fragmentation of our phenylpropanetriol glucosides (C, m/e = 167).

The phenylpropanetriols contribute to the color of the liquid sugars examined, probably as their oxidation or dehydration products. We found these products to behave like the phenyl glucosides, generating a bright yellow color when exposed to air on silica plates. The glucosides isolated most probably come from the lignin fraction of stalks of cane. It is known that acid media and high temperatures accelerate the depolymerization of the lignin with formation of phenolic material (Lundquist and Lundgren, 1972). It is not surprising that sugar syrups from beet molasses, produced without grinding and without acid treatment with phosphoric and sulfurous acid in the clarification steps, do not contain appreciable amounts of these phenolic derivatives. In the syrups from beet molasses we never detected more than 0.04% of phenolics (percent based on the total sugar content).

The knowledge of the chemical structure of the colorants suggests that anionic macroporous resins would decolorize these syrups. The resins can interact with the free phenolic groups and adsorb the organic polar compounds. Experiments until now gave satisfactory absorption only by passing the diluted and well-demineralized syrup through the resins, after regeneration with ammonia. Work is in progress to improve the decolorizing process.

The chemical and the biological significance of the presence of phenolic glucosides in liquid sugars has also been considered; trace amounts of these phenolics are very widespread in nature (Harborne, 1964), and toxic effects for human nutrition have never been reported in literature. The polyphenol derivatives can exert some antioxidant and antimicrobial effect (Harborne, 1964); we found that diluted liquid sugars from cane molasses were more microbiologically stable than the corresponding syrups from beet molasses, due, perhaps, to the higher content of phenolic compounds.

ACKNOWLEDGMENT

I thank W. V. Turner for the NMR spectra, E. Rosa for the mass spectra, and G. Simonazzi for experimental assistance.

Registry No. Lignin, 9005-53-2; 3,4-dimethoxyphenyl β-D-glucopyranoside, 84812-00-0; 3,4-dimethoxybenzyl β-D-glucopyranoside, 81381-73-9; 1-(4-hydroxy-3-methoxyphenyl)-3-hydroxy-2-propanone, 4899-74-5; 1-(3,5-dimethoxy-4-hydroxyphenyl)-3-hydroxy-2-propanone, 35263-53-7; glucose, 50-99-7; fructose, 57-48-7; (3,4,5-trimethoxyphenyl)propanetriol, 76774-03-3; (4-hydroxy-3-methoxyphenyl)propanetriol, 1208-42-0; (3,5-dimethoxy-4-hydroxyphenyl)propanetriol, 4204-29-9.

LITERATURE CITED

Adler, E.; Gustafsson, B. *Acta Chem. Scand.* **1963**, *17*, 27.
Harborne, J. B. In "Biochemistry of Phenolic Compounds"; Academic Press: London, 1964; Chapters 4 and 13.
Helferich, B.; Scheiber, H. E.; Streeck, R.; Vorsatz, F. *Justus Liebigs Ann. Chem.* **1935**, *518*, 211.
Higuchi, T.; Nakatsubo, F.; Ikeda, Y. *Holzforschung* **1974**, *28*, 189.
Horning, E. C. In "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III, p 11.
Lüdemann, H. D.; Nimz, H. *Makromol. Chem.* **1974**, *175*, 2393.
Lundquist, K.; Lundgren, R. *Acta Chem. Scand.* **1972**, *26*, and references cited therein.
Nurok, D.; Taylor, G. L.; Stephen, A. M. *J. Chem. Soc. B* **1968**, 291.
Palla, G. *J. Agric. Food Chem.* **1982**, *30*, 764.
Theander, O. *Acta Chem. Scand.* **1965**, *19*, 1972.

Received for review August 24, 1982. Accepted January 26, 1983. This work has been partially supported by the National Research Council (Italy) and by the Sugar Research Department of the Reggiane O.M.I. of Reggio Emilia (Italy).

Gas-Liquid Chromatography-Chemical Ionization Selected Ion Monitoring Assay for Glycerol Formal in Animal Tissues

William J. A. VandenHeuvel, III,* Francis P. Baylis, Joseph E. Brown, Dennis H. Wallace, David H. Minsker, Richard T. Robertson, Avery Rosegay, and Robert W. Walker

Glycerol formal, a 60/40 mixture of the two cyclic condensation products [5-hydroxy-1,3-dioxane and 4-(hydroxymethyl)-1,3-dioxolane] of glycerol and formaldehyde, is used as a nonaqueous solvent in parenterally administered animal health products. Recently this substance was reported to be a teratogen when administered to rats in large doses. We have developed an assay for glycerol formal in the edible tissues (fat, kidney, liver, muscle) and plasma of food-producing animals (cattle, swine, horses). The assay utilizes packed column GLC–chemical ionization (isobutane) selected ion monitoring (glycerol formal-d_2 serving as the internal standard) and possesses an overall lower limit of sensitivity of 0.05 ppm. The maximum glycerol formal residue found in an edible steer tissue (injection-site muscle) at 5 days postdose (14.6 mg/kg of body weight) is ~0.1 ppm. Our teratolgy studies demonstrate that doses of glycerol formal less than 150 mg/kg do not elicit a teratogenic response in rats. Thus, the pressure of the negligible residue (~0.1 ppm) in steer injection-site tissue does not appear to represent a significant hazard to the consumer.

