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Application of Microencapsulation for Toxicology Studies

II. Toxicity of Microencapsulated Trichloroethylene in Fischer 344 Rats

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Application of Microencapsulation for Toxicology Studies. II. Toxicity of Microencapsulated Trichloroethylene in Fischer 344 Rats. MELNICK, R. L., JAMESON, C. W., GOEHL, T. J., MARONPOT, R. R., COLLINS, B. J., GREENWELL, A., HARRINGTON, F. W., WILSON, R. E., TOMASZEWSKI, K. E., AND AGARWAL, D. K. (1987). *Fundam. Appl. Toxicol.* **8**, 432–442. Gelatin–sorbitol microcapsules containing 44.1% trichloroethylene (TCE) were prepared and mixed in NIH-07 rodent meal diet and provided at microcapsule concentrations of 0 (untreated control group), 1.25, 2.5, 5.0, or 10% (equivalent to 0, 0.55, 1.10, 2.21, or 4.41% TCE, respectively) to groups of 10 male F344 rats for 14 days. An additional control group received diets containing 5% empty capsules. For comparisons, TCE dissolved in corn oil was administered by gavage to different groups of 10 male rats for 14 consecutive days at dose levels adjusted to correspond to those in the feed study. Treatment-related deaths occurred only in the highest dose group of the gavage study. Body weight gain and feed consumption were reduced in high-dose groups of both the feed and gavage studies. There was no measurable loss of TCE in feed sampled from the cages during the study. Dose-related increases in organ (liver and kidney) weight/body weight ratios, individual cell necrosis in the liver, and hepatic microsomal NADPH cytochrome *c* reductase and peroxisomal palmitoyl-CoA oxidase and catalase activities were found in both the dosed-fed and gavage groups. Induction of cytochrome *P*-450 occurred only in the dosed-feed study. There were no significant compound-related pathologic lesions observed in the kidneys, the only other organ examined microscopically. Differences in lethality, cytochrome *P*-450 levels, and induction of microsomal or peroxisomal enzyme activities were attributed to differences in the method of dosing (gavage versus dosed-feed). The demonstration of no significant loss of TCE from the feed and of similar toxic effects produced by microencapsulated TCE via feed and TCE in corn oil via gavage indicate that microencapsulation can provide an excellent alternative exposure route for studying the oral toxicological properties of volatile chemicals, such as TCE, in rats.

In the preceding paper we showed that trichloroethylene (TCE) was stabilized in gelatin–sorbitol microcapsules (Melnick *et al.*, 1987). In addition, microencapsulation did not appear to interfere with the extent of absorption of TCE in male F344 rats (unpublished results). The present studies were designed to compare the toxicity of TCE administered in microcapsules mixed in rodent feed to that of TCE administered by gavage in corn oil in Fischer 344 rats.

TCE is a volatile, colorless liquid (bp = 87°C; vapor pressure at 25°C = 77 mm Hg) which has been used as a vapor degreasing agent of fabricated metal parts, an industrial solvent, a chemical intermediate, a dry-cleaning agent, and an anesthetic (Torkelson and Rowe, 1981; Merck, 1983; U.S. Environmental Protection Agency, 1985). Production of TCE in the United States in 1981 was about 235 million pounds (USITC, 1982). TCE has been found in foodstuffs and in

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drinking water in various cities in the United States (Kavlock *et al.*, 1979; U.S. Environmental Protection Agency, 1985).

Ingestion of TCE in drinking water is a potential route of human exposure. However, because of its low water solubility and high volatility, it is difficult to perform drinking water or dosed-feed toxicity studies of TCE. Microencapsulation of TCE was investigated to provide an alternative vehicle to study the oral toxicity of this chemical.

