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Fate of biocides used in leather industry and their environmental impact

Wenkai Zhang

New Zealand Leather and Shoe Research Association, Palmerston North, New Zealand

1. Introduction

Leather is a durable material created by tanning animal skin and rawhide. As a biological product, leather is susceptible to microbial attach due to its high protein composition[1]. Additionally, in term of the leather industry, the process of manufacturing finished leather product from rawhide and skin is usually segmented. Preserved hide and skin, or leather processed to intermediate stages are often traded and transported internationally. They might be stored or in transport for months before being manufactured into finished products[2]. Those intermediate products contain more sources of nutrients and water for microbial growth. Therefore, preservation of raw materials and intermediate products is critical for the leather industry[1].

Protection against microbial damage is achieved by the application of biocides which are substances intend to destroy or exert a controlling effect on harmful organism[3]. In leather industry, biocides usually refer to bactericides and fungicides. Some biocidal substances have both functionalities[1]. Bacteria are the major concern in the early stage of leather processing (e.g. curing and soaking) as they can rapidly decompose untanned proteins (in rawhide and skin)[2]. After tanning, leather is less susceptible to decomposition by bacterial attack due to alternation of protein structure. However, tanned wet leather contains several components such as ammonium salts and fat liquoring agent that enable fungal growths causing damages on the leather[4].

Currently, there are many formulated biocide products sold commercially containing various mixture of biocides. The following substances: p-chloro-m-cresol (PCMC), o-phenylphenol (OPP), octylisothiazolinone (OIT) and 2-(Thiocyanomethylthio) benzothiazole (TCMTB)

account for 95% of the active substance in those commercial products[5]. Much effort has been made in studying the direct health effect of biocide substances presence in leather product on users[6]. More studies are required to fill in the gap of knowledge of the environmental impact of biocides, especially in the content that biocides are applied in the intermediate processing stages and might be washed off or degraded in downstream processing[7].

Raw hide and skin need to be cured with salt on the day of its removal from the animal unless it is stored at low temperature or processed immediately. Curing can prevent the growth of most non-halophilic bacteria, but biocide is necessary to stop the growth of halophilic bacteria[1]. Dichlorophen had been widely used for this purpose until it was disapproved in the European Union[8]. It was then replaced by other phenolic biocides such as PCMC and OPP which are also commonly applied in pickled pelt or wet blue leather. This creates another layer of complexity for regulation compliance of not exceeding the maximum level allowed. To address this problem, this study quantified the amount of dichlorophen, PCMC and OPP applied to salted skin in the following processing stage and also to find out how much of the biocides was lost in the processing waste.

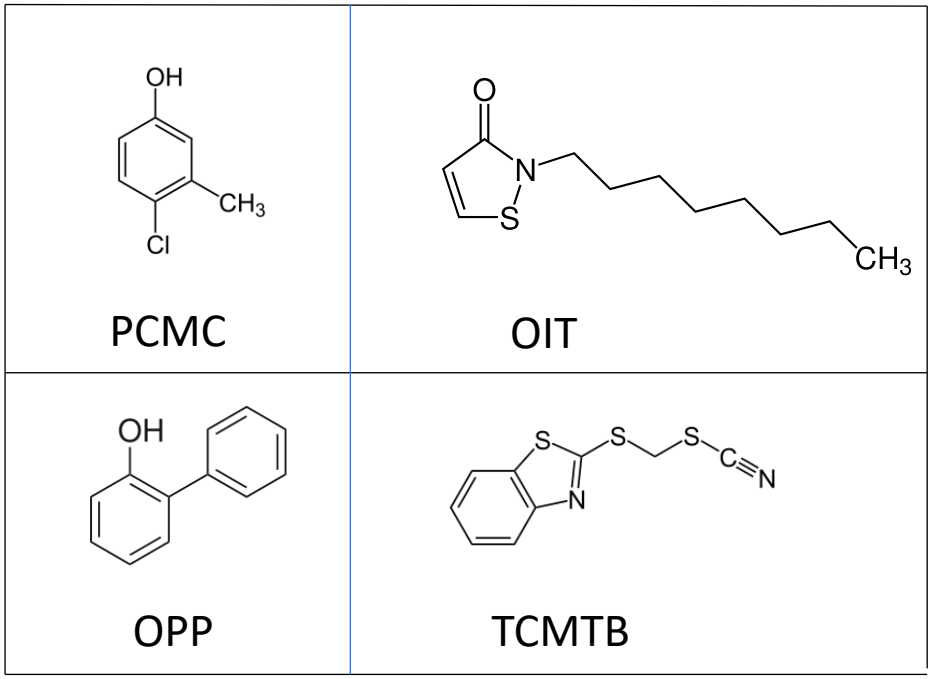

Figure 1. Chemical structure of PCMC, OPP, OIT, and TCMTB.

