylated distarch adipate (ref. 96)</td>
<td>discrete</td>
<td></td>
</tr>
<tr>
<td></td>
<td>marked</td>
<td></td>
</tr>
<tr>
<td></td>
<td>severe</td>
<td></td>
</tr>
<tr>
<td></td>
<td>same control</td>
<td></td>
</tr>
<tr>
<td></td>
<td>as above</td>
<td></td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>62/100</td>
<td></td>
</tr>
</tbody>
</table>

*5, 10, 30 and 62% modified starch in the diet correspond to daily intakes of about 2.5, 5, 15 and 30 g/kg, respectively.

**The data tabulated do not include incidence of hyperplasia of uroepithelium without calcification.
Potato starch acetylated with vinyl acetate to an acetyl content of 1.6 to 2.5 percent, and a control pregelatinized potato starch were fed to groups of 75 male and 75 female albino SPF mice, Swiss Random strain, for 89 weeks at dietary levels of 55 percent (about 80 g per kg body weight) (103). Other groups were fed diets containing 55 percent lactose or 25 percent sodium alginate. In week 80 ten mice of each sex and group were necropsied and a comprehensive microscopic examination of tissues was made. A similar examination was made of all survivors after week 89. Tissues of all animals found dead or killed when moribund were removed and examined if autolysis was not too far advanced.

Mean body weights of the treated mice did not differ significantly from those of the controls, with the exception of significant decreases in week 16, 20, 40 and 72 in males and in week 84 in females. Incidence of loose stools was no greater in the group fed starch acetate than in the control group. Death rate in the treated group was normal for the strain of mice fed but was abnormally high for males in the control group between weeks 39 and 65. At necropsy about 50 percent of the latter animals had an hemorrhagic myocarditis. Hematological indices, fasting glucose and blood urea nitrogen, were within normal limits in the test animals. Male mice, but not female mice, receiving the experimental diet had a higher content of amorphous material in their urine than controls. Analyses of urine sediment indicated about 95 percent protein; the remainder was phosphates, carbohydrate and possibly silica. Metal ions present included sodium, calcium, magnesium and potassium. No significant differences in organ:body weight ratios occurred between animals fed starch acetate and the controls except for an increased ratio for the cecum and colon of both sexes and a slight, but statistically significant, increase in ratio for the kidneys of females.

Twenty-five to 35 percent of the females in the test group had a trichobezoar in their stomach as compared to 10 percent of the female controls. Incidence in males in the test and control groups was similar and no higher than 5 percent.

Overall incidence of very slight and slight calcareous deposits in the renal pelvis that occurred in males in the test group (9/74) was greater than in the control group (0/73). However, this finding could not be tested for statistical significance because of early deaths of males in the control group. In mice that survived for a period of at least 79 weeks, frequencies were 7/49 and 0/28, respectively, a difference not statistically significant. Incidence of intratubular calcareous deposits was significantly greater in males in the test group (25/49) that survived 79 weeks or more than in like animals in the control group (5/28). In females, incidence in the two groups was 13/56 and 11/58, respectively. A slight but not statistically significant increase in calcareous material and in thickened submucosa and epithelium in the urinary bladder occurred in males fed the test diet as compared with controls. Similar renal changes occurred in mice fed the diet containing lactose. No evidence of hyperplasia of the epithelium was found. The investigators concluded that the renal and urinary bladder changes observed had little, if any, toxicological significance.
Special studies

Acetylated potato starch (1.98 percent acetyl content) was fed at the 10 percent dietary level (about 5 g per kg) in a three-generation study using groups of 10 male and 20 female rats for the P, F₁ and F₂ generations to produce two successive litters in each generation by mating at week 12 and 20 after weaning (92, 93). Ten males and 10 females of the F₂b generation were kept for 3 weeks after weaning and then sacrificed for histopathological studies. The P, F₁b and F₂b mothers were examined for implantation sites. No adverse effects were noted in fertility, litter size, resorption quotient, preweaning mortality or growth rate of pups. No gross or histological changes were noted in rats of the F₃b generation that were attributable to acetylated starch in the diet.

Starch Sodium Succinate

Absorption and metabolism

Groups of ten weanling male albino rats (strain not stated) were fed, for 4 weeks, a basal diet to which was added 1.5 or 3.0 g (about 20 or 40 g per kg of body weight) of either cornstarch or cornstarch sodium succinate; for comparison, sucrose was fed daily at levels of 0, 0.75, 1.5, 3.0, and 4.5 g (104). Assuming a caloric value of 4 kcal per g for sucrose, values of 3.78 and 3.82 kcal per g (dry basis) were estimated from the growth data for the starch succinate and cornstarch, respectively. All of the rats on the starch succinate diet were normally active and appeared in good health during the 4-week period.

Short-term studies

Weanling albino rats (strain not stated) in groups of three males and three females each were fed ad libitum, for 10 weeks, diets in which the entire carbohydrate portion (70 percent or about 60 g per kg of body weight) was provided by cornstarch sodium succinate or cornstarch (105). Average weight gain and feed efficiency of animals fed the starch succinate diet did not differ significantly from those of the control animals. Blood hemoglobin values were in the normal range. In another experiment by the same laboratory (105) similar groups of rats were pair fed the two starch diets for 10 weeks. Weight gains, feed efficiency and hemoglobin levels did not differ significantly. In both experiments, the animals were robust and healthy and no manifestations of toxicity were observed.

Long-term studies

No reports were available to the Select Committee.
Special studies

No reports on studies of mutagenicity, teratogenicity or carcinogenicity were available to the Select Committee.

**Starch Sodium Octenyl Succinate**

**Digestion and metabolism. Short-term studies**

Groups of 10 weanling male albino rats (strain not stated) were fed 2.74 g of a basal diet and 1.5 or 3.0 g (about 20 or 40 g per kg body weight) of unmodified cornstarch or cornstarch sodium octenyl succinate daily for 4 weeks (106). Growth rates of test and control animals did not differ significantly and caloric availability was not depressed in the modified starch. All rats were normally active and appeared in good health during the 4-week period.

In an 8-week feeding experiment, groups of six male and six female weanling albino rats received a ration containing 35 percent (about 30 g per kg body weight) cornstarch sodium octenyl succinate or unmodified cornstarch (107). The test group grew at a slower rate but efficiency of food utilization was equal to that of the control group. Blood cell counts, hemoglobin and blood sugar of test animals at 8 weeks were similar to those of control animals. Serum non-protein nitrogen was lower but was within the normal range. One animal each in the control and test groups died during the experiment. All other rats behaved and appeared healthy and normal throughout the experiment.

**Long-term studies**

No long-term studies were available to the Select Committee.

**Special studies**

No studies on reproduction, mutagenicity, teratogenicity or carcinogenicity were available to the Select Committee.

**Starch Aluminum Octenyl Succinate**

**Digestion and metabolism. Short-term studies**

Groups of ten weanling male albino rats (strain not stated) were fed, for 4 weeks, 2.74 g of basal diet supplemented with 1.5 or 3.0 g (about 20 or 40 g per kg of body weight) daily of either thin-boiling (acid-modified) starch or the aluminum octenyl succinate derivative of this starch (185).
Caloric value of the test starch as measured by weight gain was equal to the control starch. All of the rats were normally active and appeared in good health throughout the 4-week period. Growth was continuous and there were no deaths.

Groups of six male and six female weanling albino rats (strain not stated) were fed for 8 weeks a basal diet containing 35 percent corn-starch or diets in which 1, 10, or 25 percent (about 1, 10, and 25 g per kg body weight) of cornstarch aluminum octenyl succinate replaced an equal quantity of cornstarch (107). No adverse effects were observed on growth, food consumption, efficiency of food utilization, blood cell count, hemoglobin, sugar or non-protein nitrogen.

Long-term studies

No long-term studies were available to the Select Committee.

Special studies

No studies on reproduction, mutagenicity, teratogenicity, or carcinogenicity were available to the Select Committee.

Monostarch Phosphate

As noted in the Background Information section, potato starch contains 0.06 to 0.1 percent phosphorus present as phosphate ester groups. This may be compared with the residual limitation (Table I) of not more than 0.4 percent phosphorus in starch phosphates prepared by reaction with sodium orthophosphate. The phosphate groups are present in the dibasic form (108).

Digestion and metabolism

Wheat starch phosphate and bleached wheat starch phosphate were hydrolyzed at a faster rate than unmodified wheat starch by alpha amylase in vitro (109).

$^{32}$P-labeled starch phosphate, disodium phosphate and sodium pyrophosphate were administered by gavage to female Sprague-Dawley rats; urine was collected at 5, 22, and 47 hours and feces at 22 and 47 hours thereafter (109). At 47 hours blood samples were collected and the animals were sacrificed. Percentages of the $^{32}$P dose from starch phosphate found in the liver, kidneys, blood plasma, bone and total quantity excreted in feces and urine were similar to those for disodium phosphate and indicate that phosphorus in the form of starch phosphate is metabolized in a manner similar to that of the inorganic phosphate compound.
Short-term studies

Groups of six (experiment 1) and 20 (experiment 2) weanling male Sprague-Dawley rats were fed starch phosphate (starch source not stated) for 4 weeks at levels of 1, 5, and 10 percent (about 1, 5, and 10 g per kg body weight) in a semipurified diet (109). Efficiency of feed conversion was similar to that of control animals. Weights of testes, liver, thymus, adrenal, spleen and kidneys were not affected in experiment 1. Autopsy of all animals of experiments 1 and 2 revealed no abnormalities.

Long-term studies

No long-term studies were available to the Select Committee.

Special studies

No studies on the mutagenicity, carcinogenicity or teratogenicity of monostarch phosphates were available to the Select Committee.

Distarch Phosphate

Digestion and metabolism

In vitro amyloglucosidase digestibility of waxy maize starch cross-linked by reaction with 0.035, 0.070, and 0.100 percent phosphorus oxychloride was 96 to 98 percent of that of the unmodified starch. Both starches were gelatinized in water, then digested with enzyme for 16 hours at 50 to 55°C (110). Potato starch modified by reaction with 0.05 or 0.1 percent phosphorus oxychloride was degraded to the same extent as the unmodified starch by in vitro treatment for 1 hour with pancreatic after gelatinization in boiling water (95). In similar experiments, the in vitro digestibility of gelatinized distarch phosphate, prepared by reaction of milo starch with sodium trimetaphosphate, by salivary, pancreatic or intestinal amylase was equal to that of the gelatinized unmodified starch as measured by liberation of reducing sugars after 15 minutes (111). Dextrose equivalent values and distribution of saccharides, DP 1 through DP 6 in solutions of potato starch, waxy cornstarch, and two distarch phosphates were similar after conversion at 10 percent concentration with alpha amylase for 3 hours at 92°C (112). The distarch phosphates were prepared by treatment of waxy maize starch with 0.085 percent phosphorus oxychloride or 0.5 percent sodium trimetaphosphate. Further hydrolysis of the alpha amylase treated starches with glucoamylase for 2-64 hours at 60°C gave similar values for dextrose equivalent and quantities of dextrose, maltose and maltotriose liberated. In another experiment the in vitro pancreatic digestibility of milo starch modified by reaction with trimetaphosphate was about 80 percent that of unmodified starch as measured by degradation after 20 minutes' digestion (113). However, in vitro digestibilities of these starches in rats were similar. Groups of 10
weanling Sprague-Dawley rats were fed 1, 2, or 4 g (about 20, 40, or 80 g per kg body weight) of unmodified or trimetaphosphate-modified cornstarch for 10 days. Weight gains were similar to controls at each level of supplementation (114).

Caloric value of distarch phosphate prepared by treating milo starch with trimetaphosphate was the same as that of the unmodified starch when measured by weight gains of 50 g rats fed for 7 days on 4 g basal diets supplemented with 0.9 or 3.6 g of the two starches (115). In a similar study, two samples of commercial distarch phosphate (0.5 to 0.9 degrees of substitution) prepared by treating cornstarch with trimetaphosphate were fed to weanling Wistar-Purdue rats for 21 days at levels of 1 or 2 g daily added to a 5 g basal diet (84). Weight gains were similar to those of the control animals. Necropsy of one animal fed the higher level of modified starch showed no gross adverse effects. Caloric value of distarch phosphates prepared by treating waxy maize starch with 0.03 or 0.1 percent phosphorus oxychloride did not differ significantly from that of untreated waxy maize starch when fed for 6 weeks at the 52 percent level (about 60 g per kg of body weight) as the sole carbohydrate source in the diet of six male and six female weanling rats (116).

Acute toxicity

No deaths occurred after the administration of single oral doses of 50 percent aqueous suspensions of distarch phosphate (prepared from white milo, a waxy starch) to groups of 10 female mice (dose, 19 g per kg body weight), 10 female rats (35 g per kg), two guinea pigs (18 g per kg), two rabbits (10 g per kg), and two cats (9 g per kg). Gross autopsy findings of mice and rats conducted 16 days after treatment were negative. Microscopic examination of kidney and liver tissues of the rabbits, cats and guinea pigs showed no abnormalities (117).