TCE has a moderate to low acute oral toxicity; the oral LD50 in rats is 7207 mg/kg (Smyth *et al.*, 1969) while in mice an oral LD50 of 2402 mg/kg was reported for males and 2443 mg/kg for females (Tucker *et al.*, 1982). Central nervous system depression is the predominant response from acute inhalation exposure to TCE (Torkelson and Rowe, 1981). Liver and kidney have been identified as target organs to TCE toxicity in rats and mice (Torkelson and Rowe, 1981; NCI, 1976; Kjellstrand *et al.*, 1981; Stott *et al.*, 1982; Tucker *et al.*, 1982; NTP, 1987). Rats appear to be less susceptible to hepatic alterations induced by TCE than mice (Stott *et al.*, 1982). Some of the toxicological effects and physiological responses resulting from exposure to TCE include increase in liver weight/body weight ratio (Pessayre *et al.*, 1979; Kjellstrand *et al.*, 1981; Tucker *et al.*, 1982), increase in kidney weight/body weight ratio (Kjellstrand *et al.*, 1981; Tucker *et al.*, 1982), toxic nephropathy in rats and mice (NTP, 1987; NTP, unpublished results), hepatocellular necrosis in mice (Stott *et al.*, 1982), hepatic peroxisome proliferation in mice but not in rats (Elcombe *et al.*, 1985), increases in proteins and ketones in the urine of mice (Tucker *et al.*, 1982), decreases in rat hepatic microsomal cytochrome *P*-450 and ethylmorphine demethylase and hexobarbital hydroxylase activities, and increases in microsomal protein and microsomal NADPH cytochrome *c* reductase, aniline hydroxylase, and *p*-nitrophenol glucuronyltransferase activities (Pessayre *et al.*, 1979). The present studies were designed to compare the toxicity of microencapsulated TCE given in feed with that of TCE administered by gavage in corn oil.

METHODS

Chemical. High-purity grade TCE (99+%, obtained from Missouri Solvents, Kansas City, MO) ws encapsulated at Southwest Research Institute (San Antonio, TX) by the centrifugal extrusion process (Goodwin and Somerville, 1974; Melnick *et al.*, 1987). The concentration of TCE in the gelatin–sorbitol microcapsule preparation (Batch 07; 29.8% sorbitol/70.2% gelatin) was 44.1% (w/w). Placebo microspheres lacking TCE were prepared by the same procedure and were of the same composition as the microcapsule shells containing TCE. The preparation of test diets of ground NIH-07 rodent feed containing microencapsulated TCE and the methods for determining TCE concentrations in feed samples were described previously (Collins *et al.*, 1986).

Animals and treatment. Six-week-old male Fischer 344 rats (obtained from Charles River Breeding Laboratories, Wilmington, MA) were used in this study. The animals, quarantined for 1 week prior to treatment, were weighed, ear tagged, and randomized by weight into 10 groups of 10 rats each. The average initial group body weights ranged from 89 to 92 g. Test diets, control diets, and tap water were available *ad libitum*. Temperature in the animal room was maintained at 22 ± 2°C and relative humidity was 50 ± 10%. Fluorescent lighting was provided 12 hr per day. These conditions are consistent with the requirements of the National Toxicology Program (NTP, 1984).

Groups of 10 male F344 rats were fed diets containing 0 (untreated control), 1.25, 2.5, 5.0, or 10% of TCE-loaded microcapsules for 14 days. Since the content of TCE in the microcapsules was 44.1% (w/w), the concentrations of TCE in the diets were 0, 0.55, 1.10, 2.21, and 4.41%, respectively. A vehicle control diet containing 5% placebo microspheres was provided to an additional group of 10 animals. Freshly filled feeders were provided after 7 days of the study. A parallel corn oil gavage study was also conducted where groups of 10 male F344 rats were administered TCE dissolved in corn oil by gavage (5 ml/kg body weight) for 14 consecutive days. The dosage levels of TCE in the gavage study were adjusted five times during the 14-day treatment period to be similar to the dosage levels of TCE in the feed study.

Clinical chemistries and urinalyses. Five animals from the two highest dosed-feed groups, the two highest dose gavage groups, and the placebo, corn oil, and untreated control groups were housed individually in metabolism cages. Blood samples (2 ml) were collected from the orbital sinus of CO_2 anesthetized rats after 1, 3, and 14 days of the study, and serum samples were analyzed for sorbi-

tol dehydrogenase, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea nitrogen, creatinine, glucose and total protein with a Centrifichem 400 chemistry analyzer (Baker Chemical Co., Allentown, PA). Twenty-four-hour urine samples were also collected at those time periods and analyzed for glucose, total protein, creatinine, and blood urea nitrogen. In addition, microscopic appearance of urine sediments, specific gravity, and pH of the urine samples were recorded.

