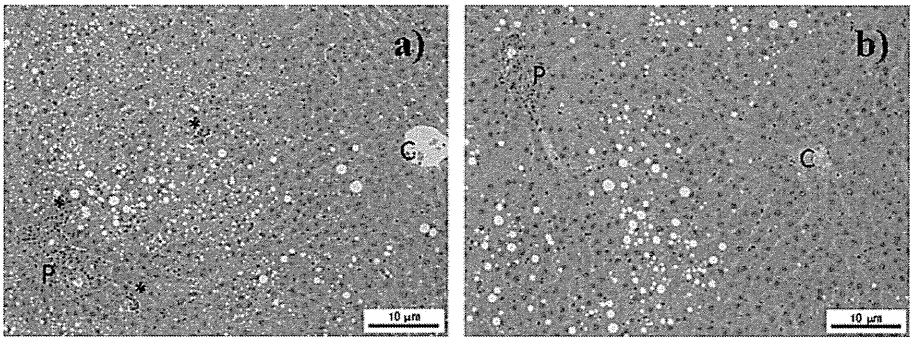

FIG. 4. Histopathological observation of the liver in the subchronic toxicity test in rats administered DOTH. (a) Control (base diet) and (b) DOTH (300 mg/[kg body weight]/day). C, central vein; P, Glisson's capsule; *, slight infiltration of inflammatory cells located around Glisson's capsule. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the total amount of the plasticizers released from the sheets was higher than that of the DEHP-PVC sheet. Conversely, although DINCH®-PVC and DOTH/DINCH® (10:49.5) sheets had less plasticizer leached, the hemolytic ratios were higher than the DEHP-PVC sheet. The data for the DINCH®-PVC sheet are in agreement with a study by Dumont et al. [26] in which DINCH®-PVC bags exhibited protective effects against RBC hemolysis that were similar to normal DEHP-PVC containers under periodic mixing conditions, but not adequate under static conditions.

The type of anticoagulant and the viability of RBCs may be closely correlated with hemolysis suppression of the plasticizer [27]. The RCC product used in Japan contains the MAP solution as an anticoagulant, whereas the SAGM solution is widely used in other countries. Hence, hemolysis suppression with SAGM/RCC was also performed, and the results are shown in Fig. 2. The hemolytic behavior using SAGM/RCC was almost identical to the result yielded from MAP/RCC described above. The DINCH®-PVC sheet exhibited significantly greater hemolysis suppression than that shown by the control (no sheet) and TOTM-PVC sheet ($p < 0.01$). The hemolysis suppression effect of DOTH/DINCH® (25:33) PVC sheet was higher than that of the DINCH®-PVC sheet ($p < 0.05$) and equal to that of DEHP-PVC sheet. Thus, the potential of the DOTH/DINCH® (25:33) PVC sheet as part of a suitable blood container was reaffirmed with SAGM/RCC.

Subchronic Toxicity of DOTH in Rats

The growth rate and feed consumption in the DOTH groups were comparable to those in the control group throughout the study. The mean dietary intake of DOTH administered to rats at the highest dose level was calculated to be 297 mg/(kg body weight)/day based on food consumption. The treatment-related macroscopic change was not detected in any of the organs except for a fatty liver observed in the control and DOTH groups. There was no significant difference in the absolute and/or relative weights shown in Tables 3 and 4. In the histopathological examination, no abnormalities were observed in the testes of rats in the control and treated groups, and all rats were normal (Fig. 3). Diffuse fatty change of hepatocytes and inflammatory cell infiltration were noted in several rats of both the control and the treated groups in the liver (Fig. 4). Although these changes are not common in rats of this age, they were not due to treatment-related toxicity. The changes may be related to the rats' diet. The prepared food included oil in the basal diet, which resulted in high calorie consumption of all groups including the control group. Other histopathological changes, such as focal inflammation in the prostate and focal myocarditis, were scattered in all groups. All of these observations are known to be common changes in rats, and their incidences or intensities were comparable among the control and the treated groups, indicating they were not treatment-related. Other organs including the epididymis and kidneys were not affected by the treatment with DOTH even at the highest dose. From the hormone assays, no significant differences in the number of sperm cells in the epididymis and the levels of TS, E2, FSH, and TSH in serum were observed between each group including control, as shown in Figs. 5 and 6. Based on the results of the study, the no-observed-adverse-effect level (NOAEL) of DOTH was found to be 300 mg/(kg body weight)/day.

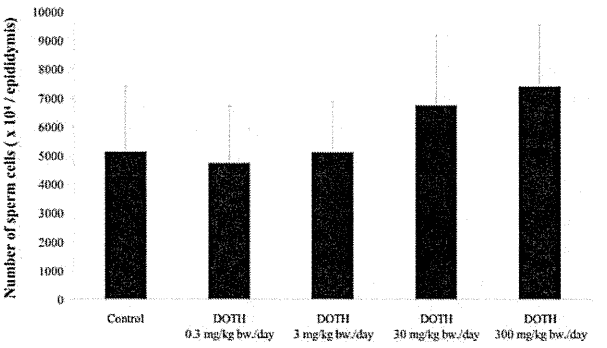

FIG. 5. Number of sperm cells in the epididymis of rats administered different amounts of DOTH for 13 weeks.