Although numerous studies aimed at detecting submicrogram quantities of drugs in the tissues of food-producing animals are carried out routinely in the development of new animal health drugs, little attention is usually given to the tissue concentration of the components of the dosing vehicle. Glycerol formal, a mixture of the cyclic condensation products of glycerol and formaldehyde, has been used as a nonaqueous solvent in parenterally administered products (Spiegel and Noseworthy, 1963) such as trivetrin ("ABPI Compendium of Data Sheets for Veterinary Products", 1978) and oxytetracycline (Green-

Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey 07065 (W.J.A.V., F.P.B., J.E.B., A.R., and R.W.W.), Merck Sharp & Dohme Research Laboratories, Fulton, Missouri 65251 (D.H.W.), and Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania 19486 (D.H.M. and R.T.R.).

0021-8561/83/1431-0548$01.50/0

baum et al., 1973) apparently with no reported toxic effects. Aliverti et al. (1980) recently reported that in the rat, glycerol formal induces embryo and fetal toxicity and is also teratogenic [leading to cardiovascular defects and misshapen (wavy) ribs]. As glycerol formal is a component of several injectable formulations presently under investigation in our laboratories, the paper by Aliverti et al. (1980) prompted us to develop and apply a sensitive and specific assay for measuring any residues of glycerol formal in animal tissue and to subject this solvent to teratogenicity studies. The results of these efforts are presented in this paper.

EXPERIMENTAL SECTION

Animal Handling. Cattle, swine, and a single horse were dosed with sterile solutions of ivermectin (an antiparasitic agent, Merck & Co., Inc., Rahway, NJ 07065) at or above the current recommended dose levels of ivermectin for the animal species in question. Each of the ivermectin solutions contained glycerol formal as one of the vehicle components at 20, 25 or 40% by volume. The formulation used in the horse study was as follows; ivermectin, 2.0% w/v; glycerol formal, 25.0% v/v; Polysorbate 80, 12.0% w/v; dibasic sodium phosphate (anhydrous), 0.1% w/v; monobasic sodium phosphate (monohydrate), 0.9% w/v; benzyl alcohol, 3.0% v/v; water, q.s. to 100.0% v/v. Formulations for the other species were similar to the above.

Cattle. In one of two experiments with cattle, a single Angus steer weighing 262.3 kg was given a single subcutaneous injection of the ivermectin solution containing 20% v/v glycerol formal (1.0% w/v ivermectin). This provided 7.3 mg of glycerol formal and 0.3 mg of ivermectin/kg of body weight. Blood was collected prior to dosing and at 0.5, 1, 2, 4, 8, 24, and 48 h postdose. The steer was slaughtered at 2 days postdose, and muscle, liver, kidneys, fat and injection-site tissue (muscle) were collected.

Nine Angus steers were used in the second experiment. These cattle (weighing 223.4–254.5 kg) were each given a single subcutaneous injection of the ivermectin solution containing 40% v/v glycerol formal (1.0% w/v ivermectin). This provided 14.6 mg of glycerol formal and 0.3 mg of ivermectin/kg of body weight. Three steers were slaughtered at 4 h, 2 days, or 5 days after dosing when muscle, liver, kidneys, fat, and injection-site tissue were collected.

Control tissues were collected from nonmedicated Angus steers.

All cattle were fed alfalfa hay cubes and a grain ration. Water was available at all times.

Swine. Thirty-five Yorkshire swine (21 barrows and 14 gilts) weighing 22.8–31.6 kg were each given a single subcutaneous injection of the ivermection solution containing 40% v/v glycerol formal (1.0% w/v ivermectin). This provided 19.5 mg of glycerol formal and 0.4 mg of ivermectin/kg of body weight. Three barrows and two gilts were slaughtered at 1, 3, 5, 7, 14, and 28 days after dosing when muscle, liver kidneys, fat, and injection-site tissue (muscle) were collected.

Control tissues were collected from nonmedicated Yorkshire barrows and gilts. The animals were fed a typical swine ration. Water was available at all times.