Biochemical measurements. Livers were removed and weighed, and portions not taken for histopathologic examination were homogenized in 3 vol of ice-cold 0.25 M sucrose. Catalase and peroxisomal palmitoyl-CoA oxidase activities in the whole liver homogenates were determined by spectrophotometric and fluorometric assay procedures (Aebi, 1974; Walusimbi-Kisitu and Harrison, 1983). The remainder of the homogenates were centrifuged at 10,000*g* for 10 min and the subsequent supernatant fractions were recentrifuged at 140,000*g* for 45 min. Measurements were made of NADPH cytochrome *c* reductase activity (Sottocasa *et al.*, 1967) and cytochrome *P*-450 (Omura and Sato, 1964) in the microsomal fractions. Protein concentrations were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as a standard.

Histopathologic examination. Liver samples and the right kidney from each animal were fixed in neutral buffered Formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically in a blind (coded) fashion for evidence of chemically induced morphological changes.

Statistical evaluation. The statistical significance of the parametric data were evaluated by analysis of variance, and group means were compared by a Student *t* test (Ryan, 1981). Histopathological evaluations were analyzed by the Mann–Whitney *U* test for nonparametric data (Sokal and Rohlf, 1969). The minimum level of probability accepted for significance was $p < 0.05$.

RESULTS

Stability of TCE in Rodent Feed during the 14-Day Study

The feed blends containing microencapsulated TCE were analyzed for TCE content after 3 and 7 days of the study to see if the rats promoted deterioration of the microcapsules. Deterioration would be expected to cause a decrease in the TCE concentration. However, as shown in Table 1, there was essentially no loss of TCE in samples taken from the surface of the feeders at any of the microcapsule concentrations. The slight elevations in TCE concentrations at the 2.5, 5.0, and 10% microcapsule concentrations may be indicative of selective feeding by the animals. The changes in TCE concentrations were generally much less between Days 3 and 7 than between Days 1 and 3 of the study.

Feed Consumption and Dosage Levels of TCE

The time-weighted average dosage levels of TCE in the feed study were 0.6, 1.3, 2.2, and 4.8 g/kg/day (Table 2). The average daily feed consumption for untreated control and placebo control animals was between 11 and 12 g per animal during the first week of the study. Similar amounts of feed were consumed by animals in the 1.25 and 2.5% microencapsulated TCE dose groups. The average daily feed consumption per animal in the 5.0 and 10% dose groups was between 8 and 9 g. The lower level of feed consumption in these groups may have been due to either a lower palatability of feed containing high concentrations of microencapsulated TCE or to a toxic effect of the TCE. Feed consumption was also reduced in the high-dose gavage group; however, this decrease may have resulted from CNS depression caused by TCE, as postgavage lethargy was noted in this group of animals. During the second week of the study, feed consumption values were elevated in all groups; however, feed consumption for the groups receiving 5 and 10% microencapsulated TCE in their diets was still 15–20% lower than those of the placebo control or untreated control groups. The two lower dose levels in the gavage study matched closely with the two lower dose levels in the feed study, while the high-dose level in the gavage study was intermediate between the two higher dose levels of the feed study. It was not practical to include a higher dose level in the gavage study since such a dose would be approaching the single-dose oral LD50 of TCE in rats.

TABLE 1

STABILITY OF TCE IN RODENT FEED DURING THE 14-DAY FEED STUDY

% Targeted microcapsule concentration[a]	TCE concentration, mg/g feed[b]		
	Day 0	Day 3	Day 7
1.25	5.7 ± 0.1	5.4 ± 0.2 (95)	5.6 ± 0.2 (98)
2.5	10.9 ± 0.6	11.0 ± 0.3 (101)	11.8 ± 0.2 (108)[c]
5.0	21.7 ± 0.5	23.7 ± 0.9 (109)[c]	24.4 ± 0.9 (112)[c]
10.0	43.5 ± 2.1	48.0 ± 2.2 (110)[c]	47.9 ± 0.9 (110)[c]

[a] The concentration of TCE in the microcapsules was 44.1% (w/w).
[b] Results are given as means ± SD; $n = 4$–8. Numbers in parentheses are percentage of Day 0 concentration.
[c] Different from Day 0 concentrations, $p < 0.05$.

