EVALUATION OF THE HEALTH ASPECTS OF BILE SALTS AND OX BILE EXTRACT AS FOOD INGREDIENTS

1975

Prepared for

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

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

This report is one of a series of evaluations of the health aspects of the Generally Recognized as Safe (GRAS) or prior sanctioned food substances 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.

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Life Sciences Research Office
FASEB
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I. INTRODUCTION

This report evaluates the health aspects of using bile salts and ox bile extract as food ingredients. The evaluation 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 1970.* 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 recognized as available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register of March 24, 1975 (40 FR 13016 and 13017) that opportunity would be provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using bile salts and ox bile extract as food ingredients. The Select Committee received no requests for such a hearing on bile salts and ox bile extract.

As indicated in the Food, Drug and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarking clearance that is required for food additives. It is stated in 21 CFR 121.1, revised April 1, 1974 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. This section of the Code also indicates 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 recognizes further (21 CFR 121.3) that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO is making its evaluations of these substances in full recognition of the foregoing provisions. In reaching its conclusions on safety the Select Committee, in accordance with FDA's guidelines, is relying primarily on the absence of substantive

*The document is available from the National Technical Information Service, U.S. Department of Commerce, P.O. Box 1553, Springfield Virginia 22161.
evidence of, or reasonable grounds to suspect, a significant risk to the public health, and realizes that a conclusion based on such reasoned judgment is expected even in instances where the available information is qualitatively or quantitatively limited. The Committee, aware that biological testing is dynamic, bases its conclusions on information now available; it cannot anticipate the results of experiments not yet conducted or those of tests that may be re-conducted, using new technologies. These 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 bile salts and ox bile extract 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

Ox bile extract, also known as purified oxgall and sodium choleate, is obtained by evaporating the alcohol extract of concentrated bile. It is a mixture of varying amounts of the salts of the bile acids, lipid materials such as cholesterol and lecithin, choline compounds, glycocol and other substances (1). The bile acids, which occur as sodium salts in the bile of most vertebrates, are derivatives of the steroid cholic acid. Cholic acid is 3, 7, 12 trihydroxycholanic acid; desoxycholic acid is 3, 12 dihydroxy-5-cholanic acid; glycocholic acid is cholyglycine; and taurocholic acid is choliyltaurine. The structures of these acids are as follows (2):

![Cholanic acid structure](image-url)
Bile salts and hence bile extract, are useful in some foods because of their emulsifying properties. Cholic acid, desoxycholic acid, glycocholic acid, taurocholic acid, and bile extract are "generally recognized as safe" when used as emulsifying agents at a level in food not exceeding 0.1 percent [21 CFR 121.101(d)(3)]. The Food Chemicals Codex (3) provides specifications for food grade cholic acid and desoxycholic acid; both should assay not less than 98.0 percent of the acid and neither should contain more than 3 ppm of arsenic, 10 ppm of lead, and 40 ppm of heavy metals as lead.

A survey of the food industry performed by a subcommittee of the National Research Council (4) indicates that ox bile extract is usually used to the extent of 0.0009 percent (weighted mean) in cheese. No other foods
are listed as containing added bile extract and there is no indication that any of the individual bile acids or their salts are used in foods.

III. CONSUMER EXPOSURE DATA

The survey by the National Research Council subcommittee has also provided information on the possible daily human intake of ox bile extract for individuals in various age groups as shown in Table I (4). The Select Committee has converted these figures to possible intakes per kilogram of body weight. Daily intake data are not available for the individual bile acids or their salts.

TABLE I

Possible Average Daily Intake of Added Ox Bile Extract by Age Group (4)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Intake</th>
<th>mg</th>
<th>mg/kg&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-5 mo</td>
<td>***&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-11 mo</td>
<td>0.024</td>
<td>0.003</td>
<td></td>
</tr>
<tr>
<td>12-23 mo</td>
<td>0.070</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>2-65+ yr</td>
<td>0.085</td>
<td>0.001</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Possible daily intake of added ox bile extract by age group estimated from weighted means of the levels of addition in cheese. Calculated intake, mg/kg body weight, based on an average weight of 60 kg for an adult (5); and the following estimated weights of infants by age groups: 0-5 mo, 5 kg; 6-11 mo, 8 kg; 12-23 mo, 11 kg (6).

<sup>2</sup>Asterisks (***<sup>2</sup>) in the table mean that there were insufficient data on which to base an estimate.
It is recognized that the figures calculated for the daily intake of ox bile extract per kg of body weight in the age group 2-65+ years could be low for some, since the majority of individuals from age 2 to maturity will probably weigh less than 60 kg; thus the daily intake of ox bile extract for children could be severalfold higher than the figures indicated.