In the leather industry, fungicides represent about 15% of the cost of chemicals in wet blue processing[4]. This study quantified the uptake of these biocides by chrome-tanned wet blue leather and the amount lost in the float. Furthermore, the four biocides mentioned above are classified into two chemical families of phenolic (PCMC and OPP) and heterocyclic

compounds (OIT and TCMTB)[4]. While the mechanism of action and chemical stability of the two phenolic biocides are well understood, heterocyclic biocides especially TCMTB gained most of the interest for its claim to have excellent and broad-spectrum antimicrobial activity in lower concentration than the phenolic biocides[4]. While the degradation of TCMTB in the content of wastewater treatment has been well studied[7, 9, 10], there is little evidence showing how it degrades on the leather product and how that affect its fungicidal activity. This report presented data tracking the degradation of TCMTB applied on wet blue leather stored in different conditions and the implication on its usage.

2. Materials and Methods

2.1 Chemicals

All solvents (water, acetonitrile, and methanol) used for HPLC analysis were Optima® grade purchased from Fisher Scientific, USA. PCMC and OPP were purchased from Lanxess AG, Germany. OIT was purchased from Rohm and Haas, Germany. TCMTB was purchased from Shamrock chemicals, New Zealand. Dichlorophen was purchased from Alfa Aesar, USA. All other chemicals used in the leather processing were technical grade.

2.2 Leather processing

2.2.1 Salting of raw skin

Raw sheep skins were salted based on their weight. 45% (w/w) sodium chloride, 0.5% (w/w) boric acid and 0.05% (w/w) dichlorophen were mixed in a drum for 5 mins before adding three pieces of raw skins to the drum. The skins were salted in the rotating drum for 1.5 hours, then stacked flesh-to-flesh in a pile and left for seven days before further processing. Another three pieces of raw skins were salted in a separated drum the same way except that dichlorophen was substituted by 0.1% (w/w) of a commercial fungicide product consist of 27-43% PCMC and 40-60% OPP.

2.2.2 Dehairing, liming and deliming and pickling

Salted sheep skins were rehydrated for 1 hour with 100% per skin weight of water. The sheep skins were dehaired by a paint consist of 200g/L sodium sulphide, 45g/L sodium hydroxide, 50g/L calcium hydroxide and 25g/L Starch thickener. Two hours after painting, the wool was pulled, and the skins were limed in 80% water (wt./skin wt.) in a drum rotating intermittently overnight. The limed skins were washed with 100% water three times. 0.2% (wt./skin wt.) of

30%(w/w) hydrogen peroxide was added to the drum to scavenge residual sulphide, before 2% (wt./ skin wt.) ammonium chloride was added to neutralise the alkaline. The skins were then delimed after 75 min rotation in the drum at 35°C.

2.2.3 Chrome tanning

6% (wt./ hide wt.) basic chromium sulphate (25% Cr2O3 equivalent, 33% basicity) was added to the hide in the pickling liquor for tanning. After 3 hours mixing, 0.25% sodium formate and 0.25% magnesium oxide were added to the liquor for basification. And the hide was chrome-tanned overnight at 40°C.

2.3 Biocides uptake experiment

Four 20cm*20cm pieces (each weighed about 1 kg) were cut from the middle of a chrome-tanned wet blue leather. 0.05%(w/w) active ingredient equivalent of fungicide products of PCMC, OPP, OIT and TCMTB were added to 1 kg of water in four different drums. The wet blue leathers and the float were sampled right before the leather was added to the float and after 0.5-hour processing and every subsequent hour until 4.5 hours.

The float samples were analysed directly after filtering through 0.45μm membrane filters. And the wet leathers were analysed following method described in section 2.5.

2.4 Storage experiment for TCMTB degradation

A storage experiment was conducted to study the durability of the fungicidal effect of TCMTB. It also aimed to study the effect of storage condition on the degradation rate of TCMTB, and also the degradation products. Three pieces of wet blue hides were treated with 0.05%(w/w) TCMTB, and then each was cut in half along the backbone. Three halves were well wrapped with black plastic and stored in NZ leather and shoe research association (LASRA)'s hide store (a room sheltered from sunlight and with little ventilation). The remaining three halves were stored outside the facility under direct exposure to sun. Samples were taken from each of the six halves in April, May, and every two months subsequently. Sample was tested for TCMTB as described in following sections. The TCMTB content was normalised to the first sample analysed in April.

2.5 Sampling and sample preparation

Samples were cut from the skin or leather in accordance with ISO 2418:2017 in the area of the bend at one side of the backbone and were prepared in accordance with ISO 4044:2017. The samples were cut into pieces of 0.5cm*0.5cm with blades instead of grinding. For wool skin samples, the wool was shaved off by blade first. The diced samples were freeze-dried instead of oven dried to avoid exposure to heat for the purpose of degradation study. The sample weights before and after drying were recorded for moisture correction.