rvival and Body Weight Changes

There were no deaths in any of the groups ated with microencapsulated TCE in the ed, while there were four deaths, all occurng in the first week, in the high-dose group the gavage study. There were no deaths in ne other gavage groups. The mean body eight gain after 14 days of treatment in the ntreated control group was nearly the same s that in the placebo control group, 65.4 and 7.8 g, respectively. Mean body weight gains of the two highest dose groups of the feed study and of the highest dose group of the gavage study were significantly lower than the mean body weight gains of the respective control groups (Fig. 1). Most of the body weight gain in these groups occurred during the second week; this probably reflects increases in feed consumption and accommodation to TCE. Postgavage lethargy was observed only in the 2.8 g/kg dose group of the gavage study.

Liver and Kidney Changes

At necropsy, liver and right kidney weights were recorded since they have been identified as target organs of TCE toxicity. There were significant and similar dose-related increases in liver weight/body weight ratios at each dos-

TABLE 2

TIME-WEIGHTED AVERAGES OF FEED CONSUMPTION AND DOSAGE OF TCE IN MALE F344 RATS DURING THE 14-DAY STUDY

Microencapsulated TCE (feed)			TCE in corn oil (gavage)		
Group	Feed/day (g)	TCE dose (g/kg/day)	Group	Feed/day (g)	TCE dose (g/kg/day)
Untreated control	12.7	0			
Placebo control	13.2	0	Corn oil control	10.6	0
1.25% microcaps.	13.0	0.6	Low-dose gavage	11.5	0.6
2.5% microcaps.	13.6	1.3	Mid-dose gavage	11.0	1.2
5.0% microcaps.	10.5	2.2	High-dose gavage	6.5	2.8
10.0% microcaps.	10.1	4.8			

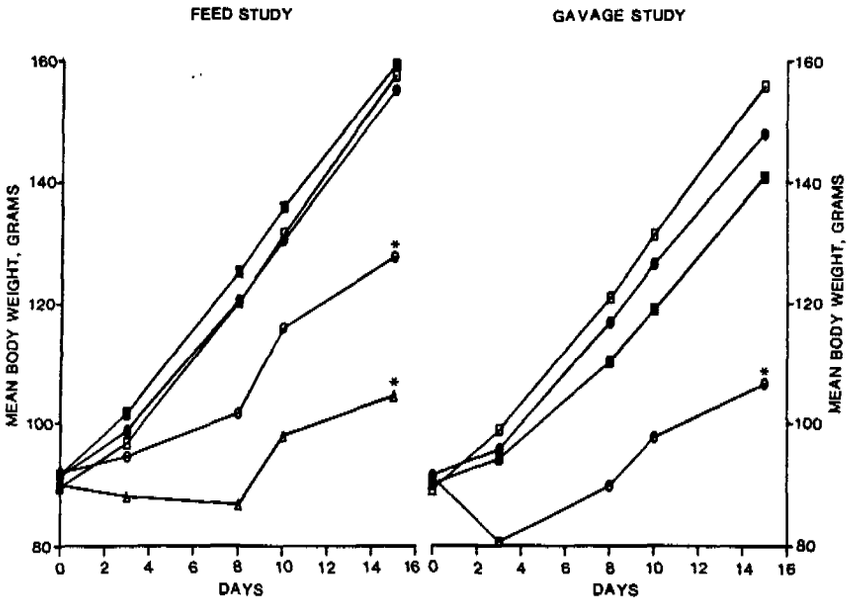
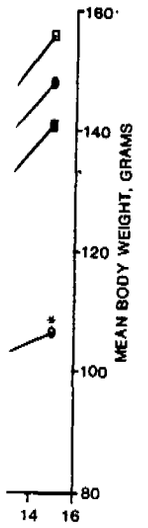


FIG. 1. Growth curves for male F344 rats treated with microencapsulated TCE (dosed-feed study) or TCE in corn oil (gavage study) for 14 days. Dosed-feed groups: ■, placebo control; □, 0.6 g/kg; ●, 1.3 g/kg; ○, 2.2 g/kg; △, 4.8 g/kg. Gavage groups: ■, corn oil control; □, 0.6 g/kg; ●, 1.2 g/kg; ○, 2.8 g/kg. Standard deviations were generally ±7% of the mean and never exceeded ±15%. *Different from controls, $p < 0.05$.

age level in the dosed-feed and gavage groups (Table 3). Relative to controls, absolute liver weights were increased only at the two lower dose levels (0.6 and 1.2 g/kg groups) of the dosed-feed and gavage studies. Neither the placebo capsules nor the corn oil treatment appeared to have an effect on liver weight. Kidney weight/body weight ratios were elevated at all TCE dose levels compared to controls in both the dosed-feed and gavage groups; however, there were no treatment-related effects on absolute kidney weight (results not shown).