Horse. A grade (unclassified breeding) gelding weighing 375.45 kg received a single intramusclar injection of the ivermectin solution containing 25% v/v glycerol formal (2.0% w/v ivermectin). This provided 3.1 mg of glycerol formal and 0.2 mg of ivermectin/kg of body weight. The horse was slaughtered 56 days after dosing (a time at which only negligible tissue residues of ivermectin were expected) when muscle, liver, kidneys, fat, and injection-site muscle were collected.

Control tissues were collected from a nonmedicated grade gelding. The horse was fed alfalfa hay cubes, long-stem hay, and a grain ration. Water was available at all times.

Preparation of Tissues. All tissues were frozen whole upon collection and shipped frozen to the laboratory. The tissues were thawed carefully, ground in a Chop-Rite meat grinder (Model 510, Chop-Rite Manufacturing Co., Pottstown, PA 19464) while still chilled, mixed throughly by hand, packaged, and refrozen in preparation for assay.

Assay Procedure. *Materials.* Glycerol formal was obtained from Chemie Linz, Linz, Austria. High-purity water, hexane ("distilled in glass"), and methylene chloride ("distilled in glass") were purchased from Burdick & Jackson. Paraformaldehyde-d_2 (98 atom % D) was obtained from Merck Sharp & Dohme, Ltd., Montreal, Canada.

Preparation of Glycerol Formal-d_2. A mixture of glycerol (2.25 g), paraformaldehyde-d_2 (0.75 g) and *p*-toluenesulfonic acid monohydrate (25 mg) in benzene (15 mL) was refluxed for 16 h by using a Dean-Stark trap to collect the water in the condensate. The benzene solution was then washed with saturated sodium carbonate (0.5 mL) and dried over magnesium sulfate. After the benzene was evaporated, the product was distilled under reduced pressure, collecting the glycerol formal-d_2 fraction at 72–74 °C/5–6 mm.

Isolation Procedure. Ten milliliters of high purity water was added to 10 g of tissue [kidney, liver, muscle, injection site (fat is treated separately; *vide infra*)] and the mixture thoroughly homogenized by using a Virtis blender. Ten microliters of the internal standard solution (1 μg of glycerol formal-d_2, which also serves as a carrier) was added to a 4-g aliquot of the homogenate (2 g equiv of tissue) or 2 mL of plasma and the mixture thoroughly agitated with a Vortex mixer. The mixture was extracted with 40 mL of methylene chloride, and the resulting organic phase was filtered to remove water droplets. The filtered organic phase was reduced in volume to 2–4 mL (NOT TO DRYNESS) on a rotovap at water-aspirator pressure and transferred to a 15-mL centrifuge tube containing 1 mL of water. The tube was warmed (with shaking) in a 60 °C water bath to remove most of the methylene chloride (0.1–0.2 mL remaining). The tube was vortexed for 15 s and centrifuged, and the upper (water) phase was removed. The remaining organic phase was washed with 0.5 mL of water, and the water phases were combined and extracted with 40 mL of methylene chloride (in a 125-mL separatory funnel). The organic phase was collected, centrifuged (to remove water droplets), and reduced in volume (under vacuum) to 1–2 mL. This methylene chloride solution was transferred to a 15-mL centrifuge tube and reduced in volume (by warming and shaking in a warm water bath) to ~20 μL; 5 μL of this solution was then subjected to analysis by GLC–mass spectrometry.

The isolation procedure for swine fat was as follows: 10 mL of water was added to 10 g of fat and homogenized thoroughly; then, as before, internal standard was added and mixed thoroughly. The mixture was extracted with 40 mL of hexane and the organic phase aspirated and discarded. At this point the isolation procedure was continued (i.e., the aqueous phase was extracted with 40 mL of methylene chloride, etc.). The isolation procedure for steer fat was as follows: 2 g steer fat was weighed into a 15-mL centrifuge tube, and 10 μL of internal standard solution and 10 mL of hexane were added. The bulk of

the sample dissolved in the hexane on shaking thoroughly with warming. The hexane solution was extracted with 2 × 1 mL of water, and the aqueous phases were combined. The water phase was equilibrated with 5 mL of hexane, and after centrifugation, the aqueous phase was transferred to a 15-mL centrifuge tube. At this point the isolation procedure was continued (i.e., the aqueous phase was extracted with 40 mL of methylene chloride, etc.).

