Applied Organometallic Chemistry (1990) 4 379–381

0268-2605/90/040379-03$05.00

COMMUNICATION

The synergistic effect of Polysorbate 80 upon the toxicity of tri-n-butyltin chloride

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Received 10 November 1989 *Accepted 5 February 1990*

Polysorbate 80 (Tween 80) was used as an emulsifying agent for administering tri-n-butyltin chloride (TBTCl) to rats below, at and above the LD_{50} for the compound. Tween 80 greatly enhanced the toxicity of the organotin compound. The LD_{50} in the presence of Tween 80 (16% of the emulsion) is less than half of the LD_{50} when the TBTCl was administered in corn oil.

Keywords: Polysorbate 80, toxicity, tri-n-butyltin chloride

Polysorbate 80 (Tween 80) (Chem. Reg. No. 9005-65-6) has been accepted as a common surfactant in pharmacological, biochemical and biological research and as an FDA-approved emulsifying agent or surfactant in the cosmetic and food industry.[1,2] Because of the assumption that it does not interfere with the systems being tested, it has wide use as a solubilizing agent. However, it has caused the release of histamine when applied to surfaces of animals[3] and increased the permeability of rabbit oral mucosa for eight non-electrolytes.[4] At levels above 6.8% in formulations, Tween 80 exhibits some toxic effects by itself,[5] e.g. some liver damage occurs at the 5–10% level[6] and at the 1–4% level may exhibit some synergistic effects.[7] Recent studies indicate that it carries inorganic ions across membrane barriers at a much higher rate than was previously anticipated.[8] Although corn oil is usually used as a carrier vehicle in most organotin (OTC) toxicity studies, especially with liquid OTC, many OTCs do not yield stable emulsions and questions exist as to the quantity of toxicant adminstered to the animals. Tween 80 was used because of its superior emulsifying properties and because it has been included in oganotin pesticide formulations to yield stable emulsions. The current work indicates that Tween 80 synergistically increases the toxicity of tri-n-butyltin chloride (TBTCl) (Chem. Reg. No. 1461-22-9).

Previous studies[9,10] on the gender-related oral toxicity of organotin compounds in Long Evans rats indicated that the most dramatic effects occurred within 24 h at 1.5 times the LD_{50} for the compounds (the LD_{50} for TBTCl in corn oil is 0.35 mmol kg^{-1} for rats.[11] To test this observation, an emulsion containing 0.5 g of TBTCl, 2.0 g of Tween 80 and 10 g of water was prepared using a tissue grinder and was administered by intragastric gavage to five male and five female Long Evans rats at a dose of 0.5 mmol kg^{-1}. [The quantity of TBTCl was gravimetrically determined.[12] The calculated concentration, based upon tin(II) oxide (SnO_2), indicated that the emulsions contained within ±4% of the amount indicated in Table 1.] One male and one female Long Evans rat were used as controls and administered the same volume of Tween 80 without the organotin compound. All rats administered the TBTCl succumbed within 17 h; neither control animal exhibited any adverse toxicity after 24 h. The probit curve for TBTCl would indicate that only 70% of the animals should have died within the first 24 h and about 90% within three days, the normal toxicity time interval for OTC studies.[9,11,12] When the experiment with the Tween 80 was repeated at the LD_{50} concentration for TBTCl using three animals (two male and one female), all animals died within 18 h.

To determine if the results, which conflicted with data in the literature, were due to an incorrect LD_{50}, a sensitive animal strain, or a synergistic effect of Tween 80 and TBTCl, parallel experiments with TBTCl in Tween 80 and in corn oil (Mazola® oil) were performed. Two male animals were initially used for each data point. Most of the rats in both trials exhibited diarrhoea (organotin compounds destroy the bacteria in the intestines), wheezing and tenderness upon palpation,

Table 1 Comparison of morbidity of Long Evans rats when TBTCl was administered in Tween 80 and corn oil