The only treatment-related lesion observed microscopically in rats from either the dosed-feed or gavage groups was individual cell necrosis in the liver. The frequency and severity of this lesion were similar at each dosage level of TCE administered microencapsulated in the feed or in corn oil by gavage (Table 4). Individual cell necrosis of the liver is a subtle lesion which was subjectively graded into one of five categories. A minimal grade was assigned for cases in which 1 to 3 necrotic hepatocytes were found in approximately 10 microscopic fields at a magnification of 200×. Similarly, 4 to 7 necrotic hepatocytes per 10 fields were graded as mild, while 8 to 12 were graded as moderate. There were no instances where more extensive lesions (i.e., marked or severe) were found. The cytological features of the individual cell necrosis were nuclear condensation and pyknosis with attendant increased cytoplasmic eosinophilia. There was individualization of affected hepatocytes manifested by shrinkage away from the surrounding parenchyma. This individual cell necrosis was randomly distributed throughout the liver with no apparent lobular distribution and the change was not accompanied by an inflammatory response. There was no histologic evidence of cellular hypertrophy or edema in hepatic parenchymal cells. There were no significant compound-related lesions



osed-feed study) or
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rent from controls,

TABLE 3

LIVER WEIGHTS OF MALE F344 RATS TREATED WITH TCE FOR 14 DAYS[a]

Microencapsulated TCE (feed)			TCE in corn oil (gavage)		
...group (g/kg)	Liver weight (g)	Liver weight/ body weight ratio × 100	Dose group (g/kg)	Liver weight (g)	Liver weight/ body weight ratio × 100
...eated ...ntrol	8.1 ± 0.8	5.2 ± 0.3			
...bo ...trol	8.4 ± 0.8	5.3 ± 0.2	Corn oil control	7.1 ± 1.3	5.0 ± 0.4[c]
...6	9.5 ± 0.5[b]	6.0 ± 0.3[b]	0.6	9.3 ± 1.2[b]	6.0 ± 0.4[b]
...3	10.1 ± 1.2[b]	6.5 ± 0.5[b]	1.2	9.1 ± 0.9[b]	6.1 ± 0.3[b,c]
...2	8.9 ± 1.3	7.0 ± 0.9[b]	2.8	7.7 ± 0.4	7.3 ± 0.5[b]
...8	7.4 ± 0.5	7.1 ± 0.5[b]			

...esults are given as means ± SD. $N = 10$ for all groups except the 2.8 g/kg dose group (gavage study) which had six surviving rats at the time of the terminal sacrifice.
[b] Increased compared to control, $p < 0.05$.
[c] Different from corresponding feed group, $p < 0.05$.

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The activity of microsomal NADPH cytochrome *c* reductase and the level of cytochrome *P*-450 were measured in the present studies (Table 5) since the rat microsomal mixed function oxidase system had been reported to undergo various changes after repeated administration of TCE (Pessayre *et al.*, 1979). Microsomal NADPH cytochrome *c* reductase activity was elevated in the 2.2 and 4.8 g/kg dose groups of rats fed diets con-

TABLE 4

INDIVIDUAL CELL NECROSIS OF HEPATOCYTES IN MALE F344 RATS TREATED WITH TCE FOR 14 DAYS

Microencapsulated TCE (feed)			TCE in corn oil (gavage)		
Dose group (g/kg)	Frequency of lesion[a]	Mean severity[b]	Dose group (g/kg)	Frequency of lesion[a]	Mean severity[b]
Untreated control	1/10	0.1			
Placebo control	0/10	0.0	Corn oil control	0/10	0.0
0.6	2/10	0.4	0.6	0/10	0.0
1.3	2/10	0.3	1.2	1/10	0.1
2.2	9/10	2.0[c]	2.8	5/6	1.8[c]
4.8	9/10	2.5[c]			

[a] Number of animals with lesion/number of animals examined.
[b] Severity of lesion: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked; 5 = severe.
[c] Different from controls, $p < 0.05$.