2.6 Extraction

The prepared samples were extracted in accordance with ISO13365-1:2020 for the analysis of PCMC, OPP, OIT and TCMTB, as well as the degradation products of TCMTB. One g of sample was extracted with 10 ml of acetonitrile in a glass vial in an ultrasonic bath for 1 hour at room temperature. For the analysis of dichlorophen, 1 g of sample was extracted with 10 ml methanol in a crimp cap glass vial in an ultrasonic bath for 1 hour at 65°C. After cooling down to room temperature, the extract was filtered through a 0.45µm membrane filter before HPLC analysis.

3. HPLC Method development

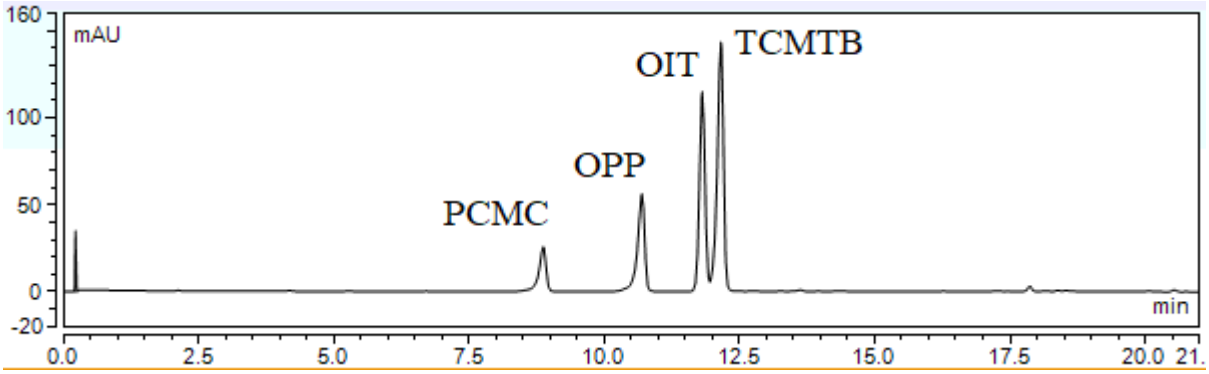

Figure 2. Chromatogram of biocides standard analysed under conditions detailed in ISO 13365-1:2020 Annex A. Column: Thermo Hypersil GOLD (C18, 250mm/4.6mm, 175Å, 5µm). Flow rate: 1.0ml/min. Mobile phase: A: water, B: acetonitrile. Gradient: 60%B for 6 min isocratic, then linear to 95%B in 9 min. Detection: 275nm.

ISO 13365-1:2020 Annex A [11] detailed conditions for chromatographic analysis of PCMC, OPP, OIT and TCMTB. Figure 2 shows a chromatogram of a mix standard solution containing the four biocides. Under these conditions, OIT and TCMTB were not fully resolved. It is to be noticed that the Annex of this ISO method serves as informative examples instead of requirements. It was used as a starting point to optimise the HPLC method. After effort on changing water/MeCN gradient made no significant improvement of the resolution of OIT and TCMTB, the option of adding a third solvent (methanol) to the mobile phase was explored.

Figure 3 shows the chromatograms of an experiment fixing water content at 50% in the eluent of isocratic run and substituting different portion of organic solvent from acetonitrile to methanol. As summarised in Figure 4, methanol significantly increases the retention of OIT in comparison to the other three analytes. Methanol is known to have slightly lesser elution strength than acetonitrile[12], thus the retention time of all analytes increased as MeCN was substituted to MeOH. Methanol as a protic solvent is also known to have different separation selectivity than the aprotic solvent acetonitrile[13].