Instrumentation. All analyses were carried out by using a Finnigan 3200 GLC–quadrupole MS instrument with a 6110 computer system. Operation was in the chemical ionization mode utilizing selected ion monitoring. MH^+ ions for glycerol formal (m/z 105) and the glycerol formal-d_2 internal standard (m/z 107) were monitored and peak height intensities obtained via computer printout. The mass spectrometric operating conditions were as follows: chemical ionization with isobutane reagent gas (0.6 torr), amplifier gain of 10^{-9} A/V, electron multiplier setting of 1200 V, electron energy of 140 eV, and emission current of 0.8 mA.

The GLC operating conditions included a 1.2 m × 3 mm i.d. glass U-tube packed with 0.2% Carbowax 1500 on 80–100-mesh Carbopack C (Supelco), isobutane carrier gas (10 mL/min), a column oven temperature of 140 °C, and a glycerol formal retention time of ~2 min. Instrumental calibration of the mass scale of the mass spectrometer was established or confirmed by use of the FC-43 standard. Examination of a mixture of glycerol formal and glycerol formal-d_2 was carried out daily to determine the m/z values (to the nearest 0.1 amu) for maximum response at m/z 105 and m/z 107. These values were incorporated into the computer programming for the day's operations. The m/z 105 to m/z 107 peak height (intensity) ratios (as established by selected ion monitoring) of a series of isolates obtained from spiked control tissue were subjected to linear regression analysis to generate equations for calculating ppm values. These samples contained different amounts of glycerol formal (0.1–0.5 μg) and 1 μg of internal standard (control tissues not spiked with the internal standard yield no response at m/z 107). Isolates from tissues were examined and the I_{105}/I_{107} ratios at the retention time of interest obtained via computer printout. The concentration of glycerol formal in the samples was determined from peak height intensity ratio values by using the slope and intercept values from the regression analyses. A 20-m methyl silicone fused silica capillary column (Hewlett-Packard) operated at 60 °C was used to separate the two components of glycerol formal (Figure 2).

Teratology. To determine the threshold for the teratogenic effects of glycerol formal in the rat, a teratogenic study of this solvent was conducted in Sprague-Dawley (Charles River, CRCD) rats that were from 12 to 13 weeks of age. Oral dosage levels of glycerol formal, administered once daily and diluted as necessary with deionized water, were 10, 75, 150, 300, and 600 mg kg^{-1} day^{-1}. There were 25 mated females per dosage group. An additional group of 24, serving as controls, received the vehicle (deionized water) in the same volume (5 mL kg^{-1} day^{-1}) as the treated groups. The treatment period was from day 6 through day 17 of gestation, with day 0 as the day spermatozoa were found in the vaginal lavage. All females had free access to Purina Certified Rodent Chow and tap water.

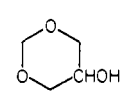
Female body weights were recorded throughout gestation. The females were killed on day 20 of gestation by cervical dislocation under chloroform anesthesia and the uterine contents were examined without knowledge of treatment group. All fetuses were examined externally immediately after laparotomy. Every third fetus and all externally malformed or dead fetuses were given a visceral examination after dissection. The head of every third fetus was sectioned after fixation in Bouin's solution. The skeletons of all fetuses were examined after being stained with Alizarin red.

Figure 1. Chemical ionization (isobutane) mass spectrum of glycerol formal.

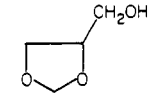
As three fetuses in the 75 mg kg^{-1} day^{-1} group were found to exhibit "wavy" ribs, a second oral teratogenic study of glycerol formal in rats was conducted at a dosage level of 10 mg kg^{-} day^{-1}. Study paradigms were identical with those of the first study; however, fetal examination was restricted to the stained skeletons.

RESULTS AND DISCUSSION

Combined gas–liquid chromatography (GLC)–mass spectrometry with selected ion monitoring is an often-employed approach for quantifying compounds of biological interest at the parts per billion level (Carlin et al., 1980; Garland and Powell, 1981; Suhre et al., 1981). No characteristic ions of high intensity were produced from glycerol formal by using electron impact ionization, but chemical ionization (isobutane reagent gas) mass spectrometry appeared to offer a potentially suitable method for detection and quantification of glycerol formal. The chemical ionization mass spectrum of glycerol formal, which is dominated by its pseudomolecular ion (MH^+) of m/z 105, is presented in Figure 1. This spectrum was obtained by using combined packed column GLC–mass spectrometry; when the MH^+ ion was monitored, a detection limit of 1 ng injected on-column was found. Glycerol formal is actually a mixture of two isomeric compounds, 5-hydroxy-1,3-dioxane and 4-(hydroxymethyl)-



5-hydroxy-1,3 dioxane
~60%



4-(hydroxymethyl)-1,3-dioxolane
~40%

1,3-dioxolane. These isomers are not separated by the Carbowax packed column employed in the assay but are separated on a 20 m × 0.25 mm i.d. methyl silicone fused silica capillary column with a peak height (m/z 105) response ratio of 57/43 (see Figure 2). Approximately the same isomer ratio is found with flame ionization detection (Miwa, 1981). Thus, the two condensation products possess similar mass spectrometric ionization yields, and a