Short-term studies

In a 90-day subacute toxicity test, groups of 25 male and 25 female Sprague-Dawley weanling rats were fed diets containing 0.20, 1.0, or 5.0 percent (about 0.2, 0.8, or 4 g per kg) distarch phosphate prepared by treating white milo starch with sodium trimetaphosphate (118). Blood and urine studies were conducted at 45 and 90 days of testing. Blood studies were done individually on five males and five females of the highest dietary group. No abnormalities were observed in hematological parameters or urinalyses of the test animals. Body weight gains and organ-body weight ratios showed only a few, randomly distributed, intergroup differences, none of which was attributed to starch ingestion. Gross pathologic findings among test animals were comparable to those observed among control animals and no adverse histopathologic changes attributed to the test starches were reported.
Groups of 10 male and 10 female rats received 5, 15, or 45 percent (about 4, 12, or 36 g per kg of body weight) of two types of distarch phosphate (0.085 and 0.128 percent esterified phosphate) in their diet for 90 days (119). No abnormalities compared to controls were seen in regard to general appearance, behavior, mortality, food consumption, hematology, serum chemistry and urinalysis which could be attributed to the test starches. No diarrhea or increased cecal weights were observed. Gross and histopathologic examination revealed no abnormalities attributable to the distarch phosphate fed.

Groups of three male and three adult female beagles were fed for 90 days a standard dog chow supplemented daily with 0.05, 0.25, or 1.25 g per kg of body weight of distarch phosphate (trimetaphosphate-treated white milo starch) administered in gelatin capsules (120). Hematological studies and urinalyses were conducted at the inception and conclusion of the feeding period and also after 45 days for the dogs fed the highest level of distarch phosphate. No significant abnormalities were observed. Mean body weight gains and organ-body weight ratios of the test animals did not differ significantly from the controls. Gross and histopathologic examination revealed no abnormalities attributable to the test substance.

Groups of eight 3-day-old Pitman-Moore miniature pigs were fed formula diets containing acid-modified waxy starch or distarch phosphate prepared by treatment of the acid-modified starch with 0.08 percent (dry weight basis) phosphorus oxychloride (72). Starch provided 24 percent of the calories in the diet and each diet was fed for 25 days. Body weight gains were similar for test and control animals. The distarch phosphate diet had no statistically significant effects on organ weights expressed as a percentage of body weight. Serum cholesterol, triglyceride, calcium, phosphorus, alkaline phosphatase, urea nitrogen, total protein, albumin and globulin levels were similar for the test and control animals.

Long-term studies

No long-term studies were available to the Select Committee.

Special studies

No studies on mutagenicity, carcinogenicity or teratogenicity of distarch phosphates were available to the Select Committee.

Phosphated Distarch Phosphate

Digestion and metabolism

In vitro pancreatic digestibility of phosphated distarch phosphate prepared from cornstarch was about 80 percent that of unmodified corn-
starch as measured by liberation of reducing sugars after 20 minutes' digestion (113). Digestibility of phosphated distarch phosphate from potato starch by in vitro pancreatic and porcine intestinal amylase also was reduced compared to the unmodified starch (87). In vivo digestibility and utilization, however, of phosphated distarch phosphate prepared from milo starch was similar to that of the unmodified starch as measured by weight gain of weanling rats fed a basal diet supplemented with 1, 2, or 4 g daily of unmodified or modified starch for 10 days (114).

Short-term studies

Groups of 10 male and 10 female weanling rats (strain not stated) were fed a diet initially containing 10 percent (about 30 g per kg) phosphated distarch phosphate (prepared from white milo starch) but increasing step-wise to 35 percent (about 70 g per kg) on the 13th day (121). At 60 days, urine and blood samples were collected and the animals were sacrificed and subjected to complete necropsy. Average weight gain of females was slightly but significantly lower than controls (p=0.05). Urine of test animals was negative for presence of reducing substances, protein and microscopic elements and hematological parameters were in the normal range. Kidney- and liver-body weight ratios were significantly lower for males in the test group; these were believed, however, to be coincidental rather than related to the ingestion of the test starch. Histopathological findings for test animals were comparable to those for controls.

Groups of 25 female Sprague-Dawley weanling rats were fed diets containing 0.2, 1.0, or 5.0 percent (about 0.2, 0.8, or 4 g per kg) phosphated distarch phosphate prepared from white milo starch for 90 days (118). No abnormalities were observed in hematological studies or urinalyses of the test animals. Body weight gains did not differ significantly from the control animals. Organ-body weight ratios showed no differences attributed to starch ingestion. Gross pathology of the test animals was similar to that of the controls. No adverse histopathological changes attributed to the phosphated distarch phosphate were observed.

Groups of 10 male and 10 female weanling rats (Wistar derived) were fed diets containing 25 or 50 percent (about 25 or 50 g per kg) phosphated distarch phosphate (0.3 percent P) prepared from potato starch for 8 weeks (88). Body weight gains were similar to those of control animals. No diarrhea occurred at either test level. Cecal weight was slightly increased in male rats at the 25 percent level but there was no consistent effect on females at either test level.

In a 90-day subacute toxicity test, groups of three male and three female beagles were fed a standard dog chow supplemented daily with 0.05, 0.25, or 1.25 g of phosphated distarch phosphate per kg of body weight (120). The modified starch was prepared from white milo starch and was admin-
istered in gelatin capsules. No effects were observed on blood components, urinalyses or liver function employing the sulfobromophthalein test. Mean body weight gains and organ-body weight ratios of the test animals did not differ significantly from the controls. Gross and histopathologic examination revealed no abnormalities attributed to the test substance.

Groups of eight 3-day-old Pitman-Moore miniature pigs were fed formula diets containing acid-modified waxy starch or phosphated distarch phosphate prepared by treatment of the acid-modified starch with 4.8 percent sodium tripolyphosphate and 0.59 percent sodium metaphosphate, both on a dry weight basis (72). Starch provided 24 percent of the calories (33 percent of the formula, dry weight basis) in the diet and was fed for 25 days. Body weight gain was similar for test and control animals. The test diet had no statistically significant effect on organ-body weight ratios. Clinical blood chemistry analyses were similar for test and control animals as were liver and carcass composition.

Twelve human volunteers ingested on each of 4 consecutive days 60 g (about 1 g per kg) of phosphated distarch phosphate containing 0.35 percent introduced phosphorus. No adverse effects were observed. No effect was noted on the frequency of defecation or on the amount of feces, fecal water or the lactic acid content of the feces (91).

**Long-term studies**

Groups of 30 male and 30 female weanling Wistar rats were fed for 2 years a basal diet containing 30 percent precooked waxy milo starch which was replaced in part or in total by 5, 10, or 30 percent (about 3, 5 or 15 g per kg) phosphated distarch phosphate (92, 122). The modified starch was prepared by cross-linking white milo starch with sodium trimetaphosphate to 0.04 percent introduced phosphorus and esterified with sodium tripolyphosphate to a total of 0.35 percent bound phosphorus. Significant growth retardation did not occur and food efficiencies were comparable to those of the control animals at all stages. Diarrhea did not occur. Hematological indices showed no significant abnormalities or treatment related differences between test and control groups at any stage of growth. Biochemical analyses of blood and serum showed no evidence of dose-related responses to the test starch. Analysis of urine samples collected at intervals from each dietary group showed no effect of the modified starch on pH, sugar, protein, occult blood, ketones or sediment. There were no statistically significant changes in organ weight except for an increased kidney-body weight ratio for females on the 30 percent test diet. No gross pathological changes were observed which could be attributed to ingestion of the modified starch. No effect was seen on tumor incidence. Non-neoplastic lesions were randomly distributed among test and control animals with the possible exception of a kidney abnormality which consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied
by calcified patches in the underlying tissues. The hyperplastic and calcified tissues often protruded into the renal pelvis and were located most often in the papilla near the junction of the papillary and pelvic epithelium. The lesion occurred to a slight degree in nine animals (5/28 males and in 4/29 females) fed the 30 percent test diet and to a moderate degree in one male on the control diet (Table V). There was not, however, a distinct relationship with feeding level. The authors considered the toxicological significance of the lesions doubtful. Reports on the occurrence of similar renal changes in rats fed other types of diets are noted in the section Starch Acetates, Long-term studies (p. 26).

Special studies. Reproduction and lactation

Groups of 10 male and 20 female weanling CIVO rats were fed, for three generations, a diet containing 10 percent (about 5 g per kg) phosphated distarch phosphate and 20 percent precooked waxy maize starch (92). The modified starch was the same as described in the preceding section. Rats were mated (P, F₁, and F₂ generations) at weeks 12 and 20 after weaning. The second litter of each generation was used to produce the next generation. The F₃b generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F₁b, and F₂b parents were used for counting implantation sites. Body weights did not differ among groups in successive generations and no treatment-related differences in mortality were observed between the test groups and controls. No adverse effects were noted regarding mortality in utero (resorption quotient), litter size, weight of pups, preweaning mortality or growth rate of pups. No gross or histological changes attributable to feeding the modified starch was observed.

Acetylated Distarch Phosphate

Digestion

In vitro digestibilities by pancreatin and porcine intestinal amylase of acetylated distarch phosphates (prepared from potato starch), 1.6 and 2.3 percent acetyl contents, were 93 and 69 percent, respectively, of that of unmodified starch (87).

Short-term studies

Groups of 10 male and 10 female weanling Wistar rats were fed, for 8 weeks, practical type diets containing 25 or 50 percent (about 30 or 60 g per kg) acetylated distarch phosphate (88). The test starch was cross-linked by treatment of potato starch with 0.02 percent phosphorus oxychloride and acetylated with 8 percent acetic anhydride (2.3 percent acetyl
content). Weight gains were similar to those of control animals fed diets containing 50 percent precooked waxy maize starch. During week 5 slight diarrhea occurred in male rats receiving the 50 percent dietary level. At sacrifice, cecal weights of both male and female rats were greater than those of controls but microscopic examination revealed no differences from controls. In a second experiment by the same investigators (88), acetylated distarch phosphate was fed at the 25 and 50 percent levels in a semipurified diet for 10 days. At day 7, cellulose was added to all diets at a level of 4 percent. Moderate diarrhea occurred at the 50 percent dietary level of the test substance. Addition of cellulose did not reduce the diarrhea.

Groups of four male and four female pigs were fed diets containing 35 or 70 percent acetylated distarch phosphate for 14½ weeks (123). Growth rate and food consumption were satisfactory. Three animals in the higher dietary group died during the test without evidence of cause of their death. Hematology, blood chemistry and urinalysis showed no treatment-related abnormalities nor did organ weight, gross and histopathological evaluations. One pig in each of the test diet groups showed evidence of neurological mal-function; the animal in the 70 percent dietary level group died; the one in the lower dietary level recovered. No histological evidence of nervous system abnormality was observed in these two nor in any other animal.

**Long-term studies**

Groups of 30 male and 30 female weanling Wistar rats were fed for two years diets containing 5, 10, or 30 percent (about 3, 5, or 15 g per kg) acetylated distarch phosphate replacing an equal amount of precooked potato starch in the control diet (92,94). The test starch was potato starch cross-linked with 0.02 percent phosphorus oxychloride and acetylated with 8 percent acetic anhydride (acetyl content 2.33 percent). Body weight gains and food efficiencies were similar to those of control animals. Hematological indices showed no significant abnormalities or treatment-related differences between test and control groups. Blood and serum analyses showed no evidence of dose-related responses to the test starch. Analyses of pooled urine samples collected at intervals from each dietary group showed no effect of the modified starch. There were no statistically significant changes in organ weight although rats of both sexes fed 30 percent of the test starch in their diet and males fed the 10 percent diet had greater cecal weights than the controls. Histopathological lesions were randomly distributed among test and control animals with the possible exception of a kidney abnormality which consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied by calcified patches in the underlying tissues. There was not a distinct relationship between incidence of the lesion and feeding level (Table V). The authors considered its toxicological significance doubtful. Reports on the occurrence of similar renal changes in rats fed other types of diets are noted in the section on Starch Acetates, Long-term studies (p. 26).
A similar protocol was followed in a 2-year rat (Wistar strain) feeding study with acetylated diamylopectin phosphate (92, 94). Amylopectin (presumably from potato starch) was cross-linked with 1.2 percent phosphorus oxychloride (0.043 percent introduced phosphorus) and acetylated with 4.5 percent vinyl acetate (acetyl content, 1.6 percent). Findings were similar to those reported in the preceding paragraph for acetylated distarch phosphate. Incidence of pelvic nephrocalcinosis in the rats at necropsy is reported in Table V.

**Special studies**

Twelve human subjects ingested on each of 4 successive days 60 g (about 1 g per kg of body weight) of acetylated distarch phosphate of either 1.6 or 2.33 percent acetyl content (91). No adverse effects were noted including effect on frequency of defecation and on the amount of feces, fecal water and the lactic acid content of the feces.

Groups of 10 male and 20 female weanling Wistar rats were fed for three generations, a diet containing 10 percent (about 10 g per kg) acetylated distarch (potato) phosphate (2.33 percent acetyl) and 20 percent precooked potato starch (92). Rats were mated (P, F1 and F2 generations) at weeks 12 and 20 after weaning. The second litter of each generation was used to produce the next generation. The F25 generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F15, and F25 parents were used for counting implantation sites. Body weights did not differ among groups in successive generations and no treatment-related differences in mortality were observed between the test groups and controls. No adverse effects were observed on the resorption quotient, litter size, weight of pups, preweaning mortality or growth rate of pups. No gross or histological changes attributed to feeding the modified starch were noted.

**Hydroxypropyl Distarch Phosphate**

**Digestion and metabolism**

In vitro digestibility of gelatinized hydroxypropyl distarch phosphate (prepared from tapioca starch, molar substitution 0.045) by hog pancreatic alpha amylase or fungal amylase was about 80 percent of that of gelatinized tapioca starch (124).