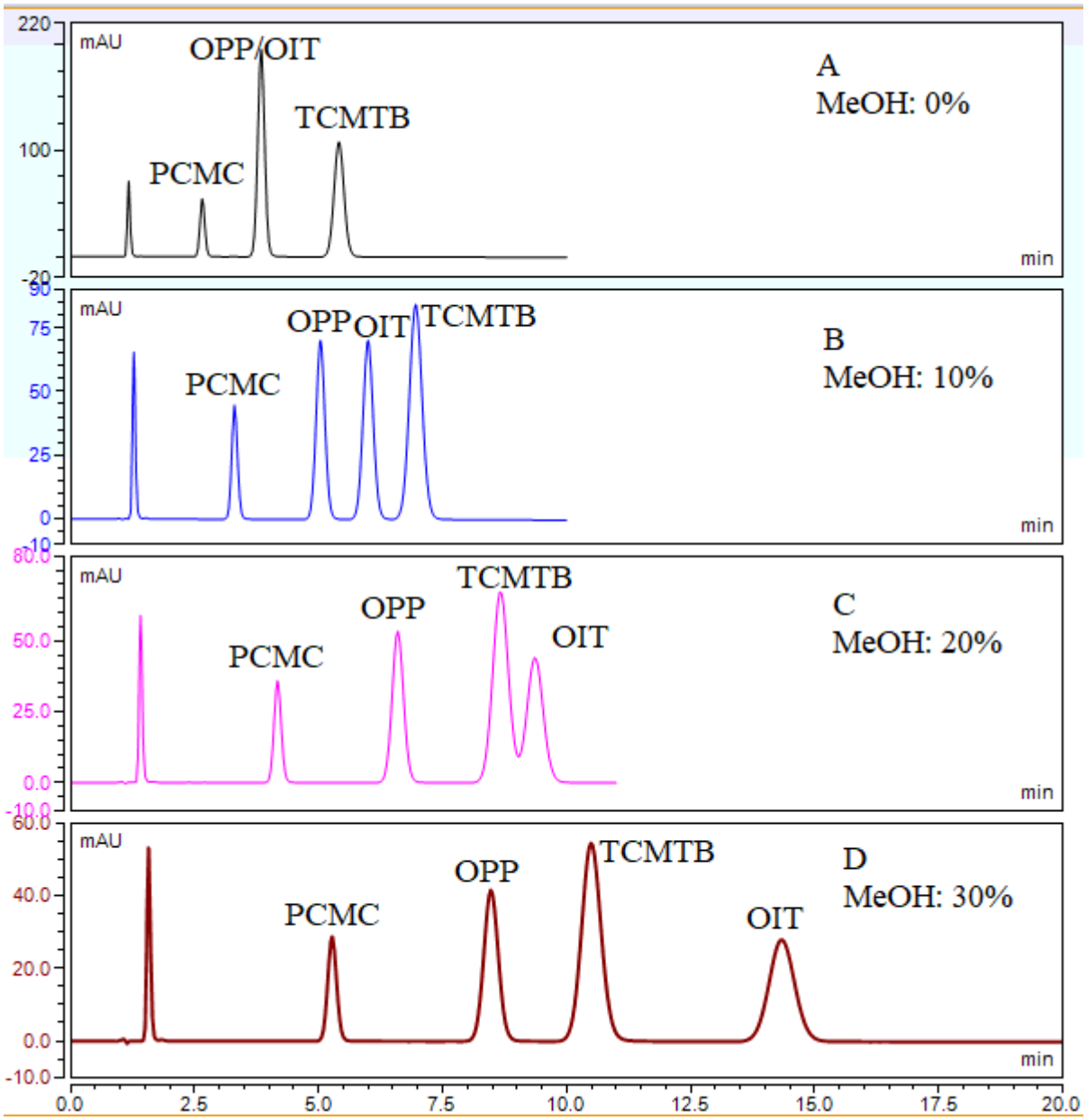

Figure 3. Effect of methanol on the separation of four biocides. Column: Phenomenex Gemini NX-C18 (30mm/4.6mm, 110Å, 3µm). Flow rate: 0.4ml/min. Mobile phase: isocratic 50% water, indicated% MeOH, (50%-indicated%) MeCN. Detection: 275nm

Mixing a portion of methanol in the eluent is shown to be a useful tool to manipulate the separation of the four biocides in regular reverse phase HPLC column, this mechanism is

applicable to any other C18 columns. To further prove the advantage of this method, separation was achieved using isocratic elution with fix 10% methanol and different water/acetonitrile ratios. The elution order and baseline-resolution were maintained, while runtime was greatly shortened with increased acetonitrile% and fixed methanol%.

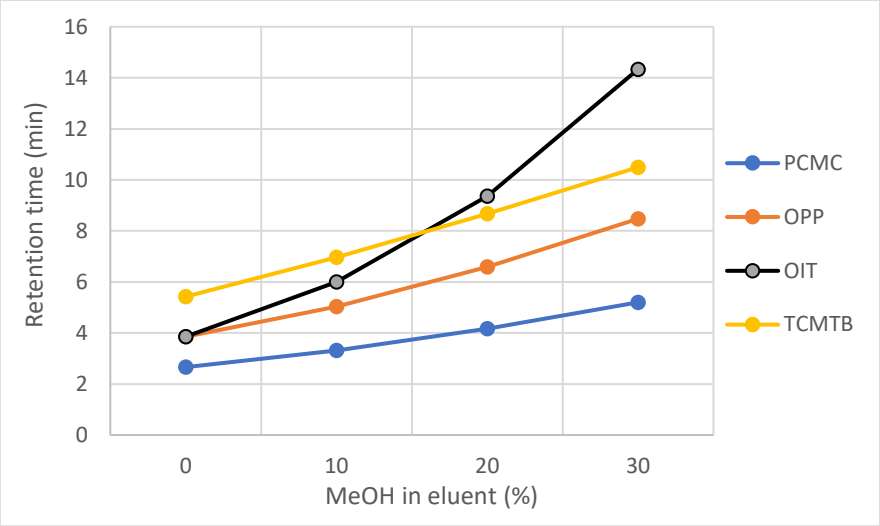

Figure 4. The retention time of biocides.

Limit of detection (LOD) and limit of quantitation (LOQ) were not calculated because analysis of calibration standard of the lowest concentration (2 mg/L) specified in ISO 13365-1:2020 gave signal-to-noise ratio well above 10 for all four analytes.