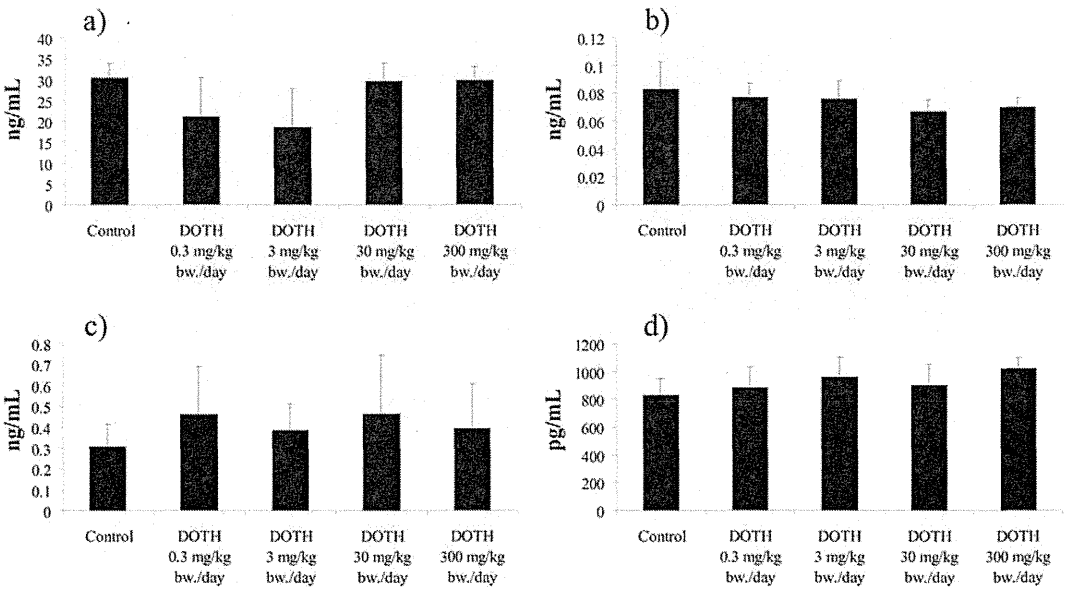

FIG. 6. The levels of hormones in the blood of rats administered different amounts of DOTH for 13 weeks. (a) TSH, (b) FSH, (c) testosterone, and (d) 17β-estradiol.

Poon et al. [28] reported that DEHP caused hepatomegaly to Sprague-Dawley rats by the administration of the diet containing the plasticizer at the concentration of 5,000 μg/g for 13 weeks. They reported there was mild to moderate seminiferous tubule atrophy and Sertoli cell vacuolation in male rats at 375.2 ± 98.5 mg/(body weight)/day, rats of both sexes showed hepatic peroxisome proliferation at the same dose. They also found that DEHP caused mild histological changes in the thyroid, consisting of reduced follicle size and colloid density, and the liver, consisting of endothelial nuclear prominence, nuclear hyperchromicity, and anisokaryosis at 5,000 μg/g. In addition to the results of other experiments, Poon et al. judged the NOAEL of DEHP in the rats to be 3.7 mg/(kg body weight)/day for testicular toxicity. In contrast, these histological changes were not observed in our study of rats treated with DOTH even at the highest dose described above. Our findings indicate that DOTH has no toxicity in the subchronic study in male rats, thereby suggesting that DOTH is a less toxic plasticizer than DEHP with the NOAEL at 300 mg/(kg body weight)/day.

The weak toxicity of DOTH was also found in another Japanese report [18], in which the NOAEL in the male rats was determined to be 100 mg/(kg body weight)/day. In this case, centrilobular hepatomegaly and a significant increase of liver and kidney weights were observed after treatment with DOTH at 300 mg/(kg body weight)/day in the repeated oral administration for 56 days. It was also confirmed that these changes were reversible in that the recovery was observed by 2 weeks after stopping the administration [18], no effect of DOTH was detected in testing for reproductive/developmental toxicity [18], bacterial reverse mutation [19], and chromosomal aberration [20].

CONCLUSIONS

Our data suggest that the DOTH/DINCH® (25:33) PVC sheet may be the most suitable material to replace the DEHP-PVC sheets used in traditional RBC storage bags because of its ability to suppress hemolysis and elute less plasticizer. A prototype of a blood container made of this sheet is now in progress: a clinical trial with the cooperation of a suitable number of volunteers will be performed in the near future. We will evaluate the chemical, physicochemical, biological, and dynamic properties required for a medical device product.

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