TABLE 5

EFFECT OF TCE ON HEPATIC MICROSOMAL NADPH CYTOCHROME *c* REDUCTASE ACTIVITY AND CYTOCHROME *P*-450 CONTENT IN MALE F344 RATS[a]

Microencapsulated TCE (feed)			TCE in corn oil (gavage)		
Dose group (g/kg)	NADPH cytochrome *c* reductase[b]	Cytochrome *P*-450[c]	Dose group (g/kg)	NADPH cytochrome *c* reductase[b]	Cytochrome *P*-450[c]
Untreated control	402 ± 48	472 ± 98			
Placebo control	347 ± 48	421 ± 95	Corn oil control	461 ± 100	461 ± 83
0.6	409 ± 83	475 ± 83	0.6	439 ± 95	433 ± 23
1.3	410 ± 89	451 ± 95	1.2	790 ± 73[d,e]	585 ± 136
2.2	732 ± 118[d]	719 ± 92[d]	2.8	680 ± 78[d]	426 ± 25[e]
4.8	775 ± 130[d]	718 ± 84[d]			

[a] Results are means ± SD; $n = 5$.
[b] Values are expressed as nmol cytochrome *c* reduced × min^{-1} × mg $protein^{-1}$.
[c] Values are expressed as pmol/mg protein.
[d] Different from controls, $p < 0.05$.
[e] Different from corresponding feed group, $p < 0.05$.

taining microencapsulated TCE and in the 1.2 and 2.8 g/kg dose groups of rats treated with TCE in corn oil by gavage. Cytochrome *P*-450 levels were elevated only in the two highest dose groups of the feed study. There were no significant decreases in cytochrome *P*-450 levels. Neither the placebo capsules nor the corn oil treatment affected the levels of NADPH cytochrome *c* reductase activity or cytochrome *P*-450.

Elcombe *et al.* (1985) suggested that TCE-induced hepatocarcinogenicity may be unique to mice and possibly may be related to a species-specific proliferation of hepatic peroxisomes resulting from treatment with TCE. At doses of 0.5 to 1.5 g/kg, TCE induced peroxisome proliferation in mice but not in rats (Elcombe *et al.*, 1985). In the present studies, rat hepatic peroxisomal palmitoyl-CoA oxidase and catalase activities were measured. Palmitoyl-CoA oxidase activity is a sensitive marker of peroxisome proliferation (Tomaszewski *et al.*, 1986). We observed dose-related increases in peroxisomal palmitoyl-CoA oxidase and catalase activities in liver homogenates prepared from rats treated with either microencapsulated TCE in the feed or with TCE in corn oil by gavage (Table 6). Increases in palmitoyl-CoA oxidase and catalase activities were observed at similar dose levels in the dosed-feed and gavage studies. Treatment with corn oil, but not with the placebo capsules, caused a slight increase in palmitoyl-CoA oxidase activity.

DISCUSSION

Microencapsulated TCE was incorporated into rodent feed to compare the toxicity of TCE administered in microcapsules mixed in rodent feed to that of TCE administered by gavage in corn oil. Previously, we had shown that microencapsulated TCE was stable when mixed in rodent feed and stored for 7 days in an open vessel at room temperature (Melnick *et al.*, 1987). In the present studies, there were no significant losses of TCE after 7 days of treatment of male F344 rats with feed mixtures containing up to 10% microencapsu-

TABLE 6

EFFECT OF TCE ON HEPATIC PEROXISOMAL PALMITOYL-CoA OXIDASE AND CATALASE ACTIVITIES IN MALE F344 RATS[a]