Dichlorophen was analysed in the following conditions:
Column: Phenomenex Gemini NX-C18 (30mm/4.6mm, 110Å, 3µm). Flow rate: 1.0ml/min. Mobile phase: isocratic 45% water, 55%MeCN. Detection: 286nm.

The metabolites of TCMTB were analysed in the following conditions:
Column: Phenomenex Gemini NX-C18 (30mm/4.6mm, 110Å, 3µm). Flow rate: 1.0ml/min. Mobile phase: A: water, B: acetonitrile, gradient: 30%B for 1.5 min, then from 30% to 90%B in 1 min, 90%B for 2.5 min. Detection: 252nm (BT, HOBT), 282nm (TCMTB, MTBT), 325 nm (2-MBT).

4. Results and discussion

4.1 Retention of biocides applied to salted skin in subsequence processing stage

This experiment aimed to find out how much biocides applied to the salted skin retains following dehairing, liming and deliming. These processes are usually carried out subsequently and the skin won't be stored until it is pickled or tanned. Further biocides would be applied if the skin is to be stored or transport as pickled pelt or wet leather. It is important to sample the delimed skin right before pickling and analyse for biocides.

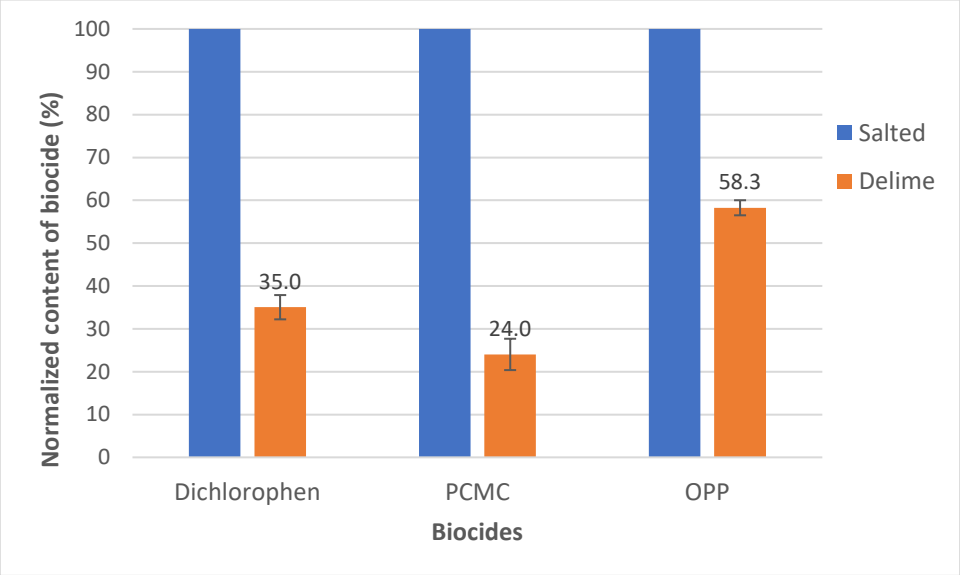

Figure 5. Biocides content in delimed sheep skins normalized to that as in the same piece of salted skin before liming.

Phenolic compounds being weakly acidic, is generally more soluble in alkaline condition. It is reasonable to propose that those phenolic biocides (dichlorophen, PCMC and OPP) will be solubilized and removed from the skin by the highly alkaline condition during the liming process. Surprisingly, a significant proportion of the biocides is retained on the skin. This might be the result of the hydrophobic phenolic biocides diffusing into the lipid of the skins. This is supported by the finding that the most hydrophobic compound OPP was the most prominent in the skins among the three.

As PCMC and OPP are commonly use in both salting and the wet blue stage of leather processing, applying the same biocides twice without knowledge of the amount carried over might lead to duplicate dosage and cause the product to exceed the regulation limit. However, looking from an economic perspective, carrying over of a portion of biocides from cured skin might have sufficient biocidal activity in pickle pelt or wet blue leather.

As for dichlorophen which is phased out in the EU market, leather manufactured from dichlorophen preserved salted skin would still contain excessive dichlorophen and be excluded from the EU market.

4.2 Uptake of biocide applied to wet blue from the processing float

As mentioned in the introduction, biocides account for a substantial cost among the processing chemicals in leather tanning. This experiment aimed to study the rate of the biocides absorbed onto the wet blue leather at recommend does and the amount remained in the processing float.