Groups of five weanling male Sprague-Dawley rats were fed 5 g per day of a basal diet to which was added 1 or 3 g of hydroxypropyl distarch phosphate (125). The test-modified starch was prepared by treating tapioca starch with 8 percent propylene oxide and 0.1 percent phosphorus oxychloride. Caloric value of the modified starch as measured by 7-day weight gains was equal to that of the unmodified control starch.
In another evaluation of the caloric value of hydroxypropyl distarch phosphates, groups of ten weanling male albino rats (strain not stated) were fed, for 10 days, 5 g daily of a basal diet to which 1, 2, or 4 g (about 20, 40, and 80 g per kg of body weight) of the modified starches was added (126). The test starches were prepared by reaction of cornstarch with 0.0123 percent phosphorus oxychloride and 3, 6, or 8 percent propylene oxide (hydroxypropyl group degree of substitution (D.S.), 0.085, 0.173, and 0.23, respectively). Caloric utilization relative to the unmodified starch control decreased slightly with increasing D.S. of the modified starch; relative caloric value for the D.S. 0.23 starch was 0.93. Diarrhea occurred when rats consumed 2 g or more of D.S. 0.23 starch or 4 g of the starches of lesser D.S.

Short-term studies

A hydroxypropyl distarch phosphate (composition not stated) prepared from cornstarch was fed ad libitum at 17, 34, 51, and 68 percent dietary levels (about 20, 40, 60, and 80 g per kg body weight) for 28 days to groups of 10 male weanling albino rats (126). Weight gains and feed efficiencies were reduced at the two highest dietary levels of the test substance. Cecum-body weight ratios were increased at all dietary levels and the increase was dose-related. No histological abnormalities were observed in the heart, liver, spleen, kidney or cecum.

Groups of 15 male and 15 female weanling FDRL-Wistar rats were fed, for 90 days, diets containing 5, 10, or 25 percent (about 4, 8, and 20 g per kg) hydroxypropyl distarch phosphate, replacing an equal quantity of unmodified cornstarch in the control diet (127). The test starch was prepared by reaction of cornstarch with 10 percent (w/w) propylene oxide and 0.1 percent phosphorus oxychloride. There were no deviations from control values with respect to growth, gains in body weight, food intakes, or efficiencies of food utilization, with the exception of a slight decrease in feed efficiency in males fed 25 percent levels of the treated starch. Hematologic, biochemical parameters and urinalyses were similar to those of control animals. No treatment-related response was observed in any organ weight except the cecum which showed a marked enlargement with contents in place. However, only males on the 25 percent diet had empty ceca significantly heavier than those of controls. Certain sections of the ceca appeared thinner but were cytologically normal. The only pathological finding of possible significance was calcareous deposits within the renal pelvis and/or the pelvic epithelium; deposits occurred only in rats of the test groups. Incidence in females at the 5, 10, and 25 percent dietary levels was 7/15, 9/15, and 11/15, respectively; in males incidence was 4/15 at each level. Focal renal tubular calcification appeared somewhat more severe than expected in the control animals; however, incidence in test (7/30 at each dietary level) and control groups (5/30) was similar.
In another 90-day study (128), groups of 15 male and 15 female weanling rats (Wistar derived) were fed pregelatinized hydroxypropyl distarch phosphate at dietary levels of 0, 5, 10, or 25 percent (about 0, 4, 8, and 20 g per kg). The modified starch was prepared by treating cornstarch with 0.1 percent phosphorus oxychloride and 5 percent propylene oxide. The product contained 96 ± 6 ppm phosphorus and less than 5 ppm propylene chlorohydrin. Degree of hydroxypropyl substitution was 0.07. Growth, food consumption, food efficiency, hematology, blood chemistry and urinalysis of test animals were comparable to those of controls fed pregelatinized cornstarch. Water content of feces was slightly higher at the 10 and 25 percent dietary levels of the test substance, but diarrhea did not occur. Organ-body weight ratios of the testicles and adrenals of males in the 25 percent group were slightly decreased (p = 0.05). The relative cecum weight, both filled and empty, was distinctly increased in both sexes at the 25 percent dietary level. Very slight or slight calcareous deposits were identified microscopically in the intercortico-medullary area of the kidneys in 11/15 females at the highest dietary level as compared with 2/15 in female controls and 0/15 for test males. The investigators did not consider the findings to have toxicological significance in view of a similar incidence usually encountered in female Wistar control rats of similar age in other studies. No other histopathological abnormalities were observed.

Long-term studies

Potato starch cross-linked with 0.1 percent phosphorus oxychloride and etherified with 5 percent propylene oxide (D.S. of treated starch, 0.075; propylene chlorohydrin content, 4.3 ppm) and a control pregelatinized potato starch were fed to groups of 75 male and 75 female weanling SPF mice of the Swiss Random strain for 89 weeks at dietary levels of 55 percent (about 80 g per kg body weight) (103). Other groups were fed diets containing 55 percent lactose or 25 percent sodium alginate. In week 80, ten mice of each sex and group were decapitated and necropsied and a comprehensive microscopic examination of tissues was made. A similar examination was made of all survivors after week 89. Tissues of all animals found dead or killed when moribund were removed and examined if autolysis was not too far advanced. Death rate in the group fed hydroxypropyl distarch phosphate was normal for the strain of mice fed but was abnormally high for males in the control group between weeks 39 and 65. At necropsy about 50 percent of the control animals that died in this period had an hemorrhagic myocarditis. Loose stools were seen in about 12 percent of the males and 5 percent of the females fed the test diet as compared to about 4 percent of males and 3 percent of females given the control diet. Body weights of males in the treated group were significantly reduced from week 16 to 48 and in females from week 40 to termination as compared to controls. Hematological indices and levels of fasting blood glucose and urea nitrogen were within normal limits. Males, but not females, fed the hydroxypropyl distarch phosphate diet had a greater amount of amorphous material in their urine than did control animals. Infrared analysis of
the sediment from the test animals indicated that it was nearly 100 percent protein. Mean relative weights (g per 100 g body weight) of the ceca and colons of the treated animals were greater than those of control animals. Frequency of intratubular calcareous deposits in the kidneys of mice that survived for at least 78 weeks was significantly greater in treated males (25/52) than in control males (5/28). Incidence of very slight calcareous deposits in the renal pelvis also was greater in treated (13/52) than in control males (0/28). A slight but not statistically significant increase in calcareous material and in thickened submucosa and epithelium in the urinary bladder occurred in treated males. Mitotic activity of the urothelium was not treatment related and there was no evidence for hyperplasia in the urinary bladder epithelium. There was no evidence for treatment related neoplastic changes. The investigators concluded that the renal and urinary bladder changes observed had little, if any, toxicological significance.

Special studies. Effect of modified starch on iron retention

Groups of 6 to 11 weanling Holtzman rats were fed for 25 to 28 days semipurified diets containing 35 percent (about 50 g per kg body weight) unmodified tapioca starch or hydroxypropyl distarch phosphate (molar substitution 0.045) prepared from tapioca starch (129). The starches were added to the diets in uncooked (experiment 1) or cooked (experiment 2) form; in a third experiment, the effect of cooking the entire diet was studied. One group of rats fed each type starch received no iron in their diet. On day 16 to 19, iron retention was estimated by whole-body counter assay after administering $^{59}$FeCl$_3$ in 5 ml of a solution containing 30 $\mu$g FeCl$_3$ and 1 g of the uncooked starch (experiment 1), 3.75 $\mu$g FeCl$_3$ and 0.125 g cooked starch (experiment 2), and 3.75 $\mu$g FeCl$_3$ and 0.35 g of cooked diet (experiment 3). Weight gains, hemoglobin levels and $^{59}$Fe retention in rats fed ironadequate diets were not affected by the starch source. Cooking the starch tended to reduce the iron retention. Rats fed the low-iron diets showed no differences in hemoglobin level that could be attributed to the type of starch that was fed. Iron retention was not affected by the type of starch when uncooked starch was used; however, retention was lower for cooked hydroxypropyl distarch phosphate (36 percent of dose) than for cooked unmodified starch (74 percent). For the cooked whole diets, respective retentions were 50 and 60 percent.

No studies on mutagenicity, carcinogenicity, teratogenicity or reproduction were available to the Select Committee.

**Hydroxypropyl Starch**

**Oxidized Hydroxypropyl Starch**

**Digestion, absorption and metabolism**

In vitro pancreatin digestibility of two hydroxypropyl wheat starches, 0.1 and 0.41 D. S. was similar to that of unmodified wheat starch
(130). The high D.S. hydroxypropyl starch (treated with 25 percent propylene oxide) had been further modified by oxidation with 0.055 pounds sodium hypochlorite per pound of dry starch and 0.45 percent active oxygen from hydrogen peroxide. In contrast Leegwater and Luten (131) reported the pancreatin digestibility of a hydroxypropyl starch, D.S. 0.04, as 80 percent of that of unmodified starch. ¹⁴C-labeled hydroxypropyl cornstarch, D.S. 0.12, prepared using labeled propylene oxide, was administered to a male rat by stomach tube (132). Over the next 50 hours, 92 percent of the radioactivity was excreted in the feces and 3.6 percent in the urine. The investigator attributed the radioactivity in the urine to propylene glycol in the test starch.

Ten 2-month-old male Wistar rats were fed, for 3 to 5 days, diets containing 56 percent of unmodified, precooked potato starch or hydroxypropyl starch (D.S. 0.025) or a 2:5 mixture of hydroxypropyl starch (D.S. 0.047) and unmodified potato starch, or a 1:6 mixture of precooked potato starch and hydroxypropyl starch (D.S. 0.106) (133). Alcohol-soluble, ether-insoluble residue in the feces per 100 g starch ingested was 0.8, 6.6, 11.6, and 19.1 g for starches of D.S. 0.025, 0.047, and 0.106, respectively, indicating that digestibility of hydroxypropyl starches decreased with increasing degree of substitution. The major component of the feces residue was identified as hydroxypropylmaltose; evidence also indicated the presence of dihydroxypropylmaltose and dihydroxypropylmaltotetraose (133). The hydroxypropylmaltose derivative was identified as 4-O-{2-O-[RS]-2-hydroxypropyl]-α-D-glucopyranosyl}-D-glucopyranose (134).

**Acute toxicity**

Groups of five male and five female Sprague-Dawley rats (about 150 and 110 g body weight, respectively) received by stomach tube doses of oxidized hydroxypropyl starch (D.S. 0.41) at dosage levels of 1, 3.16, or 10 g per kg of body weight (135). No deaths resulted. The animals exhibited normal appearance and behavior for 7 days following treatment at which time they were sacrificed. At autopsy, female rats at the highest dosage level exhibited congested kidneys; all other animals showed no significant gross pathology.

**Short-term studies**

Groups of 10 male and 10 female Sprague-Dawley weanling rats were fed diets containing 2, 5, 10, or 25 percent (about 2, 4, 8, and 20 g per kg of body weight) of oxidized hydroxypropyl (wheat) starch (D.S. 0.41) for 13 weeks (136). One male and one female rat from the 2 percent dietary-level group died from respiratory illness during the test period. Animals fed the diet containing 25 percent of the test compound exhibited mild diarrhea; this condition was not observed among animals at the lower dietary levels. Body weight gains were lower in the male rats fed diets containing
10 and 25 percent oxidized hydroxypropyl starch but only at the higher dietary level was the growth depression statistically significant. Food utilization of males and females at the 25 percent level tended to be lower than that of control animals. Hematologic and urinalysis data for test group animals were similar to those for the control group rats. No significant gross pathologic changes were observed that could be attributed to ingestion of the test compound. Liver- and kidney-body weight ratios for animals in the 25 percent dietary-level groups were significantly higher than controls but absolute weights were not significantly different. Complete histologic studies revealed no changes which were attributable to the ingestion of the oxidized hydroxypropyl starch.

Feron et al. (98) fed groups of 10 male and 10 female rats (strain not stated) diets containing 5, 15, or 45 percent (about 4, 12, or 35 g per kg body weight) hydroxypropylated acid-modified potato starch (D.S. 0.042) for 90 days. Weight gains of male rats fed the 15 and 45 percent level diets were consistently but not significantly less than those of the controls. Diarrhea occurred in the 45 percent level group but diminished during the final weeks of the experiment. Male rats fed 15 and 45 percent-level diets showed cecal enlargement whereas enlargement in females occurred only at the higher level. The enlarged ceca showed no evidence of inflammation or histological abnormalities. Hematological findings were comparable for the test and control groups. No pathological changes were observed on microscopic examination of the major organs.

Special studies

Application of hydroxypropyl starch (D.S. 0.1 or 0.41) in powdered form, or the 0.1 D.S. product in aqueous solution, produced mild irritation in rabbits' eyes (135).

Two hundred ten human subjects were patch-tested with moistened patches of powdered hydroxypropyl (wheat) starch (D.S. 0.1), allowing the patch to remain in contact with the skin for 72 hours, followed by a challenge exposure 2 weeks later (137). There was no greater irritation from the test material than from the control wheat starch and no evidence of sensitization on reexposure. There was no evidence of irritation in 23 human subjects patch-tested by the Repeat Insult Patch Test in which the subject was given nine 24-hour exposures to hydroxypropyl starch (D.S. 0.1) at intervals of 2 to 3 days (138). Challenge exposure 1 week after the last exposure gave no evidence of sensitization.
Acetylated Distarch Adipate

Digestion, absorption and metabolism

In vitro amyloglucosidase digestibility of acetylated distarch adipate prepared by treatment of waxy maize starch with 0.15 percent adipic anhydride as a 1:3 adipic:acetic mixed anhydride was 98 percent of unmodified waxy starch (110). In vitro studies of the hydrolysis of acetylated distarch adipate with pancreatin showed that the adipic acid ester linkages are not split whereas the acetate ester bonds are hydrolyzed (139).