LD_{50} in corn oil (%)	No. of animals	Day 1	Day 3	Day 8
Tween 80				
150	10 (5 m, 5 f)	10	10	10
100	3 (2 m, 1 f)	3	3	3
90	1 (1 m)	0	1	1
75	2 (2 m)	0	2	2
60	2 (2 m)	0	2	2
50	2 (2 m)	1	1	2
Total	20	14	19	20
Corn Oil				
150	4 (2 m, 2 f)	2	4	4
100	4 (3 m, 1 f)	1	2	2
90	3 (3 m)	0	1	1
75	24 (14 m, 10 f)	3	5	9
60	2 (2 m)	0	0	1
50	2 (2 m)	0	0	0
Total	39	6	12	17

F value probability

F	Meaning	Degrees of freedom	Calculated value	Significance at 0.05 probability
F_m	Difference in carrier	5×5	150.7	5.05
F_p	Difference in conc.	5×10	5.07	2.52
F_{pxm}	Carrier and conc.	5×10	1.22	2.52

which are common intoxication symptoms for organotin compounds.[9–11]

All animals were allowed to eat and drink *ad libitum* pre- and post-administration of the chemical. The food and water consumption was monitored. They consumed about 25 g of food and 30 cm^3 water per day. The majority of the animals administered Tween 80 by itself or TBTCl in corn oil returned to eating and drinking after 48 h, about 50% of the food consumed before administration of the TBTCl, whereas none of the animals administered the Tween 80 plus TBTCl resumed a normal feeding pattern within the time frame of this study and most ate or drank nothing.

As shown in Table 1, the morbidity of the animals administered the TBTCl in Tween 80 was greater than that of the animals adminstered the TBTCl in corn oil.

Since almost all the animals in the Tween 80 trial succumbed within three days, further use of the surfactant was abandoned for the project. The toxicological work on the gender effect was maintained with corn oil, primarily at 0.75 times the LD_{50}, and these data were also used in the statistical computation. Since only a small number of animals were used in the original study, all animals, which had been administered TBTCl by the same procedure in the author's laboratory, were grouped for data processing. Since most of the work was performed for other research purposes, an unusual distribution of animals and concentrations occurred. The split-plot design[13] was used to determine the *F* probability for significance (Table 1) using the six concentrations and three morbidity observations (total mortality at 24 h, three days and eight days) after normalizing for the different numbers of animals at each concentration. The F_m probability (influence of carrier upon the results) was 150.7, a very significant number. The F_p probability (influence of concentration upon the results) was 5.07 which, being greater than 2.52, is a significant value at the 0.05 level. The F_{pxm} (combination of carrier and concentration) was 1.2, an insignificant number. The first two effects are easy to rationalize; all the animals in the Tween 80 trials died whereas only 44% in the corn oil trial succumbed in eight days. An increase in concentration obviously increases the toxic effect. The lack of significance for carrier and concentration is probably due to the small number of animals in the Tween 80 sets and the smaller difference in total mortality (eight days) at any concentration.

The enhanced toxicity of TBTCl with Tween 80 may be similar to the enhanced liver damage caused by 1,1-dichloroethylene in the surfactant[14] or by the replacement of the bile acids in absorption mechanisms as previously seen with fat absorption in the presence of Tween 80.[15] Tween 80 is also reported to show synergistic effects with suspected toxic agents when compared with corn oil, but no previous synergistic effect was observed until the Tween 80 concentration was greater than 16% of solvent,[16] which is approximately the same level as used in this study.

Although the work is only preliminary, and the involvement of both sexes in the study could skew the data, nevertheless Tween 80 seems to enhance the oral toxicity of TBTCl on a time and concentration basis when compared with the same compound in a corn oil carrier. With Tween 80 as a carrier, the LD_{50} for TBTCl appears to be about 0.17 mmol dm^{-3} kg^{-1}, or half of the previous reported value. Because of the wide utilization of Tween 80 in food and organotin compounds in food packaging (more than 5 000 tons

per annum), further studies are necessary. Until better toxicological data are available, researchers should use caution in evaluating or comparing data when Tween 80 is present. Further work is definitely necessary to determine if this synergistic effect is selective to organotin compounds or is a general toxicological phenomenon shown by all substances.

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