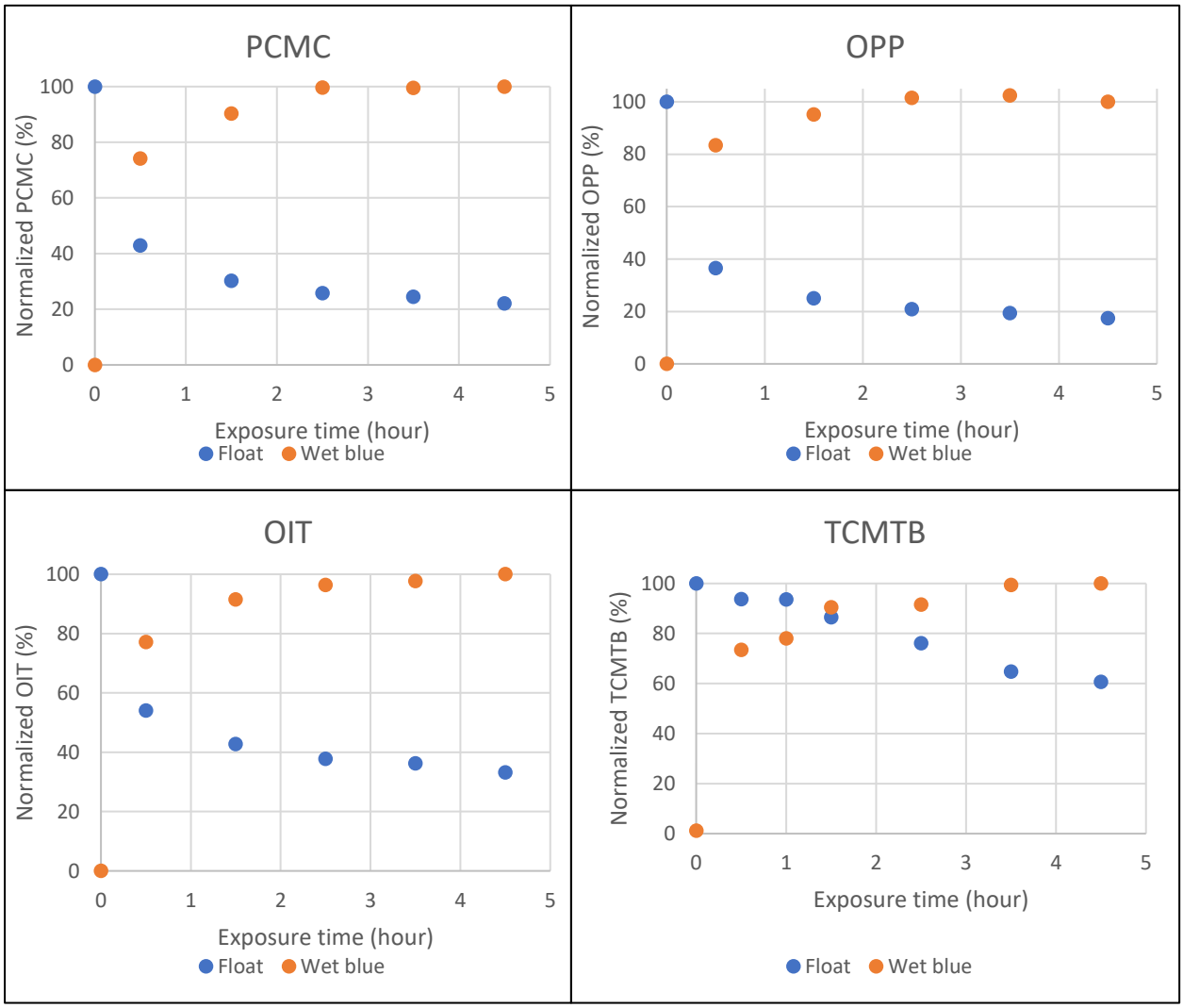

Figure 6. Uptakes of four different biocides applied to the processing float by wet blue leather. The biocides level in the float was normalized to the starting condition (before wet blue leather was put in the float). And the biocides level in the wet blue leather was normalized to the endpoint (when taken out of the processing float after 4.5 hours).

As shown in Figure 6, biocides applied to the processing float are absorbed by the wet leather rapidly. All four biocides tested (at their recommended does) reached at least 90% of their maximum concentration in the wet blue leather after 1.5 hour of processing. The distribution between the wet leather and the processing float differed depending on the biocide tested. After

prolonged processing time (4.5 hours), about 20% of PCMC and OPP remained in the processing float. This value was higher for OIT (33%) and significantly higher for TCMTB (60%). This might be due to the fact that in contrary to the other biocides which are more soluble in water, TCMTB forms an oily suspension in the processing float and sticks to the wall of the drum.

Overall, the results showed that most of the biocides applied to wet blue leather left in the processing float would go to wastewater if not recycled. With carful adjustment of the dosage, it would be both economically and environmentally wise to recycle the processing float.