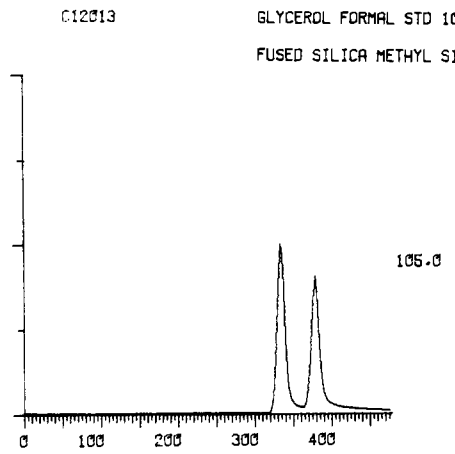


Figure 2. Fused silica capillary column (methyl silicone) separation of the two components of glycerol formal [5-hydroxy-1,3-dioxane eluted first; 4-(hydroxymethyl)-1,3-dioxolane eluted second] with detection via selected ion (m/z 105) monitoring.

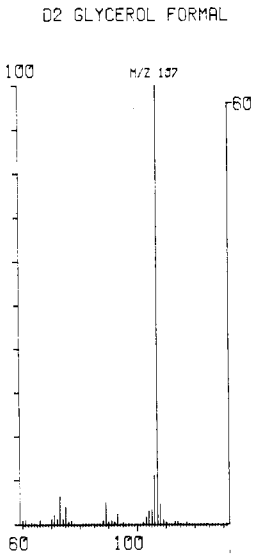


Figure 3. Chemical ionization (isobutane) mass spectrum of glycerol formal-d_2.

packed column with no isomer separation can be used with confidence to determine total glycerol formal residue levels.

Use of a mass spectrometric based detection assay system offers the opportunity for utilizing a heavy isotope labeled analogue of the compound of interest as the internal standard (Carlin et al., 1980; Suhre et al., 1981). Glycerol formal-d_2 was prepared from glycerol and perdeuterioparaformaldehyde. The chemical ionization mass spectrum of this compound is presented in Figure 3 and shows the expected base peak at m/z 107.

The isolation procedure developed for this assay involves partitioning of the extracted glycerol formal between water and methylene chloride at different volume ratios, exploiting the $K_D(CH_2Cl_2/H_2O)$ (0.13) of glycerol formal in this halogenated solvent. Approximately 5-μL aliquots of the final isolation solution ($\sim$20 μL) are subjected to quantification by selected ion monitoring. Experiments with control plasma and tissues [fat, kidney, liver, muscle (also used as the control tissue for injection site)] and these materials spiked with glycerol formal have demonstrated an overall assay detection limit of 0.05 ppm using a 2-g tissue sample and monitoring the ions of m/z 105 and 107 (intensities in digital counts).

The first extensive study involved dosing a group of swine subcutaneously at 19.5 mg of glycerol formal/kg of

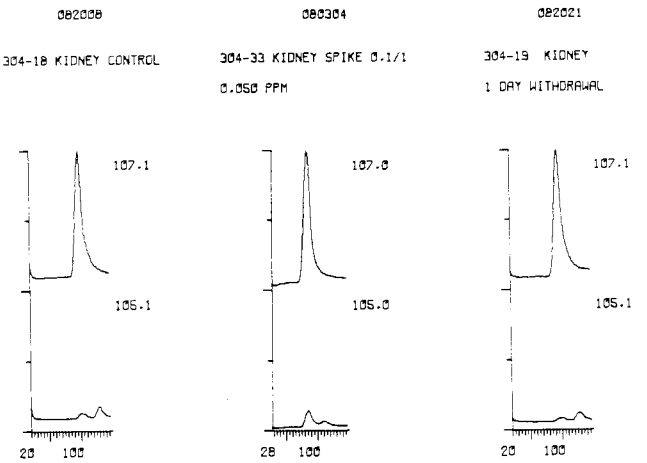


Figure 4. Selected ion (m/z 105, glycerol formal; m/z 107, glycerol formal-d_2) monitoring plots resulting from analysis of isolates from control swine kidney tissue, the same tissue spiked with glycerol formal at 0.05 ppm, and 1 day withdrawal tissue from barrow dosed with glycerol formal (left, center, and right panels, respectively).

body weight. Samples of control tissues (five samples per tissue) to which had been added different amounts (0.1–0.5 μg) of glycerol formal (equivalent to 0.05–0.25 ppm) and 1 μg of glycerol formal-d_2 were assayed. The resulting data (ion intensity ratios m/z 105/m/z 107 vs. the amount of added glycerol formal) were subjected to linear regression analysis.