Young male adult rats were administered by stomach tube suspensions of acetylated starch adipate prepared using $^{14}C$-labeled adipic acid. A physical mixture of $^{14}C$-labeled adipic acid and unmodified starch was administered to controls. Within 4 hours after dosing, 70 percent of the radioactivity administered as the free acid was recovered in carbon dioxide in the expired air whereas only 12 percent of the activity administered as the modified starch was recovered. After 25 hours 99.3 percent of the activity of the free adipic acid was recovered in the expired air, 5.8 percent in the urine and none in the feces, gastrointestinal tract, or carcass. In case of the labeled starch, 70.5 percent of the activity was recovered in the expired air, 7.2 percent in the urine, 24.5 percent in the feces and none in the carcass (139).

Caloric value of acetylated distarch adipate as measured by growth rate was equal to that of the control starch in 28-day feeding studies in which groups of 10 weanling male albino rats were fed a basal diet containing 1.5 or 3.0 g of modified or control starch, or 0.75 to 4.5 g of sucrose supplement. The modified starch was prepared by treatment of a thin-boiling waxy maize starch with 0.2 percent adipic anhydride and 5.5 percent acetic anhydride (140).

Short-term studies

Groups of 15 male and 15 female FDRL rats were fed a diet containing 50 percent (about 40 g per kg body weight) of acetylated distarch adipate or thin-boiling starch for 90 days (141). The modified starch was a thin-boiling waxy maize starch treated with 0.12 percent adipic acid and 10.5 percent acetic anhydride based on weight of starch. Weight gain of males in the test group was moderately, but significantly lower (15 percent) than males in the control group. Food intake and food efficiency also were lower in males in the test group. Full and empty cecal weights of both sexes fed the modified starch were significantly greater than those of animals in the control group. No differences were observed between groups in respect to hematolgy, blood chemistry, urinalysis, liver and kidney weights, or gross and histopathological evaluations. Corticomedullary junction
nephrocalcinosis was present in rats given either the treated or the control starch but renal pelvic nephrocalcinosis was not reported.

Long-term studies

Groups of 30 male and 30 female Sprague-Dawley rats were fed, for 2 years, diets containing 62 percent (about 30 g per kg body weight) of acetylated distarch adipate or cooked unmodified starch (starch source not stated) (142). Ten additional rats of each sex were included in the test and control groups but were necropsied at 90 days. Weight gains in test and control groups did not differ at 3 months but gain in the test group was about 15 percent lower at 100 weeks. Hematological parameters did not vary outside normal limits. Although several blood biochemical parameters of the test animals were statistically significantly different from those of the controls, only the increased serum glutamic oxalacetic transaminase (SGOT) level could be considered outside normal values. Organ-body weight ratio but not absolute weights of several organs of both sexes differed significantly from those of control animals. The investigators did not consider these differences to have biological significance. Histological findings at 24 months were hyperkeratosis of the forestomach and, in the livers of females, an increase of giant multinuclear cells. However, mean age of the females in the test group was almost 1 month greater than that of the control group because of earlier deaths in the latter group; for this reason the investigators considered the two groups not comparable and attached no toxicological significance to the observation (142). A "blind" reexamination of the stomach tissue sections by three pathologists did not demonstrate any significant differences between the test and control animals. It also was concluded that the giant multinuclear cells observed in the livers of female rats were linked with senescence and were not related to the ingestion of modified starch (143).

Kidney lesions characterized by focal hyperplasia of the epithelium lining the urinary space accompanied by calcified deposits attached to the lining epithelium were present in both treated and control animals. Authors concluded that there was no significant difference in the severity and frequency of the lesions in the two groups (142). The same conclusion was reached in a subsequent reexamination (Table V) of the kidney section (96). Reports on the occurrence of similar renal alterations in rats fed other types of diets are noted in the section Starch Acetates, Long-term studies (p. 26).

Special studies. Reproduction test

Groups of 10 male and 10 female Sprague-Dawley rats were fed for three generations 62 percent (about 30 g per kg body weight) acetylated distarch adipate (144). Each generation (P₁, F₁ and F₂) produced two successive litters with a 12 to 15 week period between matings. The
second litter was used to produce the next generation. Following weaning of F₂ β litters, the F₁ β parents were sacrificed and autopsied. The F₃ β generation was kept for 10 weeks to observe weight changes of all the young, then sacrificed and autopsied. No adverse effects were observed on fertility, litter size, weight of pups, mortality and postnatal growth. Liver weight increased in both absolute and relative value in the F₁ animals. However, no macroscopic anomalies were found. Gross and histopathologic findings of F₃ β animals revealed no differences between test and control animals.

**Distarchoxy Propanol**

**Short-term studies**

Diets containing 60 percent (about 60 g per kg body weight) of starch treated with 0.15, 0.30, 0.45, or 0.60 percent acrolein (w/w starch) were fed to groups of 10 weanling Sprague-Dawley rats for 28 days (89). Weight gains, feed efficiencies, and cecal weights of the test animals did not differ significantly from controls fed native starch. No diarrhea was observed.

**Acetylated Distarchoxy Propanol**

**Digestibility**

Digestibilities of four starches (source not stated) each cross-linked by treatment with 0.20 percent (w/w) acrolein and containing 0.46, 1.77, 2.20, and 3.50 percent acetyl groups, respectively, were determined by comparison of weights of feces from rats fed the treated starches with those fed unmodified starches in a similar basal diet. Results indicated that digestibilities of modified and unmodified starches were similar. This result was supported by similar weight gains and feed efficiencies observed for the two groups (89).

**Short-term studies**

Twenty-eight-day rat feeding studies were conducted with starches (source not stated) cross-linked by treatment with 0.15, 0.30, 0.45, or 0.60 percent acrolein and, for each level of acrolein, acetylated by reaction with 4.5, 6.0, 7.5, or 9.0 percent (w/w) vinyl acetate (89). Acetyl contents of the acetylated starches were determined by analysis. The starches were fed at the 60 percent dietary level to groups of 10 weanling Sprague-Dawley rats. Weight gains of groups fed starches treated with 9 percent vinyl acetate (3.23 percent acetyl content) were significantly (p=0.05) lower than the control animals fed unmodified starch. Groups fed starches treated with 4.5, 6.0, 7.5, or 9.0 percent vinyl acetate developed enlarged ceca and diarrhea, the incidence rates of which were significantly and progressively higher as acetyl content increased. Incidence of diarrhea increased
from 9 percent at the low level of vinyl acetate treatment to 72 percent in the groups fed starch treated with 9 percent vinyl acetate. At necropsy, cecal and large intestinal contents of all rats fed the acetylated starches were acidic. Those of the control animals were alkaline.

Groups of 10 pigs (breed not stated) were fed diets containing 66 percent unmodified starch or starch treated with 0.2 percent (w/w starch) acrolein and acetylated (2.5 percent acetyl content) with vinyl acetate (89). During a 28-day feeding period, the pigs fed the acetylated distarchoxy propanol grew slower and consumed more feed per pound of weight gain than those fed unmodified starch. The pigs fed the treated starch also exhibited a mild degree of diarrhea during the first 2 weeks of feeding after which they became adapted to the starch.

Special studies

Groups of 10 weanling Sprague-Dawley rats were fed diets containing 60 percent of acetylated distarchoxy propanol (3.4 percent acetyl groups; cross-linked with 0.13 percent acrolein) or the unmodified starch (starch source not stated) for 6 weeks (89). At necropsy, the microflora of the small and large intestines of animals fed the treated starch contained significantly fewer Escherichia coli and yeasts, but a greater number of Lactobacilli. The cecal contents were acidic. The authors noted that an acidic condition usually is associated with diarrhea. No significant histopathological changes were found in the liver, kidney, cecum, or large and small intestines of the rats fed the test starch.

In a two-generation study, groups of 20 female and 10 male Sprague-Dawley weanling rats were fed diets containing 45.7 percent acetylated distarchoxy propanol (2.5 percent acetyl groups; cross-linked with 0.20 percent acrolein) or the unmodified starch (source not stated) for 105 days (89). At this time four males were each paired with three females for breeding. The number of births, percentage survival and weanlings in the treated starch group exceeded those of the unmodified starch group. Weanlings in the modified starch group were fed the modified starch for 1 year. Weight gains and feed efficiencies were greater than those of the control animals. No diarrhea or deaths were observed in either group.

Distarch Glycerols

Digestion and metabolism

In vitro digestibility by amylglucosidase of distarch glycerol was 98.2 percent of that of unmodified starch; both starches were gelatinized by heating at 100°C for 20 minutes prior to enzymatic hydrolysis (110). The modified starch was prepared by treatment of maize starch with 0.3 percent epichlorohydrin (w/w starch).
Caloric values of unmodified starch and two distarch glycerol samples, prepared by treatment of waxy maize starch with 0.07 percent and 0.5 percent epichlorohydrin (w/w starch), respectively, were determined by feeding the starches (3 g or about 25 g per kg) to groups of 10 weanling, male FDRL rats and comparing growth response after 28 days to that obtained by feeding the basal diet supplemented with graded sucrose levels (145, 146). Differences in caloric availability of the treated and unmodified starch were not significant.

**Short-term studies**

Groups of 10 male and female weanling FDRL rats were fed, for 90 days, diets containing 71 percent (about 60 g per kg body weight) of unmodified waxy maize starch or waxy maize starch treated with 0.07 or 0.5 percent epichlorohydrin (146). Food intake and weight gains of the test groups were similar to those of animals fed the control starch. No adverse effects of feeding the test starches were observed on hematology, blood non-protein nitrogen levels, urinary parameters, or organ-body weight ratios of the liver, kidneys and adrenals. Gross pathological observations revealed no differences between the modified starch group and the controls.

**Hydroxypropyl Distarch Glycerol**

**Digestion and metabolism**

In vitro digestibility by pancreatin and porcine intestinal mucosa of a hydroxypropyl distarch glycerol (D.S. 0.04) was 86 percent of that of the unmodified starch (87).

**Short-term studies**

Groups of 25 male and 25 female Charles River rats, 105 to 150 g body weight, were fed, for 90 days, diets containing 1.0 or 5.0 percent (about 0.6 or 3 g per kg) hydroxypropyl distarch glycerol prepared by treating tapioca starch with 10 percent propylene oxide and 0.1 percent epichlorohydrin (w/w starch) (147). Growth, feed consumption and terminal body weights for the test rats were similar to controls. No adverse effects were noted on the hematological values, blood sugar, blood urea nitrogen, serum glutamic-pyruvic transaminase or urinary parameters. Organ-body weight ratios were within the normal range. Thyroid-body weight ratio for the test rats and for negative control rats (chow diet) were significantly lower than the value for the rats receiving tapioca flour. Thyroid tissue sections were histologically similar for the positive controls and the highest dose-level test rats. Neither gross nor histopathologic examination changes attributable to ingestion of the test compound were detected.
Groups of 10 male and 10 female weanling rats (Wistar-derived) were fed practical type diets containing 25 or 50 percent (about 30 or 60 g per kg) of hydroxypropyl distarch glycerol, replacing an equal quantity of unmodified starch in the control diet (88). The modified starch was prepared by treating potato starch with 0.1 percent epichlorohydrin and 5 percent propylene oxide. Weight gains at 8 weeks were similar to those of control rats fed diets containing 50 percent maize starch. Moderate diarrhea occurred in male and female rats fed 50 percent modified starch and a slight diarrhea was observed at the 25 percent dietary level. At sacrifice, cecal weights of both male and female rats were greater than those of controls but microscopic examination revealed no differences from controls. In a second experiment by the same investigators (88) the modified starch was fed at the 25 and 50 percent levels in a semipurified diet for 10 days. Diarrhea was more marked than in animals fed the practical diet. At day 7, cellulose was added to all diets at a level of 4 percent. This did not reduce the diarrhea. Hair loss was pronounced in both sexes fed modified starch at the 50 percent level and was slight at the 25 percent level.

In a 90-day subacute toxicity study (148) groups of 10 male and 10 female rats were fed diets containing 5, 10, or 30 percent (about 4, 8, and 25 g per kg) of hydroxypropyl distarch glycerol prepared by treating potato starch with 0.1 percent epichlorohydrin and 5 percent propylene oxide (w/w starch). No definite diarrhea was observed at any test level. Weight gains and food efficiencies of the test groups did not differ significantly from those of controls. Hematological parameters, blood biochemical values and urinalyses were within the normal range. Cecal weights were increased in test animals at the 30 percent dietary level but no histopathologic changes were observed in the enlarged ceca. Gross and histopathologic examination of other tissues of animals in the highest dose group did not show changes attributable to ingestion of the modified starch.