4.3 Degradation of TCMTB upon storage

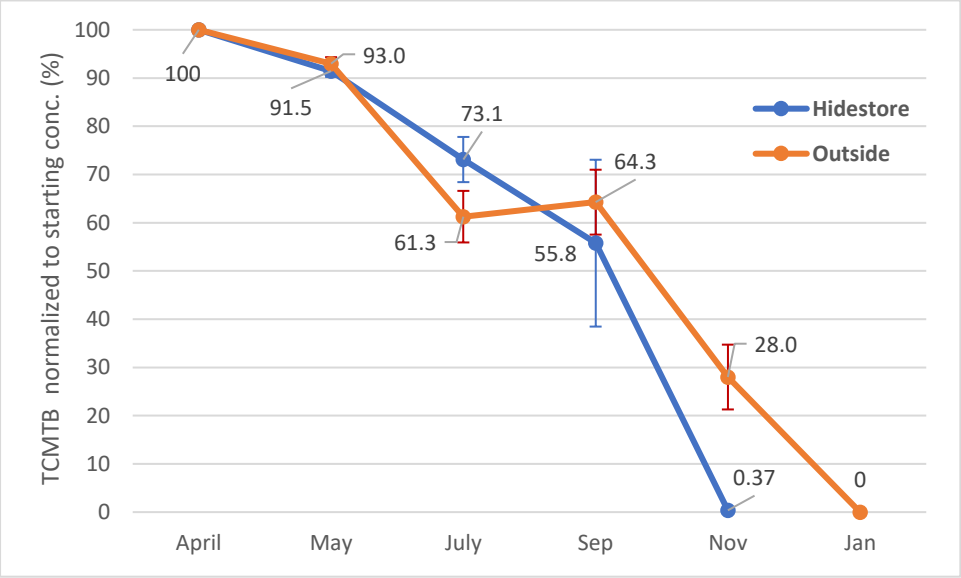

Figure 7. TCMTB concentration in wet blue hide samples normalized to initial TCMTB concentration.

As shown in Figure 7, TCMTB on wet blue leather was completely degraded in seven to nine months depending on storage conditions under this experimental setting. It wasn't until the last sampling timepoint showing fully degraded TCMTB that apparent fungal infestation was observed. The storage condition had an impact on the degradation rate of TCMTB on the wet blue leather. It was initially predicted that the environment in our hide store would slow the degradation of TCMTB. In the contrary, there was about 28% TCMTB remaining on the wet blue leather stored outside when it fully degraded on the wet blue in our hide store. When carefully examining the trend, TCMTB degraded faster initially in the wet blue leathers stored outside the facility. Then there was a halt between July and September. It is likely due to the fact that our hide store having no air conditioning is actually warmer in the winter (of southern

hemisphere). And the lower temperature outside the facility slowed down the degradation of TCMTB on the wet blue leather.

4.4 Degradation products of TCMTB

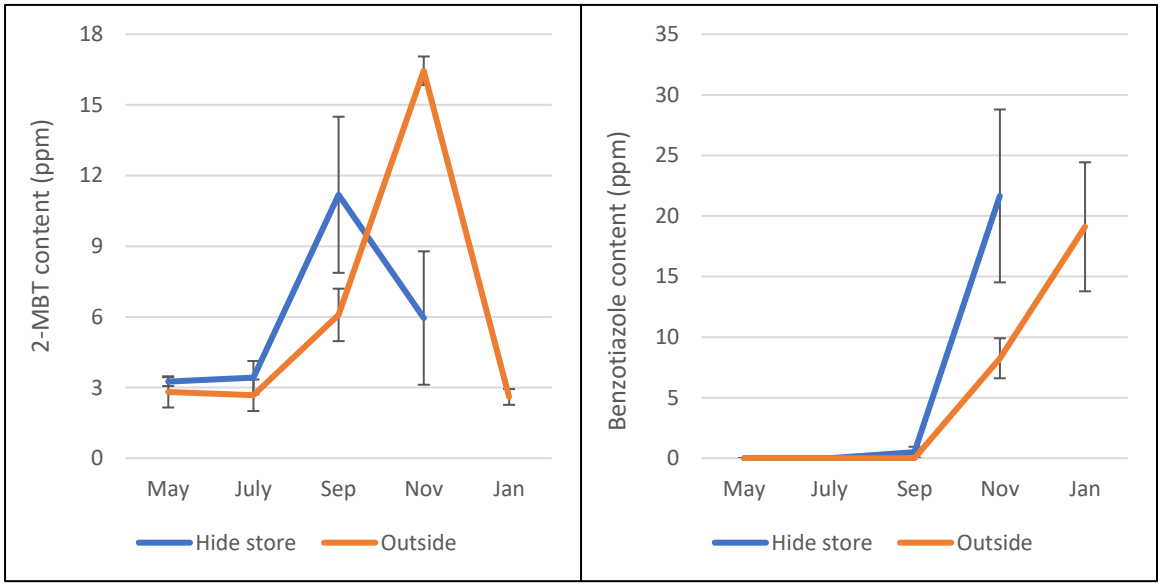

Figure 8. Degradation products of TCMTB found in the wet blue leathers.

When analysed for TCMTB, the same samples of the wet blue leathers were also analysed for the degradation products of TCMTB. Two of the proposed degradation products (2-MBT and benzothiazole) were detected in the wet blue leathers after several months of storage. And their trends provided informative evidence for the degradation pathway of TCMTB. As shown in concentration. Figure and Figure 8, a spike of 2-MBT was detected in the wet blue leather at one timepoint before TCMTB fully degraded. And then 2-MBT declined in the subsequent timepoint. Benzothiazole was barely detected until the timepoint when a significant portion of TCMTB was degraded. And the upward trajectory coincided with the decline of 2-MBT. These evidences support the proposed degradation pathway in which TCMTB degrades to 2-MBT, which further degrades to benzothiazole.

