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Title of research project	Safety assessment studies of glycidol fatty acid esters and 3-MCPD fatty acid esters
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【Abstract】

Glycidol fatty acid esters (GEs) have been recently identified as food process contaminants in refined edible oils. Although there is toxicological concern arising from potential release of glycidol from parent esters during digestion in the gastrointestinal tract, little is known about in vivo toxicity of GEs. We conducted 1) 13-week rat subchronic toxicity study and 2) three kinds of in vivo genotoxicity studies on two types of GEs, glycidol oleate and linoleate esters.

The subchronic toxicity of GEs was investigated with administration at concentrations of 0, 225, 900 and 3600 ppm (equivalent molar concentration to 800 ppm glycidol) in drinking water with 0.03% Tween 80 for 13 weeks to male and female F344 rats. For comparison, animals were also treated with 200 and 800 ppm of glycidol. Body weight gain of both sexes was markedly reduced with 800 ppm glycidol compared to the controls, and the cause was considered at least partly related to decreased water consumption. Hematological data showed significant increase of MCV in females received 800 ppm glycidol and decrease of WBC in females treated with 3600 ppm oleate ester. In serum biochemistry, increase of total cholesterol and potassium and decrease of ALT were detected in males treated with 800 ppm glycidol, males treated with 3600ppm linoleate ester and females treated with 800 ppm glycidol, respectively. Serum creatinine levels in both sexes were decreased in the group treated with 800 ppm glycidol .

Relative weights of kidney and spleen were significantly increased in males treated with 200 and females treated with 800 ppm glycidol. Increase of relative kidney weights was also found in males treated with 3600 ppm oleate ester, without any related toxicological changes. On histopathological assessment, increased cell debris was observed in the epididymal ducts of males treated with 800 ppm glycidol , but not in groups treated with ester.

No genotoxicity was observed in the micronucleus assay using bone marrow, the Pig-A assay on the peripheral blood cells, and the gpt gene mutation assay andspi- assay with the cerebrum and liver tissues of the gpt delta rats treated with these esters at the highest concentration of 3600ppm for 4 weeks. On the other hand, weak in vivo genotoxicity of glycidol was observed in the liver from the glycidol-treated rats by the gpt assay showing significant increase of GC-TA transversion.

Therefore, under the present experimental condition, glycidol oleate and linoleate esters did not show the

genotoxicity, and showed less toxicity compared with glycidol in the 13-week subchoronic toxicity test in F344 rats.]

3-Monochloropropane-1,2-diol (3-MCPD) esters are known tobe generated in various foods and food ingredients as a result of food processing. On the other hand, their hydrolytic product, 3-MCPD is regarded as a rodent renal and Leydig cell carcinogen. Since reports about toxicity of these compounds are limited, we conducted the following two studies. 1) In vivo genotoxicity studies (micronucleus assay, Pig-A assay and gpt gene mutation assays in the kidneys and testes) with male F344 gpt delta transgenic rats carrying reporter genes for mutations, treated by gavage with a carcinogenic dose of 3-MCPD (3.6 x 10-4 mol/kg body weight) or its esters, i.e., 3-MCPD palmitate diester (PD), 3-MCPD palmitate monoester (PM) and 3-MCPD oleate diester (OD) in a suspension of olive oil at the molar equivalents for 4 weeks. 2) 13-Week subchronic toxicity study of these compounds with F344 rats at three doses where the highest was the same as that in the genotoxicity study.

In the in vivo genotoxicity assays, no significant change was observed in the rat treated with 3-MCPD or its esters.

In the 13-weeks study, the absolute and relative weights of the kidney were significantly increased in rats treated with 3-MCPD at a carcinogenic dose and the esters at high and medium doses. Relative weights of the liver were significantly increased in the 3-MCPD-treated rats and the high dose ester-treated rats, except for female rats treated with PM. By 4 weeks from the start of the treatment, 1 male and 5 females of 3MCPD group died from the renal tubular necrosis. On histopathological analysis, a significantly increased incidence of apoptotic epithelial cells in the epididymis of 3-MCPD treated and high dose ester-treated male were observed as compared to vehicle control. The results suggest 3-MCPD fatty acid esters may be non-genotoxic but have potential toxicity for kidneys and epididymes of rats. NOAELs of PD, PM and OD were suggested to be 14, 8 and 15 mg/kg/day, respectively.

In order to clarify the bioavailability of glycidol or/and 3-MCPD converted from 3-MCPD fatty acid esters by hydrolysis, two metabolism studies were also performed.

For the absorption analysis, glycidol, glycidol esters, 3-MCPD or 3-MCPD esters were inoculated to 9-week-old male F344 rat by gastric tube with the same vehicle used in the repeated dose studies at the concentration that each esters were in the same molar of their hydrolyzed form. Thirty minutes after inoculation, whole blood samples were collected from the rats under isofulurane anesthesia and their serum were obtained immediately then stored at -80 ºC until analysis. For the hydrolysis analysis, glycidol, glycidol esters, 3-MCPD or 3-MCPD esters were incubated with one of the stomach-, duodenum- or cecum-contents obtained from untreated 10-week-old male F344 rat for 30 minutes at 37 ºC. The concentrations of glycidol and 3-MCPD were analyzed with GC-MS and those of esters were analyzed with LC MS/MS. The stable isotopes (glycidole-d5, 3-MCPD-d5, glydicole palmitate ester-d31, 3-MCPD-d5- palmitate monoester, 3-MCPD-d5- palmitate diester) were used as an internal standard for the error collection.

Metabolism studies indicated that not only glycidol but also 3-MCPD was detected in the rat serum which orally administered glycidol. Glycidol and 3-MCPD were also detected in the serum of rat given glycidol linoleate ester. 3-MCPD was detected in the serum of the rat which either 3-MCPD, PM, PD or OD was orally administrated. In the present condition, the serum level of 3-MCPD was higher than that of glycidol in rats. Since water-soluble vehicle including 0.03% Tween 80 carrying ester-bonds was used for glycidol and its esters analysis and oil-soluble vehicle was used for 3-MCPD and its esters analysis, the difference might be related to the induction and the efficiency of hydrolysis enzyme such as lipase and the absorption rate of compounds. Further studies are necessary for the full explanation of these differences.

In the analysis of the reaction products of the compound with one of the gastric-, duodenum- or cecum-contents of the rats, 30 minutes incubation under 37 ºC revealed that glycidol esters were changed into glycidol and also 3-MCPD, and that 3-MCPD esters were changed into 3-MCPD in all cases. These results indicated that glycidol and 3-MCPD are created in the rat body from these esters at least in a small amount.