Groups of four male and four female young adult beagles were fed for 90 days diets containing 1 or 5 percent hydroxypropyl distarch glycerol (about 0.3 or 2 g per kg body weight) prepared by treating tapioca starch with 0.1 percent epichlorohydrin and 10 percent propylene oxide (w/w starch) (149). Body weights of test animals were maintained or slightly increased except for one dog on the high level diet which had been ill (nature of illness not stated) and lost 1.5 kg (about 10 percent). No changes in hematology, blood biochemistry or urinalysis attributable to the test compound were noted. Slightly elevated kidney-body weight and testes-body weight ratios were found for the dog that had been ill during the study. Thyroid weights and thyroid-body weight ratios for two females on the 5 percent level of the test diet were slightly elevated as compared to the controls. Gross and microscopic pathologic examination showed no changes attributable to ingestion of the modified starch.
In a second 90-day subacute toxicity study with dogs (150), groups of four male and four young adult female beagles were fed diets containing 10 percent (about 4 g per kg body weight) cornstarch or hydroxypropyl distarch glycerol prepared by treating cornstarch with 0.1 percent epichlorohydrin and 25 percent propylene oxide. Animals were normal in appearance, behavior, appetite and elimination. No significant weight difference between test and control animals was observed. Food consumptions were comparable. Hematologic and blood chemical findings, urinalyses and organ-body weight ratios showed no test compound-related effects. Gross and microscopic pathologic findings showed no abnormalities.

Groups of eight 3-day-old Pitman-Moore miniature pigs were fed liquid diets containing 28.8 percent (dry basis) hydroxypropyl distarch glycerol prepared by treating a thin-boiling waxy maize starch with 0.009 percent epichlorohydrin and 4.2 percent (dry weight basis) propylene oxide (72). Hydroxypropyl group degree of substitution was about 0.06. Growth rates were similar during a 25-day test period. At sacrifice, blood biochemical values including hemoglobin, cholesterol, triglycerides, calcium, phosphorus, alkaline phosphatase, urea nitrogen, total protein, albumin, and globulin, were similar for treated and control animals. Relative organ weights as well as carcass and liver composition were similar for test and control animals.

Twelve adult human volunteers consumed 60 g hydroxypropyl distarch glycerol on each of 4 successive days. No adverse effects were observed nor were there abnormalities in frequency of defecation, quantity of feces, fecal water or its lactic acid content (91).

**Long-term studies**

In a 2-year study, hydroxypropyl distarch glycerol was fed to groups of 30 male and 30 female weanling CIVO rats at 5, 10, and 30 percent (about 3, 5, and 15 g per kg body weight) dietary levels, replacing an equal amount of precooked control potato starch (92, 93). The test starch was a potato starch cross-linked with 0.1 percent epichlorohydrin and etherified with 5 percent propylene oxide (D.S. 0.04 to 0.05). Body weight gains of females fed the 30 percent dietary level were significantly lower than those of control animals; females at this dietary level also showed a slight reduction in hemoglobin concentration but the values were within the normal range. Blood chemical findings and urinalysis showed no evidence of a dose-related response to the test substance. Cecal weight was increased at the 30 percent dietary level in males and at the 10 and 30 percent levels in females. No change in cecal tissue was found by microscopic examination. No distinct compound-related gross or microscopic pathologic changes were observed in any of the organs examined. Renal calcification accompanied by focal hyperplasia of the pelvic epithelium was more marked in
males receiving the 30 percent test diet than in the control animals (Table V). However, because there was no distinct relationship with either the feeding level, or with the type of chemically modified starch among the five fed in the study (Table V, CIVO/TNO Studies, p. 27), the investigators considered the toxicological significance doubtful. Reports on the occurrence of similar renal changes in rats fed other types of diets are noted in the section Starch Acetates, Long-term studies (p. 26).

Special studies on reproduction

Groups of 10 male and 20 female weanling CIVO rats (Wistar-derived) were fed, for three generations, a diet containing 10 percent (about 5 g per kg body weight) hydroxypropyl distarch glycerol and 20 percent precooked potato starch (92). The test starch was potato starch which had been cross-linked with 0.1 percent epichlorohydrin and etherified with 5 percent propylene oxide (w/w starch). Rats were mated (P, F₁ and F₂ generations) at weeks 12 and 20 after weaning. The second litter of each generation was used to produce the next generation. The F₃b generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. Implantation sites were counted in the P, F₁b and F₂b parents. Body weights did not differ among groups in successive generations and no treatment-related differences were observed in the test groups. No adverse effects were observed regarding resorption quotient, litter size, weight of pups, preweaning mortality or growth rate of pups. No gross or histological changes attributable to feeding the modified starch were observed.

In a modified 13-week subacute one-generation reproduction study, cornstarch and hydroxypropyl distarch glycerol (25 percent propylene oxide, 0.1 percent epichlorohydrin-treated cornstarch) were fed at a dietary level of 10 percent to Charles River rats from 2 weeks prior to mating of the F₁a generation until the sacrifice of the parental females for the caesarean delivery of the F₁b generation (151). Twenty-five F₁a males and 25 F₁a females were selected and placed on the 90-day study at the 10 percent dietary level. The number of conceptions, litters, live births, still births and preweaning deaths, weights of pups at 24 hours and at weaning for the F₁a litters, were comparable for the test and control groups. Necropsies on 10 male and 10 female pups of the F₁a litter at weaning revealed no gross or microscopic pathologic changes attributable to the test compound. Skeletal and visceral examination of F₁b fetuses delivered by caesarean section on day 18 or 19 did not reveal any compound-related abnormalities. Test and control groups were comparable in respect to number and placement of implantation and resorption sites and number and weight of live and dead fetuses. Terminal necropsy of the P₁ animals did not reveal any consistent compound-related alterations. Females had kidney- and spleen-body weight ratios that were significantly greater (15 and 20 percent, respectively) than those of the controls. However, microscopic examina-
tion revealed no abnormalities. Physical appearance, behavior, growth and food consumption of the F₁₄ test and control groups fed 90 days were comparable. Survival was 100 percent in both groups. Hematological determination and urine determinations were within normal limits and were comparable between the groups. Kidney-body weight ratios were significantly greater for test animals of both sexes than for controls and the thyroid-body weight ratio was significantly less. However, gross and microscopic examination of the kidneys and thyroids and other organs showed no pathological alterations which could be attributed to ingestion of the modified starches.

**Acetylated Distarch Glycerol**

**Digestibility and metabolism**

In vitro amyloglucosidase digestibilities of two gelatinized acetylated distarch glycerol samples, 1.16 and 2.58 percent acetyl content, were 80.4 and 67.6 percent, respectively, of that of gelatinized cornstarch (86). Digestibility of cornstarch cross-linked by the same treatment with epichlorohydrin, but not acetylated, was 98.2 percent of that of the control.

Caloric values of two acetylated distarch glycerol preparations, one treated with 0.1 percent epichlorohydrin and 5.5 percent acetic anhydride and the other with 0.3 percent epichlorohydrin and 5.5 percent acetic anhydride, were estimated from the 28-day growth response of groups of 10 weanling albino rats (strain not stated) fed low-calorie basal diets to which 1.5 or 3 g (about 12 or 25 g per kg body weight) of the test starch was added (152). Comparison was made with the growth response of similar groups fed the basal diet to which graded amounts of sucrose were added. All animals were normally active and in good health. Caloric availability of the modified and control starches was similar and in no instance was the caloric estimate from the high dose less than that from the lower one.

**Short-term studies**

Groups of 15 male and 15 female weanling FDRL rats were fed, for 90 days, semipurified diets containing 50 percent (about 40 g per kg body weight) of thin-boiling starch or acetylated distarch glycerol prepared by treating the thin-boiling waxy maize starch with 0.3 percent epichlorohydrin and 10.5 percent acetic anhydride (w/w starch) (153). Because diarrhea was observed in the test group, the level of cellulose flour was increased to 4 percent from the second week on after which diarrhea disappeared. Weight gain in males of the test group was significantly lower (13 percent) than that of the control group but weight gains of females in the two groups showed no difference. Food intake and efficiency of utilization were slightly but not significantly different in the test group than in
controls of the same sex. Blood chemical and hematologic analyses, urinalysis, organ weights, and gross and histological examinations were normal.

Two groups of 3-day-old Pitman-Moore miniature pigs were given liquid diets containing 32.8 percent (dry basis) thin-boiling waxy maize starch or this starch after treatment with 0.05 percent epichlorohydrin and acetic anhydride to an acetyl content of 1.1 percent (73). Each diet was fed ad lib for 25 days. Growth was less for pigs fed the modified starch diet but feed intake was about 20 percent less than that of the control group which was attributed to the viscous nature and relative unpalatability of the diet. Cecum-body weight ratios were greater for animals in the control group. With the exception of higher (10 percent) serum concentrations of inorganic phosphorus in pigs fed the modified starch, no differences due to treatment were observed in any of the serum chemical values.

Long-term studies

In a 2-year feeding study, groups of 30 male and 30 female Sprague-Dawley rats were fed diets containing 62 percent (about 30 g per kg body weight) of acetylated distarch glycerol or cooked unmodified starch (142). Ten additional rats of each sex were included in the test and control groups, but were necropsied at 90 days. No gross or histopathologic abnormalities were observed in these animals. Weight gains of males and females fed the test diet for 100 weeks were 13 and 20 percent lower than controls of the same sex. Organ-body weight ratios, but not absolute weights, of the brain and kidneys of both sexes and the heart of males differed significantly from those of control animals. The investigators did not consider these differences to have biological significance. Hematological parameters measured after 3, 12, and 24 months of treatment did not show variations outside normal limits; among biochemical parameters measured after these periods of treatment, only the level of SGOT at 24 months in the test group appeared to be outside (higher) normal limits. Incidence of tumors in the treated animals did not differ significantly from that in the control groups. Kidney lesions characterized by focal hyperplasia of the epithelium lining the urinary space accompanied by calcified deposits on the lining epithelium were present in both control and treated animals. The authors concluded that there was no significant difference qualitatively or quantitatively in the lesions in the two groups (142). The same conclusion was reached in a subsequent reexamination (96) of the kidney sections (Table V).

Abnormal histological findings in the test animals at 24 months were hyperkeratosis of the forestomach and, in females, an increase of giant multinuclear cells in the liver. The investigators considered the first finding to be of no toxicological significance to man and the second to be of doubtful significance because the mean age of the treated group was
almost 1 month older than that of the control group, and the animals were not truly comparable, as a result of earlier deaths of the controls (142). A "blind" reexamination of the stomach tissue sections by three pathologists did not demonstrate any significant differences between the test and control animals. It also was concluded that the giant multinuclear cells observed in livers of female rats were linked with senescence and were without relationship to the ingestion of modified starch (143).

Special studies. Reproduction test

Groups of 10 male and 10 female Sprague-Dawley rats were fed for three generations 62 percent (about 40 g per kg body weight) acetylated distarch glycerol (144). Each generation (P, F₁, F₂) produced two successive litters with a 12- to 15-week period between matings. The second litter was used to produce the next generation. Following weaning of the F₂₅ litters, the F₁₅ parents were sacrificed and autopsied. The F₃₅ generation was kept for 10 weeks to observe weight changes of all the young, then sacrificed and autopsied. No adverse effects were observed on fertility, litter size, weight of pups, mortality and postnatal growth. Liver weight increased in both absolute and relative values in the F₁ animals; however, no macroscopic anomalies were found. Gross and histopathologic examination of F₃₅ animals revealed no differences between test and control animals.

Succinyl Distarch Glycerol

Short-term studies

In a 90-day subacute feeding study, groups of 15 male and 15 female weanling albino rats were fed semisynthetic diets containing 50 percent succinyl distarch glycerol (about 50 g per kg) or control starch (154). The test starch was prepared by treating an acid-modified waxy maize starch with 0.3 percent epichlorohydrin and 4 percent succinic anhydride (w/w starch). Diarrhea occurred in some of the rats in the test group during the first week and the level of cellulose flour in the diet was increased from 2 to 4 percent, which corrected this condition. Growth of females of test and control groups was equivalent, but that of males of the test group was significantly lower (P<0.01) than that of the control group. Food efficiency of males of the test group also was significantly lower. Clinical examinations showed no treatment-related effects on the hematological or blood biochemical values. Urine of males of both test and control groups was alkaline. Cecal weights of rats of both sexes in the test group were significantly greater than those of the control group. Kidney and liver weights were normal and comparable in animals fed the test and control diets. Microscopic examination of tissue sections revealed no abnormalities attributable to the test diet. The kidneys of a number of female rats fed the
control (7/15) and test (8/15) diets exhibited varying degrees of apparent calcification in the region of the corticomedullary junction. This condition did not occur in males.

**Long-term and special studies**

No reports on long-term studies, mutagenicity or teratogenicity were available to the Select Committee.

**Sodium Hydroxide Gelatinized Starch**

No information on the biological properties of starches gelatinized by treatment with 1 percent sodium hydroxide was available to the Select Committee.

**Biological Properties of Possible Residues in Chemically Derivatized Starches**

**Propylene oxide.** The oral LD$_{50}$ (14 days) of propylene oxide in male Wistar rats, 90 to 120 g body weight, was 1.14 g per kg body weight; in guinea pigs, 250 to 300 g body weight, it was 0.69 g per kg (155). Repeated oral doses of 0.1 or 0.2 g per kg body weight administered as a 10 percent solution in olive oil to groups of five young adult female rats, 5 days a week for 24 days, produced no toxic effects (156). In vapor inhalation studies, groups of three to 17 rats, eight guinea pigs, and one rabbit of each sex and one female monkey were exposed 7 hours daily, 5 days a week, to 457 ppm propylene oxide vapor. Male rats received 79 exposures in 112 days, female rats 138 exposures in 198 days, both male and female guinea pigs 110 exposures in 157 days, and rabbits and monkey 154 exposures in 218 days. The monkey and rabbits showed no adverse effects including gross and histopathological findings. Irritation of eyes and respiratory passages occurred in the rats and guinea pigs; a slight growth depression and increase in lung weights also were observed for the guinea pigs. All four species tolerated 102 ppm propylene oxide without effect for 183 to 198 days.