		r^2
fat	ppm = 0.53R − 0.01	0.9984
kidney	ppm = 0.49R − 0.01	0.9996
liver	ppm = 0.49R − 0.01	0.9957
muscle	ppm = 0.49R − 0.01	0.9973
injection site	ppm = 0.49R − 0.01	0.9973

$$R = \frac{I_{105}}{I_{107} - 0.85\% \, I_{105}}$$

The equation includes a correction factor of 0.85 I_{105} for the varying contributions of glycerol formal to I_{107} and an intercept value of 0.01 arising from the nonvarying contribution of the internal standard to I_{105}. A statistical analysis of the data arising from the spiked control tissues resulted in the following detection limits which provide a 5% false positive and a 5% false negative error: fat, 0.029 ppm; kidney, 0.022 ppm; liver, 0.038 ppm; muscle, 0.031 ppm.

Isolates from 13 control tissue samples spiked with glycerol formal at the 0.05-ppm level gave a mean I_{105}/I_{107} ratio of 0.11 (cv % = 6.5, n = 13), leading to a concentration of 0.05 ppm. This empirically demonstrated detection limit of 0.05 ppm is used throughout this work. Tissues from barrows and gilts obtained at slaughter 1 day postdose were assayed for glycerol formal residues by using the slope and intercept values from the regression analyses. No glycerol formal (detection limit of 0.05 ppm) was found in the 1 day withdrawal tissues. Selected ion monitoring plots arising from analysis of kidney tissue (control, control spiked with glycerol formal at the 0.05-ppm level, and 1 day withdrawal tissue) are shown in Figure 4.

In a similar study in cattle, glycerol formal levels in fat, kidney, liver, muscle, and the injection site of animals (three per time point) dosed subcutaneously with 14.6 mg of glycerol formal/kg of body weight and slaughtered at 4 h, 2 days, and 5 days postdose were determined. For samples from the latter two withdrawal times (as with the swine study), ion intensity ratios from control tissues spiked (0.05–0.25 ppm) with glycerol formal provided data

Table I. Glycerol Formal Levels in Tissues of Animals Dosed with This Solvent

species	animal no.	dose of glycerol formal, mg/kg of body wt	time postdose		ppm of glycerol formal[a] fat	kidney	liver	muscle	inj site
cattle	steer 2787	7.3[b]	2 days		0	0	0	0	0
	steer 2821	14.6[b]	4 h		1.6	14.0	12.0	12.2	21.2
	steer 2824	14.6[b]	4 h		1.4	12.0	9.5	11.4	19.3
	steer 2833	14.6[b]	4 h		1.1	13.5	10.1	12.2	26.9
				$\bar{x}$:	1.4	13.2	10.5	11.9	22.5
	steer 2823	14.6[b]	2 days		0	0.05	0	0.06	0.16
	steer 2826	14.6[b]	2 days		0	0.13	0.07	0.16	0.11
	steer 2829	14.6[b]	2 days		0	0.06	0	0.07	0.13
				$\bar{x}$:	0	0.08	0	0.10	0.13
	steer 2825	14.6[b]	5 days		0	0	0	0	0.05
	steer 2831	14.6[b]	5 days		0	0.07	0	0.07	0.11
	steer 2834	14.6[b]	5 days		0	0.06	0	0.08	0.09
				$\bar{x}$:	0	0	0	0.05	0.08
horse	gelding 294	3.1[c]	56 days		0	0	0	0	0
swine	barrow 304-7	19.5[b]	1 day		0	0	0	0	0
	barrow 304-11	19.5[b]	1 day		0	*d*	0	0	0
	barrow 304-19	19.5[b]	1 day		0	0	0	0	0
	gilt 304-47	19.5[b]	1 day		0	0	0	0	0
	gilt 304-58	19.5[b]	1 day		0	0	0	0	0

[a] Total glycerol formal (both isomeric forms); "0" indicates < 0.05 ppm. [b] Subcutaneous. [c] Intramuscular. [d] This tissue showed evidence for the presence of glycerol formal at an estimated level of 0.03 ppm.

