Approximately 80 percent of the fatty acids in soybean lecithin is unsaturated, the bulk coming from linoleic acid (72). This lecithin is commonly treated with hydrogen peroxide to produce bleached preparations widely used commercially. The so-called "bleached lecithin," "double bleached lecithin," and "hydroxylated lecithin" all employ hydrogen peroxide in their preparation. The "double bleached" and "hydroxylated" lecithins utilize benzoyl peroxide as an additional bleaching agent. For preparation of hydrogen peroxide bleached lecithin, 0.05 to 2.0 percent of 35 percent hydrogen peroxide is added to a lecithin emulsion (20 to 50 percent water) at 60° to 93°C and the moisture removed by heating under reduced pressure. Typically, 1.2 percent of 35 percent hydrogen peroxide is added, based on lecithin dry weight. Bleached lecithins have peroxide values ranging from 30 to 90 meq per kg (1.0 to 3.0 g per kg) (73). The estimated intake of lecithins modified by hydrogen peroxide (based on poundage data) is 4 mg daily (13) or a daily intake from this source of less than 0.1 mg peroxide. Hydroxylated lecithin with peroxide values of 52 meq per kg was fed to weanling Sprague-Dawley rats at levels of 5 and 10 percent of their diet. The 10 percent group received about 17.0 mg peroxide per kg body weight daily and was maintained on this diet for 8 weeks. The rats on the 5 percent levels (8.5 mg per kg) were kept for 52 weeks. Weight gains were normal for both groups and no abnormalities were detected in the vital tissues (74). Verrett (75) found no increase in teratogenic effects in the developing chick embryo when 10 to 200 mg per kg egg weight of double bleached lecithin were administered in the air cell at 0 and 96 hours or via the yolk at the same time intervals.

The effect of feeding epoxidized oil was investigated by Kieckebusch et al. (76). Soybean oil was epoxidized with hydrogen peroxide and fed to rats at various levels. The toxicity of the treated oil increased with increasing epoxidation, presumably due to the action of diepoxystearic acid produced from linoleic acid, which comprises the bulk of the unsaturated fatty acids in soybean oil. Male rats receiving the equivalent of about 250 mg per kg diepoxystearic acid daily for 8 weeks gained 8 percent less than the control animals. When this amount was tripled (750 mg diepoxystearic acid per kg daily) 80 percent of the rats died within 8 days. The investigators estimated a tolerance level for diepoxystearic acid of 380 mg per kg per day. The greatest changes in the rats receiving the larger dose were a marked increase in water consumption per unit body weight, and testicular atrophy. The investigators speculated that the observed signs might be manifestations, at least in part, of an essential fatty acid deficiency rather than direct epoxide toxicity.

Epoxy acids are natural constituents of some seed oils, including 9:10 epoxystearic acid (77), cis 12:13 epoxyoleic acid (78), cis 9:10 epoxy octadec-12-enoic acid (79), and cis 15:16 epoxylinoleic acid (80). Thus, the monoepoxides of oleic, linoleic, and linolenic acids are all natural constituents of seed

oils. Smith et al. (79) speculated that epoxy acids may be widely distributed in the plant kingdom, derived biogenetically through the action of specific epoxidases. The most abundant natural source of epoxy acids appears to be Vernonia anthelmintica, whose oil contains about 69 percent of 12:13 epoxyoleic acid. Chalvardjian and coworkers (81) fed male, weanling Sprague-Dawley rats a diet containing 10 percent of the Vernonia oil, representing an estimated epoxy acid consumption of approximately 7 g per kg body weight per day. The growth curves of these rats were not significantly different from those of control rats receiving olive oil. The animals were killed after 28 days and the tissues examined. No gross or histological damage was detected.

The epoxides of some of the fatty acids have been tested for carcinogenicity. Van Duuren (82) reported 9:10 epoxystearic acid (from oleic acid) and 9:10, 12:13 diepoxystearic acid (from linoleic acid) to be noncarcinogenic when applied on the skin of mice. The former compound was also shown noncarcinogenic after subcutaneous injection in the mouse and rat (83). Swern et al. (84), however, reported the production of six sarcomas at the injection sites of 70 mice (BALB/C and Swiss) receiving epoxystearic acid subcutaneously. They considered the compound to be "marginally active" as a carcinogen. No reports are available on the carcinogenicity of those epoxides that might conceivably be produced from the small amounts of other unsaturated fatty acids in the diet (palmitoleic, linolenic, arachidonic).