**Reverse mutations were induced in macroconidia of the purple adenine requiring mutant 38701 of Neurospora crassa after treatment with 0.5 M propylene oxide in water for 15 minutes (157).** Propylene oxide also induced recessive lethal mutations in Drosophila melanogaster (158).

Groups of 12 rats were administered by subcutaneous injection 1.5 g per kg total dose of propylene oxide in water or arachis oil over a period of 325 days. Sarcomata occurred at the site of injection after 507 to 739 days in eight mice injected with the arachis oil solution; and in three mice treated with the water solution after 158 to 732 days (159).
Propylene chlorohydrin. Propylene oxide reacts with chloride ion in foods to form propylene chlorohydrin (160). Two isomers are formed, 1-chloro-2-propanol and 2-chloro-1-propanol and both isomers have been identified in foods treated with propylene oxide (161). Propylene chlorohydrin has been found in starches modified by hydroxypropylation. Wesley et al. (160) found that volatilization reduced the ethylene chlorohydrin content of food about 50 percent after prolonged cooking in an open vessel but the combination of high temperature and prolonged time of cooking in a closed autoclave caused little change in the ethylene or propylene chlorohydrin content.

Groups of 10 male and 10 female 5-week-old rats were fed for 25 weeks diets to which 0, 0.1, 0.25, 0.5, and 1.0 percent propylene chlorohydrin (75:25 mixture of the 1-chloro and 2-chloro isomers) was added (162). However, analysis of the 1.0 percent-level diet showed loss by volatilization during 20 minutes' mixing in an open mixer reduced the level to 0.36 percent. After 7 days' exposure of this diet to laboratory conditions, the level of propylene chlorohydrin was 0.0838 percent (about 90 mg per kg body weight at week 1) of which 32 percent was the 2-chloro isomer. Growth rates of animals fed the 0.5 and 1.0 percent dietary levels were lower than those of controls but food consumption was less and food efficiency was similar to those of controls. Organ-body weight ratios were comparable to those of control animals. No adverse effects were observed on hematological values, mortality, gross or histopathologic findings.

In another test (162) propylene chlorohydrin was administered to groups of 10 male and 10 female 8-week-old rats by stomach tube in doses of 25, 50, 75, and 100 mg per kg of body weight daily for 22 weeks. The dose for the high level group was increased to 150 mg per kg in the 11th week, to 200 mg per kg in the 14th week and to 250 mg per kg in the 16th week. Weight gain in the high dietary level group was moderately depressed in males and slightly depressed in females while the dose was not above 150 mg per kg. Both sexes lost weight when the dose was increased to 200 mg per kg. All rats died within 3 weeks after increasing the dose to 250 mg per kg; only one death had occurred on the high level diet before the 16th week. Weight gain was slightly, but not significantly, depressed in both sexes given doses of 75 mg per kg daily. The liver-body weight ratio was increased at the 75 mg per kg dose for both sexes and for males at the 25 mg per kg dose. However, no hematological changes or gross or histopathologic changes were observed in treated rats at dose levels of 75 mg per kg or less. Microscopic examination of tissues of the rats dosed at higher levels was not conducted.

Oral LD₅₀ of propylene chlorohydrin in the rat was reported as 218 mg per kg body weight. No deaths occurred in dogs administered 150 mg per kg orally; 1/7 deaths occurred at 200 mg per kg and 6/6 deaths resulted from oral administration of 250 or 300 mg per kg (162).
Propylene chlorohydrin (75 percent 1-chloro-2-propanol and 25 percent 2-chloro-1-propanol) was mutagenic to Salmonella typhimurium TA 1530 but not for TA 1538 when tested in agar containing 1.1 to 22 mg propylene-chlorohydrin per plate (163).

Epichlorohydrin. The oral LD₅₀ of epichlorohydrin in male ICR mice and male Sprague-Dawley rats was 240 and 260 mg per kg of body weight, respectively. Administered intraperitoneally, the LD₅₀ ranged from about 120 to 170 mg per kg for mice, rats, guinea pigs, and rabbits. Cause of death in acute experiments is generally attributed to depression of the respiratory center. Epichlorohydrin produces extreme irritation when tested intradermally, dermally or intravascularly in rabbits (164).

In a subacute toxicity study, groups of 12 male Sprague-Dawley rats received 0, 11, 22, or 56 mg epichlorohydrin per kg body weight in cottonseed oil by intraperitoneal injection 3 days per week for 12 weeks. There was a dose-related decrease in hemoglobin values, segmented neutrophils were increased in the high dose group and lymphocytes were decreased in the two highest dose groups (164).

Sprague-Dawley rats, 240 to 260 g body weight, became infertile after daily oral administration of 15 mg epichlorohydrin per kg body weight. The effect was reversible within 1 week. Histological examination of testes, epididymides, prostates and seminal vesicles after 12 days of treatment showed no differences compared with untreated controls (165).

Epichlorohydrin did not induce dominant lethal mutations in ICR/Ha Swiss mice when 150 mg per kg was administered intraperitoneally (166). Reverse mutations were induced in macronconidia of the purple adenineless mutant 38701 of Neurospora crassa by treatment for 30 minutes in a 0.15 molar aqueous solution of epichlorohydrin (157). Dissolved in ethanol and adjusted to pH 7.2, epichlorohydrin induced reverse mutations from tryptophan dependence to tryptophan independence when tested in Escherichia coli strain B/r (167). Epichlorohydrin was four- to five-fold less mutagenic than the polyfunctional alkylating agent tris(1-aziridinyl)-phosphine oxide when tested on human lymphocytes in vitro at concentrations of 10⁻⁶ to 10⁻¹¹ M. The 10⁻⁴ M concentration was toxic and only the 10⁻⁶ M concentration increased significantly the number of cells with chromosomal aberrations and number of aberrations and breaks per hundred cells (168).

Sarcomas at the site of injection occurred in two of 50 6- to 8-week-old female ICR/Ha Swiss mice given weekly subcutaneous injections of 1 mg epichlorohydrin in 0.05 ml tricaprylin for 300 days (169). In a later test, 1 mg epichlorohydrin in 0.05 ml tricaprylin was administered weekly by subcutaneous injection to 50 6- to 8-week-old female ICR/Ha Swiss mice for 580 days. Median survival time was 486 days; six animals developed
sarcomas at the site of injection and one had a local adenocarcinoma. One of 50 mice had a local sarcoma in the control group given tricaprylin only (170).

In mouse-skin initiation-promotion experiments, 30 female ICR/Ha mice were injected with 2 mg epichlorohydrin in 0.1 ml acetone followed 14 days later by applications of 2.5 μg phorbol myristate acetate in 100 μl acetone three times a week until the experiment terminated at 385 days. Nine mice developed skin papillomas (first one observed at 92 days) and one developed a skin carcinoma. Three of 30 control animals treated with phorbol myristate acetate alone developed papillomas, the first being observed at 224 days (170).

Preliminary reports (171, 172) on rat inhalation studies with epichlorohydrin in which 40 rats were exposed to a concentration of 100 ppm for 6 hours daily for 30 days identified squamous cell cancers of the nasal epithelium in three animals that died at 460, 490, and 596 days and a squamous cell papilloma in a fourth animal that died at 391 days. In another study in which groups of rats were exposed at concentrations of 10, 30, and 100 ppm, two of 12 rats, alive after an unstated period of exposure to 100 ppm epichlorohydrin, had developed a mass on the nose suspected to be cancerous.

In 2-year rat feeding studies carried out with two starches cross-linked with epichlorohydrin, hydroxypropyl distarch glycerol (5, 10, and 15 percent levels in the diet) and acetylated distarchglycerol (62 percent in the diet), no evidence of carcinogenicity of the treated starches was observed (92, 142).

3-Chloro-1,2-propanediol. This chlorohydrin is formed by the reaction of epichlorohydrin with water and may be present in trace amounts in epichlorohydrin cross-linked starches. The oral LD₅₀ of this chlorohydrin in rats is reported as 150 mg per kg body weight and in mice 160 mg per kg (173). Like epichlorohydrin, 3-chloro-1,2-propanediol produces reversible male infertility in rats (174). Minimum effective oral dose for young, mature Spartan and Upjohn male rats was approximately 6 to 7 mg per kg of body weight. No change was detected in sperm numbers or motility. Return to fertility was complete within 1 week post-treatment. Histological examination of the testis and epididymis from the males treated with one to two times the minimum effective dose for 8 to 49 days showed no deviations from normal. However, doses five times the minimum effective dose produced a lesion in the caput epididymis of the rat.

1,2-Dichloropropanol and 1,3-dichloropropanol. These chlorohydrins may be formed by reaction of epichlorohydrin with traces of chlorides present in starch or the reaction medium. The oral LD₅₀'s for
these compounds in rats are 90 mg per kg for the 1,2-isomer (173) and 0.11 ml (150 mg) per kg for the 1,3-isomer (175).

Vinyl acetate. The oral LD$_{50}$ for vinyl acetate in rats is reported as 2.92 g per kg body weight (173).

Vinyl acetate was nonmutagenic when tested in the Salmonella/microsome test using tester strains TA 1535, TA 1537, TA 100 and TA 98 both with and without the S-9 fraction of rat liver homogenate (176). No tumors were detected in Sprague-Dawley rats exposed to atmospheres containing 2,500 ppm vinyl acetate 4 hours per day, 5 days per week, for 12 months (177).

Acetaldehyde. Acetaldehyde is a byproduct of the reaction of vinyl acetate with starch. Only traces are expected to remain in starch after washing starch acetylated by treatment with vinyl acetate. Acetaldehyde occurs naturally as a constituent of many fruits and vegetables including grapefruit (juice, 1.45 ppm), oranges (juice, 1 to 10 ppm), grapes (0.36 to 1.8 ppm), pears (3 to 119 ppm), broccoli (2 to 15 ppm), and peas (2.4 ppm) (178).

The oral LD$_{50}$'s for acetaldehyde in rats and mice are reported as 1.9 and 1.2 g per kg of body weight, respectively (173).

Twenty hybrid rats received subcutaneous injections, one to two times per week, of 0.5 to 1.0 ml of 0.5 percent solutions of acetaldehyde for a total of 26 to 41 injections each followed by injections of 1.0 to 1.5 ml of 1 percent solutions, also 1 to 2 times per week, for a total of 40 to 50 injections. Four rats developed spindle cell sarcomas near the site of injection within 554 days, the duration of the experiment (179).

Acrolein. The oral LD$_{50}$ for acrolein in the rat is 46 mg per kg and in the rabbit, 7 mg per kg of body weight (173). Acrolein was mutagenic in Drosophila (180) and S. typhimurium, strains TA 1538 and TA 98 (181) but was nonmutagenic in the dominant lethal assay in the mouse when doses of 1.5 or 2.2 mg per kg body weight were administered intraperitoneally (166). Injected subcutaneously in 15 partly inbred albino mice (a strain susceptible to subcutaneous sarcoma) at weekly intervals for 24 weeks, doses of 0.2 mg acrolein in 0.1 cc of sesame oil were not carcinogenic (182). Two of 15 albino S strain mice developed skin papillomas within 33 weeks after 10 weekly applications of a 0.5 percent acrolein solution in acetone (12.6 mg total dose) (179).

Succinic anhydride. Injected subcutaneously in rats, the TDL$_{0}$ (lowest toxic dose) of succinic anhydride was 2.6 g per kg (173). The compound was not mutagenic in the Salmonella test using strains TA 100, TA 98,
TA 1535, and 1537 with the S-9 fraction of liver homogenate (176). However, the chemical toxicity of succinic anhydride limited the dose that could be tested. Six rats, each weighing 100 g, were injected subcutaneously with arachis oil containing 2 mg succinic anhydride twice weekly for 65 weeks (183). Three rats developed local tumors at the site of injection after 93, 104, and 106 weeks, respectively.

**Incidence of Kidney Stones in Age Group 0 to 20**

The occurrence of uroliths in rats fed high levels of chemically derivatized starches in long-term feeding experiments raised the question whether an increased incidence of kidney stones may have occurred since the introduction of these starches in baby foods in the 1950's. Winters (184) examined the available information on the incidence of stones in the U.S. population group age 0 to 20. It was concluded that there was no evidence of increased incidence in this age group over the past 10 to 20 years and that no effects of specific dietary components have been shown to be etiologically related or even correlated with the occurrence of stones in children of this country.
Unmodified and Pregelatinized Starches  
(pages 18-21 in report)

The digestibility of unmodified cereal and tapioca starches used commercially as food ingredients, both raw and after cooking, is almost complete. Potato and arrowroot starches are less completely digested when fed raw but their digestibility is similar to that of the cereal starches after cooking. Pregelatinized starches (dried, cooked starches) generally are highly digestible. Consumption of excessive quantities, pounds per day, of raw starch has resulted in obesity and iron-deficiency anemia in human subjects. Most of the foods to which starch is added by the food industry are cooked in processing or are cooked before serving. Moreover, the total quantity of unmodified and pregelatinized starch added to processed foods is insignificant compared to the natural starch content of the American dietary, some of which is eaten in its native form in raw vegetables. No adverse effects have been attributed to these starches as added food ingredients. It is suggested, however, that specifications for food grade unmodified starches be developed in order to distinguish them from the starches that are used in non-food applications.