However, such a possible deviation from the figures in Table I must be considered in the light of the NRC subcommittee's statement that its calculations of intakes in most cases are overstated, often by considerable margins.* That the intakes of ox bile extract indicated in Table I are probably overstated is borne out by comparison with intake figures calculated from the amount of ox bile extract used annually in food in the United States (4). The food industry reported the use of 470 pounds of ox bile extract in 1970 which the NRC subcommittee regards as about 60 to 70 percent of the total actually used in that year. Assuming 60 percent and a U.S. population of 210 million, the per capita daily intake would be about 4 μg rather than the 85 μg indicated in Table I. On the basis of these considerations, the Select Committee regards the figures in Table I as levels that are not likely to be achieved by any of the age groups.

The Joint FAO/WHO Expert Committee on Food Additives has established the acceptable daily intake for man of cholic and deoxycholic acid and their salts as 0 to 1.25 mg per kg body weight (7).

IV. BIOLOGICAL STUDIES

Absorption, metabolism, excretion

Bile acids are formed in the liver from cholesterol, conjugated with taurine and glycine, excreted in bile, and deconjugated by intestinal flora; 90-95 percent are reabsorbed via the portal circulation (8). Bile acids are absorbed in the cecum of rats (9), lower small intestine of

*An explanation for such overstatements is detailed in Section XI, "Significance and Use of Data in Safety Evaluations," of the NRC subcommittee's report (4). The Select Committee finds this explanation reasonable, and concurs in the first recommendation in Section XII of the same report, that "In order to conduct a more accurate survey of the intake of substances used in food processing, food consumption data collected specifically for this purpose are needed."
guinea pigs (10) and man (11), and the large bowel of man (12). The entero-
hepatic circulation conserves most of the bile acids secreted (12). Bile
acids are excreted only in the feces (8).

The reported mean half-life of cholic acid in man on a regular diet,
based on feeding cholic acid-24-\textsuperscript{14}C, was estimated to be 2.8 days (13). The
half-life and the size of the cholic acid pool were found to be dependent on
the amount of fat in the diet. In later studies, conducted on a single subject,
cholic acid half-life was found to be 8.8 days on a butterfat diet, 3.7 days on
a corn oil diet and 4.2 days on a fat-free diet (14). The cholic acid pool
sizes were 1.67 g, 1.49 g, and 1.59 g, respectively.

The bile acids are strongly bound to plasma albumin, cholic acid
being more avidly bound than its taurine conjugate. There is reported to be
one primary binding site per albumin molecule and several sites of weaker
affinity (15). In diets rich in cholesterol, cholic acid or glycocholic acid,
but not desoxycholic acid, enhance the absorption of the cholesterol in rabbits.
This results in a hypercholesterolemia. Although the precise mechanism is
unknown, cholic acid stimulation of the esterification of cholesterol in the gut
wall was suggested (16).

**Acute toxicity studies**

From a summary of some of the acute toxicity data on cholic acid
and its derivatives (Table II), desoxycholic acid appears to be comparati-
vely more toxic than cholic acid, although the oral LD\textsubscript{50} is greater than
1 g per kg for both. The signs of intoxication produced by cholic acid and
its derivatives are similar. Bronchoconstriction, probably related to
histamine release, was most prominent following the intravenous adminis-
tration of desoxycholic acid to cats, glycocholic acid to guinea pigs and
cats, and taurocholic acid to cats.

**TABLE II**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oral LD\textsubscript{50} (17) (mg/kg)</th>
<th>Intravenous LD\textsubscript{50} (18) (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholic acid</td>
<td>1520</td>
<td>350</td>
</tr>
<tr>
<td>Desoxycholic acid</td>
<td>1060</td>
<td>150</td>
</tr>
<tr>
<td>Glycocholic acid</td>
<td>No data</td>
<td>370</td>
</tr>
<tr>
<td>Taurocholic acid</td>
<td>No data</td>
<td>330</td>
</tr>
</tbody>
</table>
Chronic toxicity studies

a. Cholic acid - Chicks fed 0.34 percent cholic acid in the diet (estimated to be about 400 mg per kg body weight per day) for 14 days exhibited a slightly greater weight gain than control animals (19). A level of 0.2 percent cholic acid in the diet of chicks for four weeks caused no adverse effects other than a slight increase in fat absorption (20). There was a temporary decrease in egg production after feeding 0.2 percent cholic acid to laying hens (21).