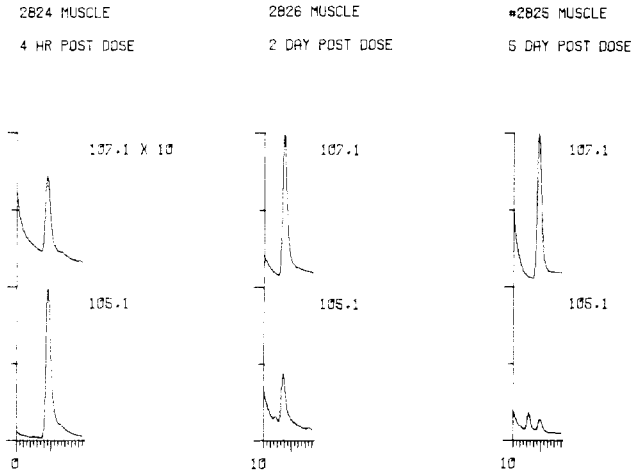


Figure 5. Selected ion (m/z 105, glycerol formal; m/z 107, glycerol formal-d_2) monitoring plots resulting from analysis of isolates from steer muscle tissue [4-h, 2-day, and 5-day withdrawal (left, center, and right panel, respectively)] from animals dosed with glycerol formal.

which were subjected to linear regression analysis to generate the equations

		r^2
fat	ppm = 0.51R − 0.03	0.9997
kidney	ppm = 0.50R − 0.02	0.9995
liver	ppm = 0.50R − 0.08	0.9987
muscle	ppm = 0.50R − 0.01	0.9996
injection site	ppm = 0.50R − 0.01	0.9996

$$R = \frac{I_{105}}{I_{107} - 0.85\% \, I_{105}}$$

Isolates from kidney control tissues spiked at the 0.05-ppm glycerol formal level gave a mean I_{105}/I_{107} ratio of 0.13 (cv % = 8.8, n = 5), leading to a glycerol formal concentration of 0.05 ppm. A standard working curve covering the concentration range 0–20 ppm was employed with the 4-h samples. Isolates from the three sets of withdrawal tissues were examined by selected ion monitoring and the I_{105}/I_{107} ratios at the appropriate retention time obtained. The

Table II. Levels of Glycerol Formal and of Its Two Isomeric Forms in Plasma of Steer Dosed Subcutaneously with Glycerol Formal Using an Aqueous Based Formulation (7.3 mg of Glycerol Formal/kg of Body Weight)

time postdose, h	ppm total glycerol formal	5-hydroxy-1,3-dioxane	4-(hydroxymethyl)-1,3-dioxolane
0.5	8.9	5.5	3.4
1.0	7.5	5.3	2.2
2.0	6.4	5.5	0.9
4.0	4.0	3.9	0.1
8.0	1.8	1.8	0[a]
24.0	0	–[b]	–
30.0	0	–	–
48.0	0	–	–

[a] Zero value indicates < 0.05 ppm. [b] Samples not assayed for isomeric forms.

glycerol formal residues were determined by using the slope and intercept values from regression analyses as shown above and are reported in Table I. Not surprisingly, the highest residues are found in the injection-site tissues at each withdrawal time, but the decay is rapid. The mean concentration of glycerol formal in muscle tissue is just at the lower limit of sensitivity at 5 days postdose. Selected ion monitoring plots arising from analysis of muscle tissues (4 h, 2 days, and 5 days postdose) are shown in Figure 5.

A second cattle study involved dosing of a steer with glycerol formal at a level of 7.3 mg/kg of body weight, with slaughter at 2 days postdose. None of the tissues examined contained glycerol formal (detection limit of 0.05 ppm). In another single animal experiment, glycerol formal was found to be absent (<0.05 ppm) from tissues obtained from a gelding 56 days following administration of glycerol formal (dosed at 3.1 mg/kg of body weight). Residue levels from the four studies discussed in this paper are given in Table I.

Plasma samples were collected during the single animal steer residue study and subsequently assayed for glycerol formal. As can be seen from the data presented in Table

Table III. Reproductive Status and Results of Fetal Examination of Rats Dosed with Glycerol Formal

	glycerol formal, mg^{-1} kg^{-1} day					
	control	10	75	150	300	600
no. fetuses examined	308	310	313	300	262	288
no. litters examined[a]	24 (0)	24 (1)	24 (1)	24 (1)	21 (4)	24 (1)
% fetal wastage (resorption + dead fetuses)	6	4	1	2	3	15
mean fetal weight, g	3.68	3.60	3.69	3.38[b]	3.16[b]	2.81[b]
% fetuses with external alterations (anasarca, atresia ani, tail malformation)	0.0	c	0.3	0.0	2.0	7.7
% fetuses with cardiovascular malformations (ventricular septal defect, malformed atrial and ventricular cavaties, aortic dysplasia, displaced aorta, and pulmonary artery)	4.4	c	0.0	2.2	4.5	48.5
% fetuses with skeletal alterations (skull, sternebral, scapular, and vertebral malformations + hypoplastic and fused ribs)	0.3	0.06	0.0	0.7	0.0	2.5
% fetuses with wavy rib	0.9	0.0	1.0	4.7	17.6	22.3

[a] Numbers in parentheses denote the number of mated but nonpregnant females in each group (total of 25 mated females per dosage group). [b] Significantly different from control ($P \leq 0.05$), based on rankit adjusted mean values. [c] Not recorded in this dosage group.