In light of the foregoing, the Select Committee concludes that:

There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo (also called grain sorghum starch), rice, potato, tapioca or arrowroot starch 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.

There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo, rice, potato, tapioca or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from paper and paperboard packaging.

There is no evidence in the available information on unmodified or pregelatinized corn, high amylose corn, waxy maize, wheat, milo, rice, potato, tapioca or arrowroot starch that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from cotton and cotton fabrics used in dry food packaging.
Modified Starches

Lack of intake data for individual starches is common to all of the modified starches. Consequently, in evaluating their safety as a food ingredient, it was assumed that the per capita total daily consumption of modified starches, about 17 mg per kg body weight, applied to each individual starch. Similarly, it was assumed that the maximum daily intake, 1.7 g per kg, reported for the modified starches used in infant foods (distarch phosphate, acetylated distarch phosphate, and acetylated distarch adipate) also represented the possible intake for each of these modified starches.

An observation common to the six modified starches for which long-term rat feeding experiments have been conducted, and noted with another modified starch in 90-day studies, was the occurrence of renal lesions with similar morphology in all cases. To minimize repetition, it is convenient to discuss this renal alteration and its significance at this point. The renal alteration consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied by calcified patches in the underlying tissues or calcified deposits attached to the lining epithelium. The condition occurred in some control animals and, although there was no clear dose-response relationship, highest incidence appeared at the highest dietary levels of the modified starches. The renal lesion does not appear to be related to the chemical composition of the modified starches as evidenced by the different derivatives which are associated with this condition. Similar lesions have been reported in rats fed high levels of lactose, sodium alginate, magnesium oxide and in uninephrectomized rats fed high levels of sodium chloride. A similar pathological process of unknown etiology also has been described in Sprague-Dawley rats. Hyperplastic changes and mineral deposits have been associated with the presence of the urinary tract parasite, Trichosomoides crassicauda, frequently found in rats. The mineralization observed in case of the modified starches did not appear to be associated with formation of kidney or bladder calculi although occurrence of microlithiasis was not excluded. In long-term studies with mice fed two modified starches at a single relatively high dietary level (80 g per kg body weight) a slightly increased incidence of intratubular nephrocalcinosis, concrements in the renal pelvic space and urinary bladder stones was observed in males of the treated groups. No evidence of hyperplasia of the epithelium was found. High early mortality of males in the control group complicated evaluation of the findings.

Although there is no evidence that the use of modified food starches in baby foods has increased the incidence of kidney or ureter calculi in infants or young adults that have eaten such foods, or that other adverse effects have resulted, it would be desirable to undertake studies, in due time, to determine the toxicological significance of the renal alterations observed in animal feeding studies. In any experiments repeated with rats, care should be taken to insure parasite free animals. Because mineral balance in the diet may be
a factor contributing to the renal lesion in rats, this dietary factor deserves consideration. Other species should be studied to find whether they also exhibit similar kidney lesions when fed one or more of the modified starches at levels (g per kg body weight) comparable to, and above, those of infants that receive processed baby foods.

The conditions given for starch treatment in the specifications for several of the modified food starches are insufficient to define the extent of reaction with the respective reagents. It is suggested that limitations be placed on the content of groups introduced by monofunctional reagents as is done in the case of the acetyl and phosphate derivatives.

**Acid-modified starches** (page 21-22 in report)

Hydrolysis of glucosidic linkages occurs during acid modification of starch resulting in molecular fragmentation similar to that which occurs in the production of glucose syrups and maltodextrins. The extent of hydrolysis is limited in the acid-modified starches and is comparable to that of the malto-dextrins, the main difference being that the granular form is retained in the acid-modified starches. No adverse effects were noted in feeding acid-modified starch to 3-day-old pigs nor in 90-day rat feeding tests. The evidence indicates that the acid-modified starches are without hazard as food ingredients.

Based on consideration of the available evidence, the Select Committee concludes that:

There is no evidence in the available information on acid-modified starches 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.

There is no evidence in the available information on acid-modified starches that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from paper and paperboard packaging.

There is no evidence in the available information on acid-modified starches that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are substances migrating to food from cotton and cotton fabrics used in dry food packaging.
Bleached starches (page 22 in report)

The principal change in starch effected by the approved bleaching treatments is the removal of color due to traces of plant pigments. Bleaching treatments also help to reduce the microbiological count in starches. Although no feeding studies with bleached starches have been reported, no adverse effects would be expected because the permitted concentrations of the bleaching agents, all oxidants, are so low that few, if any, carboxyl or carbonyl groups would be introduced to affect the biological properties of bleached starches.

In light of the foregoing, the Select Committee concludes that:

There is no evidence in the available information on bleached starches 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.
Hypochlorite oxidized starches (pages 22-24 in report)

Digestibility and caloric value of starch oxidized with the maximum permitted level of sodium hypochlorite, as determined in rat feeding tests, were similar to those for unmodified starch. No adverse effects were observed in gross pathology or histopathology, hematology, serum chemistry or urinalyses in 90-day feeding tests when the oxidized starch was fed at levels an order of magnitude higher than the per capita consumption of all modified starches as indicated by available consumption data. However, starch treated with about eight times the permitted level of hypochlorite caused diarrhea and marked growth depression and cecal enlargement in rats in 21-day feeding studies, demonstrating the adverse effects of starches containing high levels of oxidized groups. In view of this indication of possible toxicity of starch oxidized with permitted levels of hypochlorite when ingested at high levels of intake, and the lack of long-term chronic toxicity tests and data on actual intake levels, the Select Committee recommends that information on either one or both of the latter be obtained in order to adequately evaluate the safety of hypochlorite oxidized starch as a direct food ingredient. This modified starch is not currently used in infant foods.

On basis of the above considerations, the Select Committee concludes that:

While no evidence in the available information on sodium hypochlorite oxidized starch demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.
Starch acetate (pages 25-29 in report)

Although the in vitro rate of digestion of acetylated starches is lower than that of the corresponding unmodified starch, no difference in digestibility was found in rat feeding experiments with starches containing 2 percent acetyl groups which is near the maximum permitted level of 2.5 percent. No adverse effects were observed in short-term animal feeding studies except for enlargement of the ceca and slight diarrhea in some studies at high intake levels (50 g per kg body weight). Cecal enlargement also was observed in long-term feeding studies with rats and mice but, as in the short-term studies, no cecal tissue abnormalities were found and the enlargement is not considered a significant finding. No adverse effects were noted in fertility, litter size, resorption quotient, preweaning mortality or growth rate of pups in a three-generation rat feeding study of starch acetate. In the 2-year feeding study, suburothelial deposits of calcium accompanied by hyperplasia of the epithelium lining the renal pelvis occurred slightly more frequently in test males at the highest treatment level than in control animals. Slightly increased incidence of intratubular nephrocalcinosis and concrements in the renal pelvic space of males were observed in a 79-week mice feeding study but hyperplasia did not occur. The toxicological significance of the renal changes observed in these studies needs clarification. This modified starch is not currently used in infant foods.

Based on the foregoing considerations, the Select Committee concludes that:

There is no evidence in the available information on starch acetate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is 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.
The nutritional properties of starch sodium succinate, starch sodium octenyl succinate and starch aluminum octenyl succinate are similar to those of unmodified starch as indicated by caloric values and growth rates observed in animal feeding experiments. No adverse effects were noted on growth rate or on hematology in short-term rat feeding experiments. However, these experiments were of less than 90 days duration and no gross or histopathological examinations were made. The evidence available is insufficient to answer questions concerning the possible chronic toxicity of these succinates particularly in view of the lack of information on their consumption levels. The Select Committee considers it desirable to undertake long-term animal feeding studies with these modified starches. Information on consumption levels by the U.S. population is also needed. These modified starches are not currently used in infant foods.

In view of the foregoing, the Select Committee concludes that:

While no evidence in the available information on starch sodium succinate, starch sodium octenyl succinate and starch aluminum octenyl succinate demonstrates a hazard to the public when they are used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.
Because of their close structural relationship, monostarch phosphate, distarch phosphate and phosphated distarch phosphate are considered as a group. Digestibilities of these modified starches were similar to those of the corresponding unmodified starches as measured by caloric values and growth rates determined in rat feeding experiments. No differences were noted between distarch phosphates cross-linked by treatment with trimetaphosphate or phosphorus oxychloride. No significant gross or histological changes or dose-related responses in clinical chemical indices were observed in 90-day rat feeding studies with distarch phosphate or phosphated distarch phosphate. Neither were adverse effects observed in a 3-generation reproduction and lactation study with phosphated distarch phosphate. A possible exception in the 2-year rat feeding study was a kidney abnormality which consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied by calcified patches in the underlying tissues. Incidence of the lesion was not distinctly dose related and, as discussed earlier, is considered to be of doubtful toxicological significance.

Other than the occurrence of the renal changes which are of doubtful biological significance, no adverse effects were found in the long-term feeding experiments with phosphated distarch phosphate. Because of the relationship in structure, the results for this starch derivative would appear also to apply to distarch phosphate. Studies with monostarch phosphate have shown that the phosphorus in the $^{32}$P-labeled starch derivative administered orally to rats is metabolized in a manner similar to the phosphorus in radiolabeled disodium phosphate, a substance evaluated in another report of the Select Committee. Thus, the monophosphate ester group would be expected to be removed in the digestion of phosphated distarch phosphate, presenting fragments of starch chains cross-linked by phosphate linkages such as would be present in distarch phosphate.

In view of the foregoing evidence, the Select Committee concludes that:

There is no evidence in the available information on monostarch phosphate, distarch phosphate, and phosphated distarch phosphate 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.
Although in vitro rate of digestion of acetylated distarch phosphate by pancreatin or porcine intestinal amylase was less than that of unmodified starch, this was not reflected in growth rate or feed efficiency in animals fed diets containing high levels of this starch derivative. Slight diarrhea and increased cecal weights were noted in short-term rat feeding experiments at a dietary level of 60 g per kg body weight, an intake level much greater than the highest indicated current consumption levels. No cecal tissue changes were observed. No significant effects related to treatment with the possible exception of a renal alteration were observed in long-term rat feeding studies of acetylated distarch phosphate and acetylated diamylopectin phosphate. The former derivative was acetylated with acetic anhydride and the latter with vinyl acetate. The renal alteration consisted mainly of focal hyperplasia of the renal papillary and pelvic epithelium accompanied by calcified patches in the underlying tissues. There was not a distinct relationship between incidence of lesions and feeding level for either acetylated distarch phosphate or acetylated diamylopectin phosphate. The toxicological significance of this renal alteration needs clarification.

On the basis of the foregoing, the Select Committee concludes that:

There is no evidence in the available information on acetylated distarch phosphate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is 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.
Hydroxypropyl distarch phosphate (pages 39-42 in report)

A reduction of 7 percent in caloric value as compared with unmodified starch in a 10-day rat feeding test was demonstrated for hydroxypropyl distarch phosphate prepared by treating a phosphate cross-linked cornstarch with 8 percent propylene oxide. However, no reduction in growth rates or feed efficiencies were found in 90-day rat feeding studies with hydroxypropyl distarch phosphate prepared by treatment with 5 or 10 percent propylene oxide. Increased cecal weights were observed in both studies at dietary levels of 20 g per kg but no tissue abnormality was found. The only possible pathologic change noted was calcareous deposits within the renal pelvis and pelvic epithelium in rats fed the starch treated with 10 percent propylene oxide. In a 79-week feeding test with mice, a slightly increased incidence of intratubular nephrocalcinosis, concrements in the renal pelvic space and urinary bladder stones were observed in males. As discussed in the introduction to the opinion on modified starches, this renal alteration is considered to be of doubtful biological importance, but the Select Committee suggests that experiments should be undertaken in due time to clarify its toxicological significance.

As pointed out in the opinion on hydroxypropyl starch, treatment of starch with propylene oxide to introduce hydroxypropyl groups may also result in the formation of propylene chlorohydrin by reaction with chloride ions that may be present. Propylene chlorohydrin has been shown to be mutagenic to Salmonella typhimurium TA 1530 but not to TA 1538. Short-term (22-week) rat feeding experiments have revealed no pathologic changes at dietary levels up to 75 mg propylene chlorohydrin per kg, nor have long-term feeding experiments with hydroxypropyl distarch glycerol shown an increase in tumor incidence in rats. A long-term mice feeding study with hydroxypropyl distarch phosphate containing 4.3 ppm propylene chlorohydrin revealed no increased incidence of neoplastic lesions. Intake of propylene chlorohydrin was about 350 μg per kg body weight. This would be about four orders of magnitude greater than per capita human exposure assuming that hydroxypropylated starches are the only modified starches used in processed foods. In view of the mutagenicity of propylene chlorohydrin in the Ames test, however, the Select Committee suggests that the limits on this residue be reduced to the lowest level consistent with feasible manufacturing practice and that long-term feeding studies be undertaken with graded levels of propylene chlorohydrin to clarify whether the mutagenic activity observed in a bacterial system is an indication of potential mutagenic activity in animals. This modified starch is not currently used in infant foods.