Lithocholic acid (3-hydroxycholanic acid) increased liver size and raised plasma cholesterol when fed as a hypercholesterolemic agent at a level of 0.2 percent in the diet (about 250 mg per kg of body weight) to growing chicks (22). These effects were partially reversed when cholic acid was fed.

Cholic acid fed to female mice at levels up to 1.0 percent (up to about 1,500 mg per kg of body weight) for 8 months caused cholesterol stones and gall bladder lesions (23). The effects appeared to be dose related.

Male Goffmoor rats fed cholic acid at a level of about 250 mg per kg of body weight for 26 days had smaller body weights and exhibited, at autopsy, increased heart weight and decreased liver weight per 100 g body weight (24). Weanling male Holtzman rats fed 0.1 and 2.0 percent of sodium cholate (about 100 and 2,000 mg per kg of body weight, respectively) for periods up to 28 days developed diarrhea. Rats at the 0.1 percent level showed no growth depression. However, rats fed at the 2 percent level had a high mortality (none survived longer than 21 days) and at autopsy, hyperemia of the distal small intestine was observed (25).

b. Desoxycholic acid - Chicks fed up to 0.2 percent desoxycholic acid (about 200 mg per kg of body weight per day) in the diet for as long as 21 days did not present adverse signs (19, 26) and the chicks fed 0.1 percent showed a slightly greater weight gain than control animals (19).

Holtzman-Rolfsmeyer male and female rats fed 100 mg of desoxycholic acid per rat per day (about 1 g per kg of body weight) for one to four weeks achieved higher serum cholesterol levels than did control animals. Desoxycholic acid was found to be much more toxic for male rats than for females (27). In other studies, male rats received 0.15 percent desoxycholic acid in the diet (about 150 mg per kg of body weight per day) for up to four weeks without adverse effects (24, 28). In a longer term study, male Wistar rats were fed diets containing 0.005, 0.025, or 0.125 percent desoxycholic acid (about 3 to 80 mg per kg of body weight) for 20 months without adverse effects (29).
c. Glycocholic acid - Growth of male Wistar rats on an essential fatty acid-deficient diet was impaired (about 66 percent of the controls) when 1.0 percent of sodium glycocholate (about 1 g per kg of body weight per day) was added to the diet for five weeks (30).

d. Taurocholic acid - Male Holtzman rats received 0.1 and 2.0 percent of sodium taurocholate in their diets for 28 days. The 0.1 percent level diet (about 200 mg per kg of body weight) appeared to stimulate growth, whereas the 2.0 percent level depressed growth (25).

e. Ox bile - Male Holtzman rats were administered 0.5 and 1.0 percent ox bile in their diet for 28 days to study the effects on efficiency of food utilization. Weight gain was about 70 percent of the controls at the 0.5 percent level, and about 50 percent of the controls at the 1.0 percent level (25). The mechanisms responsible for the growth depression were not reported; the authors did not suggest that ox bile was toxic at these levels.

Special studies

a. Carcinogenesis - Cholic acid fed to rats at a level of 2.0 percent (about 1 g per kg of body weight) for 8 months was not tumorigenic (31). Cholic acid or desoxycholic acid fed to rabbits at a level of 100 mg per animal daily (about 50 mg per kg of body weight) for 90-120 days and to mice at a level of 20 mg daily (about 1 g per kg of body weight) for 84 days produced no evidence of tumorigenicity (32). Desoxycholic acid (a total of 70 mg in 15 injections in 300 days) injected intramuscularly in sesame oil resulted in malignant tumor development in mice (33). However, male Wistar rats fed up to 0.125 percent desoxycholic acid in the diet (about 80 mg per kg of body weight) for 20 months did not develop malignant tumors nor did the rats or C3H mice receiving subcutaneous implants of pellets averaging 16.5 mg of desoxycholic acid (29). Chicks fed 0.25 percent cholic acid (about 300 mg per kg of body weight) in their diet for 16 days developed three times as many tumors as control chicks following the injection of Rous sarcoma virus (34). In these experiments tumor response was also stimulated by folic acid, nicotinamide, pantothenate, and riboflavin in the diet, and the significance of these data with respect to the possible carcinogenicity of cholic acid is not clearly demonstrated.

b. Interaction with cholesterol - Male rats fed a diet containing 5 percent cholesterol and 2 percent sodium cholate (about 2 g of sodium cholate per kg of body weight) for 35 weeks developed hypertension and hyperlipemia. Occurrence of multiple thrombi in small vessels of the heart and aorta, and nephrotic lesions were considerably increased in comparison with controls receiving no added cholesterol or cholate (35).
Young weanling rats fed a diet containing 1 percent cholesterol and 0.3 percent cholic acid (about 300 mg of cholic acid per kg of body weight) for 28 days showed a decreased weight gain compared with control rats. At autopsy, these rats exhibited sudanophilia of the left ventricular valves and aorta and edematous kidneys. The sudanophilia was believed to reflect a condition where entrapped leukocytes contained minute fat droplets that stained red with the Sudan IV tissue stain (36).