Van Duuren et al. (83) points out that even those epoxides found to be carcinogenic are effective only at large doses, and that none has induced gastric tumors when given by mouth or gavage. Seelkopf and Salfelder (85) fed epoxy- and diepoxystearic acid to Holtzman rats and to C57BL/6 mice for 3 to 5 months at daily dose levels varying from about 50 to 150 mg per kg body weight. None of the 45 rats or 41 mice on the monoepoxide diet which survived the test period developed gastric tumors, nor did any of the 31 mice surviving the diepoxystearic acid regimen. One rat (of 46) receiving diepoxystearic acid developed a gastric carcinoma and one of 29 control mice had an early "pregastric" carcinoma.

Van Duuren et al. (83) attributed the relative tolerance of animals to orally administered epoxides to their rapid degradation in vivo, especially in the stomach where acid-catalyzed hydrolysis would be expected. The extent of such epoxide hydrolysis in the stomach is unknown, but the study by Chalvardjian et al. (81) indicates that complete destruction is unlikely when large amounts are fed. These investigators detected epoxyoleic acid in the tissues and feces of rats fed Vernonia oil. No quantitative analyses were performed, but some of the epoxy acid from the oil obviously survived contact with the hydrochloric acid of the stomach. Little is known of the action of the intestinal contents upon the epoxide fraction surviving hydrolysis in the

stomach. Ivie (86) recently reported that epoxides are reduced to their corresponding olefins in ruminal fluids and suggested that such bacterial action in the intestine may represent a significant detoxifying mechanism in mammals. Once absorbed, epoxides are subject to enzymatic action in the liver with conversion to their vicinal glycols (87).

Lipid peroxides are also poorly absorbed from the intestinal tract. Andrews et al. (68) and Glavind and Tryding (88) failed to detect peroxides in the lymph of rats after feeding lipoperoxides and only very small amounts could be recovered in the feces. Since pancreatic juice, bile, and lymph had little effect on the peroxides, the latter investigators suggested that the essential site of peroxide destruction was the intestinal mucosa.

In addition to acting upon unsaturated fatty acids, hydrogen peroxide might be expected also to form oxidation products with sterols. The structure, concentration, and significance of such oxidation products remain largely unknown because of the complexity of the resulting mixture. In a recent symposium, it was stated that about 50 oxidation products of cholesterol had been identified, but an additional 50 compounds were possible theoretically (89). Claims that the oxidized derivatives of cholesterol are carcinogenic have focused chiefly on 5,6 α-epoxy-5-cholesten-3β-ol (cholesterol-(α)-oxide) as the putative agent (90). Bryson and Bischoff (91) reported this compound to be carcinogenic in Evans rats and in Marsh mice following subcutaneous injection, but inactive upon intraperitoneal injection. Seelkopf and Salfelder (85), however, found no increase in tumor frequency above that of control groups among 45 Holtzman white rats of both sexes or in an equal number of C57BL/6 mice fed 50 to 150 mg per kg body weight of cholesterol-(α)-oxide daily for 3 months. Thirty-three rats and 27 mice survived the test period. Smith and Kulig (92) obtained a yield of 0.2 percent of cholesterol-(α)-oxide upon treatment of cholesterol (1 mg per ml) for 6 hours at 50°C with 0.015 percent hydrogen peroxide. From the cholesterol content of milk (0.15 percent) about 0.3 mg cholesterol-(α)-oxide per liter of milk theoretically could be produced by this treatment.

Recent evidence has indicated that some oxidative products of cholesterol may also have angiotoxic or atherogenic effects (93,94). Preliminary experiments suggest that large amounts of the active products are necessary to induce these changes. A concentrate of products of cholesterol oxidation given by gavage to rabbits at levels of 250 mg per kg body weight increased the frequency of dead smooth muscle cells of the aorta (93).

Carbohydrates. The Subcommittee is not aware of any toxic substance produced by the treatment of carbohydrates with hydrogen peroxide. Hydrogen peroxide is known to oxidize simple aldehydes to the corresponding acids and is employed in wine production for this purpose (6). In the presence of ferrous salts

it will oxidize aldoses and ketoses to their corresponding osones (95) and with high concentrations, it will degrade carbohydrates stepwise to products containing one less carbon atom (96).

Proteins. Modification of protein structure by hydrogen peroxide treatment has been discussed in an earlier section. There is no evidence that the altered protein is toxic (56). Treatment of fish protein with 20 to 80 g hydrogen peroxide per kg dry weight at 50°C for 2 hours oxidized small amounts of methionine and cystine to methionine sulfoxide, methionine sulfone, and cysteic acid (61). Methionine sulfoxide was as effective nutritionally as methionine in rat feeding experiments. Methionine sulfone and cysteic acid were ineffective nutritionally but had no apparent toxic effect.

Alarcon (97) demonstrated that acrolein, a volatile toxic unsaturated aldehyde, can be produced by vigorous treatment of various amino acids and polyamines with hydrogen peroxide. The reaction was carried out at 100°C for 10 to 60 minutes with 20 mmoles hydrogen peroxide and 5 mmoles of amino acid. Less drastic treatment was not reported.