The other two transformation products proposed by Brownlee et al.[9] (MTBT and HOBT) were not detected. Consistent with the proposed mechanism (Figure 9), methylation of 2-MBT to MTBT if mediated by bacteria found in soil. And oxidation of benzothiazole to HOBT require reactive oxygen species. Either conditions were not present in our experiment setting. Therefore, our studies suggest that benzothiazole is the degradation end-product of TCMTB applied to wet blue leather.

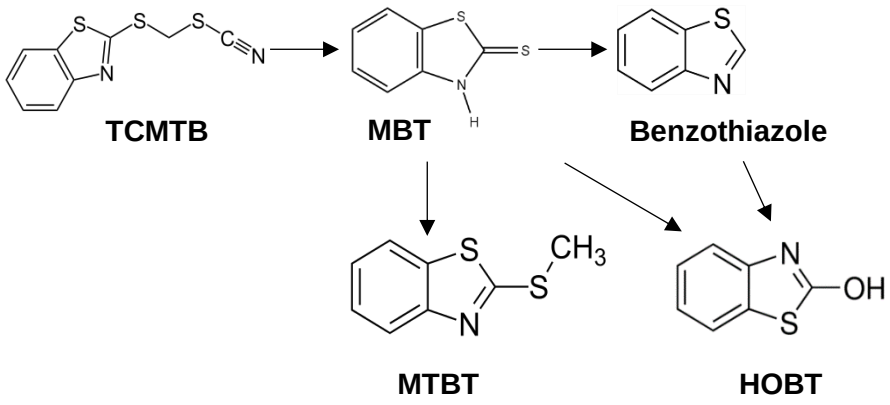

Figure 9. Proposed degradation pathways of TCMTB. (modified from Brownlee et al.)[9]

5. Conclusion

This study showed an optimised HPLC method for the analysis of PCMC, OPP, OIT and TCMTB under ISO 13365:2020, and more importantly a transferable way to optimize the HPLC parameters for improved separation and reduced runtime. Using this analytical method, this study showed the partial retention of some biocides applied to salted skins following downstream processing, which could be beneficial for cost saving but also imposes challenge for regulation compliance. By quantifying the absorption in four commonly used biocides by wet blue leather we found that TCMTB is most likely to be wasted in the processing float. By recycling the processing float and carefully modifying the dosage, less biocide will be discarded in wastewater. Further, the temperature of the storage condition of the wet blue leather affected the degradation rate of TCMTB. It also showed that the antimicrobial activity of TMCTB was retained until its degradation. Finally, the study provided data supporting the degradation pathway of TCMTB confirming benzothiazole as the degradation end-product in wet leather.

6. Suggestion for Future Work

Biocides applied in salted skins shall be tracked further down the processing stages (i.e. chrome-tanned). Biocides applied in wet blue leather shall also be tracked in further processing stage (crust). Recycling biocides containing processing float shall be conducted as suggested by results in section 4.2. And a gas chromatography method detecting benzothiazole will be developed as an indicator for TCMTB degradation.

7. Reference

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New Zealand Leather & Shoe Research Association Inc.

LASRA®

Fitzherbert Science Centres, Dairy Farm Road, Palmerston North
PO Box 8094, Hokowhitu, Palmerston North 4446
Ph: (06) 355 9028 Fax: (06) 354 1185
Email: info@lasra.co.nz

Fate of biocides used in leather industry and their environmental impact

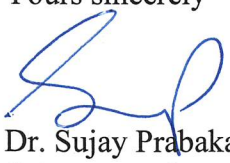
Biocides are essential for the leather industry as it preserves the value of raw skin and hide as well as processed wet leather prone to microbial deterioration. However, the fate of biocides needs to be tracked during processing stages for environmental and legislative purposes. This study focusses on quantifying the uptake of common phenolic biocides (PCMC and OPP) and heterocyclic biocides (OIT and TCMTB) in chrome-tanned wet blue leather and the float.

I would like to thank the selection committee of the 2020 IULTCS/IUR Young Leather Scientist Grant for giving Wenkai Zhang the opportunity to conduct this work.

The major findings can be summarized below.

- The partial retention of PCMC and OPP applied to salted skins by following downstream processing was observed using optimised HPLC methods.
- Quantifying the absorption of PCMC, OPP, OIP and TCMTB in wet blue leather showed that TCMTB is most likely to be wasted in the float.
- The temperature of the storage condition of the wetblue leather affected the degradation rate of TCMTB.

Yours sincerely



Dr. Sujay Prabakar
Science Leader
Leather & Shoe Research Association of New Zealand
E-mail: sujay.prabakar@lasra.co.nz