Cebus monkeys fed casein diets containing 5 percent cholesterol and 1 percent sodium cholate (about 500 mg per kg of body weight) for a year lost weight and developed hypercholesterolemia and aortic atherosclerosis (37). However, in these experiments other animals, consuming diets containing other proteins, also became hypercholesterolemic and atherosclerotic whether or not cholic acid was fed.

Mice fed a diet containing 1 percent cholesterol and 0.5 percent desoxycholic acid and/or 0.5 percent cholic acid for 4 months, developed cholesterol gallstones, fatty degeneration of the liver and increased liver and serum cholesterol levels (38). Apparently, control animals on diets without added bile acids were not studied. Similar findings were later reported for the mongolian gerbil (39).

Tepperman et al. fed mice that had previously received a single intraperitoneal injection of 0.8 mg of aurothioglucose, on a diet containing 1 percent cholesterol and 0.5 percent cholic acid for 9 months. These animals developed gallstones and fatty livers. Gallstones developed only under the following conditions: cholesterol and cholic acid had to be fed simultaneously, food intake had to be sufficiently high and liver function had to be unimpaired (40).

Male Wistar rats on a diet containing 1 percent cholesterol and 0.4 percent cholic acid (about 180 mg per kg of body weight) for 11 weeks developed yellow livers, hypercholesterolemia, and adrenal enlargement at 5 weeks (41). In another study, Wistar rats fed a 1.29 percent cholesterol - 1.0 percent cholic acid diet (about 500 mg per kg of body weight) for 10 weeks developed hypercholesterolemia and elevated liver lipid levels (42).

The feeding of a 1 percent cholesterol - 0.5 percent cholic acid diet to hamsters for 4 months produced fatty livers, gallstones, enlarged spleens, fatty renal changes, and cholesterol crystals in the adrenal cortex, lung, spleen, and lymph nodes (43).

c. Other studies - Cholic acid in doses of 0.25 g three times a day for one week served as an effective cathartic in four of five female patients in a controlled, single blind study (44). The fifth patient did not
respond to this dose but obtained a cathartic effect at a total dose of 2.25 g per day. With human subjects it was estimated that the effective dose is 20 mg per kg per day, which is approximately twice the daily synthesis rate of cholic acid in a healthy person.

Bile acids have been reported to control and suppress appetite in obese humans (45). Desoxycholic acid or cholic acid was taken in three daily doses totalling 1200 mg per day for two weeks and the body weights of the nine patients were compared with placebo administration trials. Desoxycholic acid decreased the desire for food and caused some weight loss, but cholic acid was without effect. Because bile is essential for normal digestion and absorption, ox bile extract was formerly prescribed for patients with digestive disorders in doses of 300 mg two or three times a day (46). Ox bile extract is not commonly prescribed in modern medical practice.

V. OPINION

The amounts of the bile acids ingested as constituents of ox bile extract used as a food additive are relatively insignificant, compared to the amounts normally present in the bile of man. The available information indicates that orally administered cholic acid, desoxycholic acid, glycocholic acid, and taurocholic acid are readily absorbed and excreted without accumulation. They exhibit a relatively low toxicity for several animal species tested.

The Select Committee has no information to indicate that the individual bile acids or their salts are used in foods. However, the intake estimates available for ox bile extract, of which bile acids and their salts are major constituents, indicate that average daily consumption of ox bile extract added to foods is small, amounting to 0.1 mg or less. The intake of individual bile acids or their salts would be, commensurately, very small. Such biological effects as have been reported in animal studies, have been elicited at levels of administration that are several orders of magnitude greater than the levels to which man is now exposed in his daily diet.

In the light of the information contained in this report, the Select Committee concludes that:
There is no evidence in the available information on ox bile extract, or its constituents - cholic acid, desoxycholic acid, glycocholic acid, and taurocholic acid - 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 future.
VI. REFERENCES CITED


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June 19, 1975

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