Based on the foregoing considerations, the Select Committee concludes that:

- 71 -
While no evidence in the available information on hydroxypropyl distarch phosphate demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.
Hydroxypropyl starch and oxidized hydroxypropyl starch (pages 42-44 in report)

Incomplete digestibility of hydroxypropyl starch was demonstrated by the isolation of hydroxypropyl maltose in feces of rats fed this modified starch. This residue increased as the degree of hydroxypropylation of the starch was increased. Other experiments in which labeled hydroxypropyl starch was fed indicated that over 90 percent of the hydroxypropyl groups were excreted in the feces. Thus high degrees of substitution as permitted by the present specifications for hydroxypropylated food starches may reduce the digestibility and caloric value of the starch significantly. Usage of starches of high hydroxypropyl content as compared to a more completely digestible starch could result in a significant reduction of caloric value of the food. It is suggested that a limit be placed on the content of hydroxypropyl groups introduced into a hydroxypropylated modified starch.

Hematological findings were normal and no pathological changes were observed in the major organs in 90-day rat feeding studies of starch containing 1.53 percent hydroxypropyl groups. Weight gains were consistently but not significantly lower than controls at dietary levels of 12 g per kg and above, and diarrhea was observed at 35 g per kg. In a similar study with an oxidized hydroxypropyl starch containing 14.9 percent hydroxypropyl groups, no pathological findings were reported. However, growth rate and food efficiency were reduced at dietary levels of 8 and 20 g per kg and the depression in growth rate was significant at the higher dietary level. Diarrhea also occurred at the 20 g per kg level. Because this starch was both oxidized (i.e. carboxyl and carbonyl groups introduced) and hydroxypropylated, the growth depression and diarrhea cannot be attributed solely to either treatment.

In the hydroxypropylation of starch by treatment with propylene oxide, propylene chlorohydrin may be formed by reaction with chloride ions that may be present. Short-term (22-week) experiments in which propylene chlorohydrin were administered to rats by gavage showed no hematological or histopathologic changes in treated animals at dose levels of 75 mg per kg, the highest dose level at which histological examination of rats was made. However, the liver-body weight ratio was increased at the 25 mg per kg dose level for males and at the 75 mg per kg dose levels for both sexes. Propylene chlorohydrin was mutagenic to Salmonella typhimurium TA 1530 but not for TA 1538 when tested in agar containing 1.1 mg propylene chlorohydrin per plate. Present specifications for hydroxypropyl starches permit 5 ppm of residual propylene chlorohydrin in the product. Long-term rat feeding experiments with hydroxypropyl distarch glycerol etherified by treatment of starch with 5 percent propylene oxide showed no increase in tumors as compared to control animals. Three generation reproduction and lactation studies with
the same hydroxypropylated starch revealed no adverse effects. No increase in neoplastic lesions was observed in long-term studies with mice fed hydroxypropyl distarch phosphate at a level providing about 350 µg propylene chlorohydrin per kg body weight. This is four orders of magnitude greater than per capita human exposure assuming that hydroxypropylated starches are the only modified starches used in processed foods. Although no adverse effects have been observed which have been attributed to residual propylene chlorohydrin, the Select Committee suggests that the limits on this residue be reduced to the lowest level consistent with feasible manufacturing practice, and that long-term animal feeding studies be undertaken with graded levels of propylene chlorohydrin to clarify whether the mutagenic activity observed in a bacterial system is an indication of potential for similar activity in animals. This modified starch is not currently used in infant foods.

In view of the foregoing considerations, the Select Committee concludes that:

> While no evidence in the available information on hydroxypropyl starch and oxidized hydroxypropyl starch demonstrates a hazard to the public when they are used at levels that are now current and in the manner now practiced, uncertainties exist requiring that additional studies should be conducted.
Acetylated distarch adipate (pages 45-47 in report)

Studies with radio-labeled acetylated distarch adipate in the rat showed that adipic acid entered the metabolic pool more slowly but followed normal pathways for free adipic acid. Body weight gains were about 15 percent lower than controls but no significant pathological changes were observed in 90-day and 2-year rat feeding studies at dietary levels of 40 and 30 g per kg body weight, respectively. Kidney lesions, characterized by focal hyperplasia of the epithelium lining the urinary space accompanied by calcified deposits in the lining of the epithelium, were observed in both treated and control animals but there was no significant difference in severity or frequency of the lesions in the two groups. The Select Committee considers these lesions to be of doubtful biological importance but suggests that studies be undertaken in due time to determine their toxicological significance. Cecal enlargement occurred in both feeding studies but without associated histopathological change and is considered to have no toxicological significance.

Based on the foregoing evidence, the Select Committee concludes that:

There is no evidence in the available information on acetylated distarch adipate that demonstrates or suggests reasonable grounds to suspect a hazard to the public when it is 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.
Distarchoxy propanols (pages 47-48 in report)

Two modified starches, distarchoxy propanol and acetylated distarchoxy propanol are included in this group. Twenty-eight-day feeding studies with distarchoxy propanol prepared by treatment with up to the maximum permitted level of acrolein showed no adverse effect on weight gains, feed efficiencies or cecal weights when fed at relatively high levels (60 g per kg) compared to probable human consumption. Similar studies with acetylated distarchoxy propanol prepared by acetylation with vinyl acetate and cross-linking with acrolein showed a significant reduction in growth rate and a high incidence of diarrhea with products containing 3 percent or more acetyl groups. However, no adverse effects were observed on reproduction or lactation in dams or weight gains or feed efficiencies of the pups in a two-generation study of the acetylated derivative (2.5 percent acetyl groups) fed at a level of 45 g per kg to the weanling rats. Both generations were fed for 1 year. Thus it appears advisable to limit the acetyl content of these starches to 2.5 percent; this level is consistent with the permitted treatment with a maximum of 7.5 percent vinyl acetate. Some evidence indicates that acrolein, the cross-linking agent used in preparing distarchoxy propanols, is mutagenic indicating that residues of acrolein in the modified starches should be reduced to minimum feasible levels. Long-term feeding studies on distarchoxy propanols also are suggested since those conducted have been limited to 1 year duration and no histopathological or other examinations have been reported. However, it is understood that manufacture of these modified starches was discontinued several years ago.

In view of the foregoing the Select Committee concludes that:

While no evidence in the available information on distarchoxy propanol and acetylated distarchoxy propanol demonstrates a hazard to the public should they be used at former levels and in the manner formerly practiced, uncertainties exist requiring that additional studies should be conducted.
Distarch glycerols (pages 48-56 in report)

Included in this group of modified starches are distarch glycerol, hydroxypropyl distarch glycerol, acetylated distarch glycerol, and succinyl distarch glycerol. No adverse effects were observed in short-term rat feeding studies with distarch glycerols cross-linked by treatment with almost twice the permitted level of epichlorohydrin (0.5 percent as compared to 0.3 percent) and fed at levels (60 g per kg) much greater than probable human intake.

Cecal enlargement appeared to be the only significant change observed in short-term rat feeding studies with hydroxypropyl distarch glycerols in which the modified starch was fed at levels up to 25 g per kg; no significant changes were noted in similar studies with dogs fed at levels up to 4 g per kg. In a long-term rat feeding study weight gain was reduced in females at the highest feeding level but necropsy revealed no change except for cecal enlargement and renal calcification accompanied by focal hyperplasia of the pelvic epithelium. No histological abnormality was associated with the former change and it is considered to have no toxicological significance. The latter condition was most marked in males but was not clearly dose related. A three-generation reproduction study revealed no adverse effects of feeding this modified starch.

Short-term and long-term rat feeding studies with acetylated distarch glycerol revealed no abnormalities in gross or histopathological findings. No adverse effects were observed in a three-generation reproduction test. Growth rate was somewhat reduced in males in the short-term study at the high dietary level fed (40 g per kg) but not at the level (30 g per kg) fed in the long-term study. Kidney lesions characterized by focal hyperplasia of the epithelium lining the urinary space accompanied by calcified deposits on the lining of the epithelium were present in both control and treated animals. The Select Committee considers these lesions observed with both the hydroxypropyl and acetyl distarch glycerols to be of doubtful biological importance but suggests that studies be undertaken in due time to determine their toxicological significance.

A 90-day feeding study of succinyl distarch glycerol showed no abnormalities attributable to diets in blood analyses, gross or histopathological findings. Growth rate was somewhat reduced in males but this result is of doubtful significance in view of the high dietary level (50 g per kg) fed. However, the Select Committee considers that additional information should be obtained on the chronic toxicity of succinyl distarch glycerol or the related modified starch, sodium starch succinate.

Although there is no evidence of adverse effects resulting from unreacted epichlorohydrin or its reaction by-products in the distarch glycerols, the
mutagenic properties of epichlorohydrin and the indication that it induces cancer of the respiratory system in animals on exposure by inhalation, and local sarcomata by subcutaneous injection, suggests that this substance may also be carcinogenic when ingested in food. Although analyses reported for starches cross-linked with epichlorohydrin showed no detectable residue and two long-term feeding studies of the treated starches gave no evidence of carcinogenicity, it would be prudent to discontinue use of such cross-linked starches in foods until animal feeding experiments with graded levels of epichlorohydrin have been carried out. It is understood that industry has voluntarily adopted this precaution.

On basis of the above evidence, the Select Committee concludes that:

The evidence on distarch glycerol, hydroxypropyl distarch glycerol, acetylated distarch glycerol, and succinyl distarch glycerol is insufficient to determine that the adverse effects reported are not deleterious to the public health should they be used at former levels and in the manner formerly practiced.
Sodium hydroxide gelatinized starch (page 56 in report)

No information was available to the Select Committee on the biological properties of starch gelatinized with 1 percent sodium hydroxide. The lack of specification of temperature and time of treatment with sodium hydroxide makes it difficult to assess what chemical changes may occur in addition to the physical disruption of the granule structure. Such specifications should be developed.

Based on the foregoing considerations, the Select Committee concludes that:

In view of the deficiency of relevant biological studies and product specifications, the Select Committee has insufficient data upon which to base an evaluation of starch gelatinized with sodium hydroxide when it is used as a food ingredient.
VI. REFERENCES CITED


- 80 -


172. Memorandum dated 28 March 1977, from N. Nelson, New York University Medical Center, Institute of Environmental Medicine, New York, to J.F. Finklea, NIOSH, Washington, D.C.

Fertil. 31:267-273.

175. Smyth, H.F., C.P. Carpenter, C.S. Weil, U.C. Pozzani, and
J.A. Striegel. 1962. Range-finding toxicity data: list VI. Am.

Detection of carcinogens as mutagens in the Salmonella/microsome
test: assay of 300 chemicals. Proc. Natl. Acad. Sci., USA 72:5135-
5139.

which have been tested for carcinogenic activity: 1972-1973 volume.
DHEW No. (NIH) 75. U.S. Government Printing Office, Washington,
D.C.

178. Flavor and Extract Manufacturers' Association of the United States.
1974 (as supplemented December, 1975). Scientific literature review
of aliphatic primary alcohols, esters, and acids in flavor usage.
Section IV. Presence and levels of reviewed substances in foods.
A. Natural occurrence. Prepared under Contract No. 73-162 with
the Food and Drug Administration, Department of Health, Education,
and Welfare, Washington, D.C.

179. Shubik, P. and J.L. Hartwell. 1969. Acetaldehyde, page 34 and
acrolein, page 35 in Survey of compounds which have been tested for
carcinogenic activity: supplement 2. PHS Publication No. 149.

by L. Fishbein. 1977. Structural parameters associated with
carcinogenesis (halogenated olefins, vinyl and allyl analogs and
epoxides). Pages 8-21 in I.M. Asher and C. Zervos, eds. Structural
correlates of carcinogenesis and mutagens. Proceedings of the
second summer symposium. The Office of Science, Food and
Drug Administration, Washington, D.C.

181. Bignami, M., G. Cardamone, P. Comba, V.A. Ortoli, G. Morpurgo,
and A. Carere. 1977. Relationship between chemical structure and
mutagenic activity in some pesticides: the use of Salmonella typhi-
murium and Aspergillus nidulans. Mutat. Res. 46:243-244.

182. Steiner, P.E., R. Steele, and F.C. Koch. 1943. The possible
carcinogenicity of overcooked meats, heated cholesterol, acrolein,
and heated sesame oil. Cancer Res. 3:100-107.

- 97 -
183. Dickens, F., and H.E.H. Jones. 1965. Further studies in the
carcinogenic action of certain lactones and related substances in the

and Chemical Corporation, Bridgewater, N.J. in Attachment M in
Submission dated September 15, 1976 to Federation of American
Societies for Experimental Biology, Bethesda, Md.

Food and Drug Research Laboratories, Inc., Maspeth, N.Y. Sub-
mitted to National Starch and Chemical Corporation, Plainfield, N.J.
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. La Du, 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 decisions 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

The Select Committee expresses its appreciation to the following organizations who contributed information and data:

Corn Refiners Association, Inc., 1001 Connecticut Ave.,
Washington, D.C. 20036.

National Starch and Chemical Corporation, 10 Finderne
Ave., Bridgewater, N.J. 08807.

A. E. Staley Manufacturing Company, 2200 Eldorado St.,
Decatur, Ill. 62525.

Corn Products, Moffett Technical Center, P.O. Box 345,
Argo, Ill. 60501.

Stein, Hall and Co., Inc., 605 Third Ave., New York,
N.Y. 10016.

American Maize-Products Company, 113th Street and
Indianapolis Blvd., Hammond, Ind. 46326.

Centraal Instituut voor Voedingsonderzoek, Utrechtseweg
48 Zeist, The Netherlands.

Report submitted by:

July 30, 1979

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