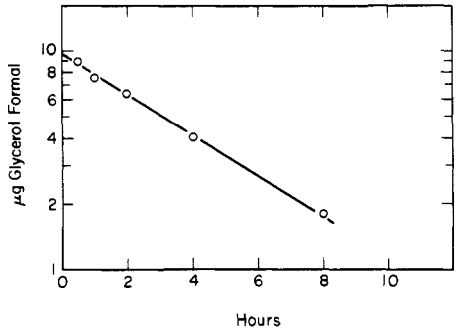


Figure 6. Plot of glycerol formal plasma (from a steer dose with glycerol formal) concentrations (μg/mL) vs. time postdose.

II, the glycerol formal concentration was greatest soon after administration and dropped below the lower limit of sensitivity between 8 and 24 h postdose. A plot of plasma concentrations vs. time postdose is presented in Figure 6; the apparent $t_{1/2}$ is ~3.5 h. As indicated earlier, the two isomeric forms of glycerol formal are separable on a capillary column. Assay of the plasma isolates using selected ion monitoring with capillary column GLC gave the data presented in Table II. It is clear that the 5-hydroxy-1,3-dioxane disappears at a slower rate than does the 4-(hydroxymethyl)-1,3-dioxolane (apparent $t_{1/2}$ values of ~3.8 and ~0.6 h, respectively), presumably because of a slower rate of excretion and/or metabolism.

With respect to teratogenicity studies, no clinical signs of toxicity or adverse effects on mean weight gains were noted among mated females in any glycerol formal treated group. Glycerol formal is teratogenic in rats at dosage levels of 150, 300, and 600 mg kg^{-1} day^{-1}; a no-effect level of 75 mg kg^{-1} day^{-1} was observed (Table III). There were dose-related increases in the incidence of wavy ribs at 150, 300, and 600 mg kg^{-1} day^{-1}. A dosage of 600 mg kg^{-1} day^{-1} resulted in fetal anasarca, anal atresia, tail malformations, and cardiovascular malformations. Anal atresia and tail malformations also appeared in fetuses in the 300 mg kg^{-1} day^{-1} group. The cardiovascular and skeletal malformations we observed confirm the findings of Aliverti et al. (1980). Evidence of fetotoxicity was observed as a dose-related significant decrease in average live fetal weight in the three highest doses and an increase in the numbers of dead fetuses and resorptions at the highest dose level.

The maximum glycerol formal residue found in cattle tissue at 5 days postdose is ~0.1 ppm (Table I), whereas a treatment-related increase in the occurrence of teratology (wavy ribs) in rats requires 150 mg of glycerol formal/kg (Table III). Thus, a 70-kg person consuming 500 g of the residue-containing tissue would be exposed to ~0.7 μg of glycerol formal/kg, 0.0005% of the teratogenic dose in the rat.

ACKNOWLEDGMENT

We thank Dr. T. P. Capizzi and Dr. L. Oppenheimer for calculating the statistically analyzed assay detection limits and Dr. F. J. Wolf for his interest and helpful suggestions.

Registry No. Glycerol formal, 5464-28-8.

LITERATURE CITED

"ABPI Compendium of Data Sheets for Veterinary Products"; Pharmid Publications, Ltd.: London, 1978; p 422.
Aliverti, V.; Bonanomi, L.; Giavini, E.; Leone, V. G.; Mariani, L. *Toxicol. Appl. Pharmacol.* **1980**, *56*, 93–100.
Carlin, J. R.; Walker, R. W.; Davies, R. O.; Ferguson, R. T.; VandenHeuvel, W. J. A. *J. Pharm. Sci.* **1980**, *69*, 1111–1115.
Garland, W. A.; Powell, M. L. *J. Chromatogr. Sci.* **1981**, *19*, 392–434.
Greenbaum, S. B.; Schaaf, K. H.; Klein, H. C. U.S. Patent 3 712 949, 1973.
Miwa, G., Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065, personal communication, 1981.
Spiegel, A. J.; Noseworthy, M. M. *J. Pharm. Sci.* **1963**, *52*, 917–927.
Suhre, F. B.; Simpson, R. M.; Shafer, J. W. *J. Agric. Food Chem.* **1981**, 29, 727–729.

Received for review April 8, 1982. Revised manuscript received November 29, 1982. Accepted January 25, 1983.