Microencapsulated TCE (feed)			TCE in corn oil (gavage)		
:oup :)	Palmitoyl-CoA oxidase[b]	Catalase[c]	Dose group (g/kg)	Palmitoyl-CoA oxidase[b]	Catalase[c]
ited :ol	270 ± 12	8.49 ± 0.81			
) :rol	242 ± 17	7.98 ± 1.62	Corn oil control	318 ± 27[e]	8.59 ± 0.91
	298 ± 64	8.49 ± 1.92	0.6	369 ± 26	10.10 ± 1.82
	424 ± 55[d]	8.59 ± 1.31	1.2	413 ± 40[d]	12.83 ± 3.43[d,e]
	651 ± 148[d]	13.03 ± 2.02[d]	2.8	1002 ± 273[d,e]	13.54 ± 2.32[d]
	999 ± 266[d]	15.76 ± 1.11[d]			

sults are given as means ± SD; $n = 5$.
lues are expressed as nmol H_2O_2 produced × min^{-1} × g $liver^{-1}$.
lues are expressed as mmol H_2O_2 consumed × min^{-1} × g $liver^{-1}$.
fferent from controls, $p < 0.05$.
ifferent from corresponding feed group, $p < 0.05$.

ed TCE. Thus, rats feeding on these diets not cause deterioration of the microcap- ies. Since feeders are normally changed eekly in toxicity studies conducted by and or the National Toxicology Program, there oes not appear to be a concern that deterio- ration of the capsules and loss of the chemical would occur. The slight increase in TCE concentration in the feed blends during the study may be indicative of selective feeding by the rats. This increase, however, was less apparent between Days 3 and 7 than between Days 1 and 3 and had no impact on the outcome of the study. The decrease in feed consumption at the 5 and 10% dose levels of microencapsulated TCE (equivalent to 2.21 and 4.41% TCE in the feed) may be due to diminished palatability of the dosed-feed at these concentrations or to a toxic effect at these doses of TCE (2.2 and 4.8 g/kg/day). The present study does not clearly distinguish between these two possibilities. Feed consumption was also decreased in the corresponding high-dose gavage group; however, CNS depression was evident in these animals and might have contributed to their reduced level of feed consumption.

Administration of microencapsulated TCE in the diet of F344 rats for 14 days at levels up to 10% microcapsules (the time-weighted average dosage level of TCE at this concentration of microcapsules was nearly 5 g/kg/day) caused no deaths, while the highest dose level of TCE in the gavage study caused four deaths during the first week of the study; the time-weighted average dose of TCE during that time was nearly 3 g/kg/day. The dose level of TCE in the highest dose group of the feed study is nearly equal to the single dose oral LD50 of 7.2 g/kg for TCE in rats (Smyth *et al.*, 1969). Therefore, microencapsulation can provide a means of administering higher daily dose levels of unstable chemicals than is possible by single daily gavage treatments. Higher peak blood levels of TCE would be expected in animals administered single daily bolus doses of TCE by gavage than in those fed diets containing TCE. Thus, the method of dosing was probably the cause for the differences in lethality and clinical signs of toxicity (lethargy in only the high-dose gavage group) in TCE-treated rats. The use of microencapsulation in a feed study also eliminates potential problems of gavage trauma

and of bolus dosing effects, and in the case of TCE, more closely resembles the pattern of human exposure to this chemical. The decrease in body weight gain at the higher dose levels in the feed and gavage studies may be due to a toxic manifestation of the TCE treatment, CNS depression in the high-dose gavage group, decreased feed consumption, or a combination of these factors.

The liver and kidney were evaluated for evidence of TCE toxicity since they have been identified as target organs of this chemical (Torkelson and Rowe, 1981; NCI, 1976; Pessayre *et al.*, 1979; Kjellstrand *et al.*, 1981; Stott *et al.*, 1982; Tucker *et al.*, 1982; Elcombe *et al.*, 1985; NTP, 1987). In the present studies, microencapsulated TCE and TCE in corn oil produced dose-related increases in liver weight/body weight ratios and in kidney weight/body weight ratios. The effects of TCE on the liver, but not the kidney, are considered to be toxicologically significant since there were also increases in absolute liver weight and dose-related histopathologic alterations in this organ. The effects of TCE on liver weight and the frequency and severity of hepatic lesions were similar at comparable dosage levels in the feed and gavage studies. A similar increase in liver weight/body weight ratio was reported in male Osborne–Mendel rats treated with TCE by gavage for 3 weeks at a daily dose level of 1.1 g/kg (Stott *et al.*, 1982) or for 10 days at daily dose levels of 0.5, 1.0, and 1.5 g/kg (Elcombe *et al.*, 1985). Stott *et al.* (1982) did not observe any increase in the kidney weight/body weight ratio. Neither Stott *et al.* (1982) nor Elcombe *et al.* (1985) observed any histopathological changes in the livers of rats treated with TCE, whereas in the present studies individual cell necrosis was discernible in animals in the 2.2 or 4.8 g/kg dose groups of the feed study and in the 2.8 g/kg dose group of the gavage study. At dose levels similar to those used by Stott *et al.* (1982) and Elcombe *et al.* (1985), the frequency and severity of this lesion were not significantly different from those of controls.

Individual cell degeneration and necrosis in this study were characterized by nuclear pyknosis and karyorrhexis and cytoplasmic condensation of scattered single cells. These changes are morphologically compatible with what has been described as "piecemeal" necrosis as well as apoptosis (Zimmerman, 1980; Wyllie *et al.*, 1980). Apoptosis is the death of single isolated cells occurring with normal cell turnover in healthy tissues, during embryonic development, in endocrine-dependent atrophy and hyperplasia, or after exposure to cytoxic agents (Wyllie *et al.*, 1980). The observation of an increased number of necrotic cells in TCE-treated rats may reflect a perturbation in the kinetics of normal cell survival such that proportionally more cells are dying than in the normal steady state.

The induction of microsomal NADPH cytochrome *c* reductase activity in livers of rats treated with TCE in corn oil by gavage was similar to that reported by Pessayre *et al.* (1979). The minimal effective dose for induction of NADPH cytochrome *c* reductase activity was higher in animals treated with microencapsulated TCE in feed (2.2 g/kg) than in those that were administered TCE by gavage (1.2 g/kg). This difference may be due to the method of administration of TCE, since higher peak blood levels and perhaps higher tissue levels of TCE would be expected after the gavage treatment. Cytochrome *P*-450 levels were elevated in rats treated with microencapsulated TCE in the feed, but not in rats treated with TCE in corn oil by gavage. Pessayre *et al.* (1979) reported a decrease in cytochrome *P*-450 levels in rats treated with TCE by gavage. The extent of destruction of cytochrome *P*-450 following TCE treatment may be related to the tissue concentration of active metabolites of TCE. Low concentrations of TCE may lead to induction of cytochrome *P*-450, while high concentrations may also cause induction of microsomal enzymes, but in addition, result in levels of reactive metabolites of TCE which inactivate cytochrome *P*-450. Higher peak liver concentrations of TCE

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epatic peroxisomal enzymes catalase
itoyl-CoA oxidase were measured
combe *et al.* (1985) reported that in-
of peroxisome proliferation by TCE
in $B6C3F_1$ mice but not in Os-
lendel rats, and that this difference
nse may be related to the species
es in hepatocarcinogenicity of TCE.
esent studies, TCE produced dose-
ncreases in the activities of these en-
liver homogenates prepared from
s, particularly for palmitoyl-CoA ox-
duction of catalase and palmitoyl-
dase activity was slightly more pro-
in gavage-treated animals than in
which received TCE in the feed. This
ce may be related to the method of
rather than to the vehicle used for dos-
imals with TCE. The increase in he-
eroxisomal palmitoyl-CoA oxidase ac-
n F344 rats was not as great as that re-
by Elcombe *et al.* (1985) in $B6C3F_1$
. Furthermore, the doses of TCE used in
resent study included levels 2–3 times
ter than the highest level used by El-
mbe *et al.* (1985). The present study shows
t TCE-induced peroxisomal proliferation
ot specific for mice, but may be more pro-
unced in this species than in rats.

In conclusion, the stability of TCE in feed
s not altered by rats feeding on diets contain-
ng microencapsulated TCE. For the most part, the toxicological profile of microencapsulated TCE administered in feed is similar to that of TCE administered in corn oil by gavage. Differences in lethality, levels of cytochrome *P*-450, and induction of microsomal or peroxisomal enzymes were attributed to the method of dosing (gavage versus dosed-feed) rather than the dosing vehicle. Microencapsulation affords a high level of stability to TCE so that homogeneous mixtures of this volatile chemical can be prepared in rodent feed and used for toxicology studies (Melnick *et al.*, 1987). Microencapsulation provides an excellent alternative for studying the oral toxicological properties of volatile chemicals, such as TCE, in laboratory animals.

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