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Adjustment of the Conditions Required for Complete Decontamination of T-2 Toxin Residues with Alkaline Sodium Hypochlorite

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The trichothecenes are extremely potent toxins produced by several fungal species, including *Fusarium*, *Stachybotrys*, *Myrothecium*, and others (Bamburg and Strong 1971). Due to the frequent contamination of foods and feeds with these toxins, extensive research is being performed in several regions of the world on subjects such as analytical methodology, taxonomy of the toxigenic fungal species, experimental toxic effects and so on. Obviously, for all the laboratories involved in this type of research the availability of effective methods for decontamination of toxin residues is a matter of prime practical importance.

A previous report from Thompson and Wannemacher (1984) stated that T-2 toxin (I, Fig 1), one of the most potent trichothecenes, can be effectively destroyed (nearly 100 % inactivation) by treatment with dilute alkaline hypochlorite (0.25 % NaOCl + 0.025 M NaOH) during 4 hours. These data were derived from the loss of toxic effect in a protein synthesis inhibition bioassay *in vitro*.

However, in an attempt to apply the reported method to laboratory glassware highly contaminated with T-2 toxin, we found that at 4 hours a significant proportion of the toxin still remained intact (average 4 %). Moreover, a chemical analysis revealed that the breakdown products give a positive reaction with the 4-nitrobenzyl pyridine (NBP) reagent, which has been used to characterize the 12,13-epoxy group of trichothecene mycotoxins (Takitani et al 1979). This suggested that these breakdown products might retain some degree of toxicity (Wei and McLaughlin 1974).

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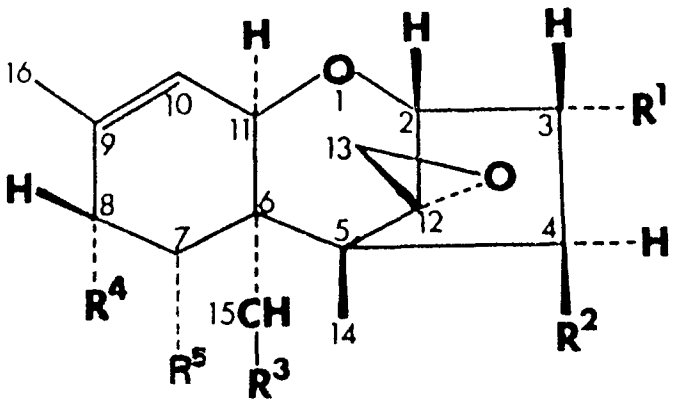

	R1	R2	R3	R4	R5	
I	OH	OAc	OAc	ISV	H	(T-2 toxin)
II	OH	OH	OAc	ISV	H	(HT-2 toxin)
III	OH	OH	OH	ISV	H	(T-2 triol)
IV	OH	OH	OH	OH	H	(T-2 tetraol)
V	OH	OAc	OAc	OH	H	(Neosolaniol)
VI	H	OH	OH	H	H	(Verrucarol)
VII	OH	H	OH	=O	OH	(Deoxynivalenol)

Figure 1. Structure of T-2 and related trichothecenes.

MATERIALS AND METHODS

T-2 toxin was prepared in our laboratory by a modification of the method of Burmeister (1974). It was considered that, for the purpose of the present work, partially purified toxin was adequate. Therefore preparations of about 80-90 % purity were used throughout.

Concentrated sodium hypochlorite solutions (8 - 10 %, technical grade) were used for preparing the decontaminating mixtures. The actual concentrations of dilute hypochlorite solutions were checked by iodimetric titration (Castegnaro 1980).
4-Nitrobenzyl pyridine (NBP) and tetraethylene-pentamine (TEPA) were purchased from Aldrich Chemical Co., Wisc., USA. All other chemicals were of AR grade.

About 400 µg of T-2 toxin was used for each experimental condition tested. The toxin was dissolved in 200 µl of methanol and mixed with 9 ml of decontaminating solutions in ground glass-stoppered test tubes, and left at room temperature. After the appropriate reaction times, the decontamination mixtures were saturated

with sodium chloride and the residual toxin and/or its breakdown products were exhaustively extracted by vigorous shaking with 2 x 3 ml of ethyl acetate. The organic phases were then transferred to small glass vials and evaporated to dryness in a vacuum oven at 40 °C. The residues were redissolved in 100 µl of methanol and used for TLC analysis.

Aliquots (5 - 10 µl) of the methanolic extracts were spotted on TLC plates (0.25 mm Silicagel 60 pre-coated aluminum sheets, Merck, Darmstadt, Germany, Art.5553). Developing solvent was ethyl acetate/ toluene (3:1).

The toxin and breakdown products were visualized by means of the NBP/TEPA reaction, as described by Takitani et al (1979). Quantitation was performed by visual comparison of the colour intensity of the sample spots with those of standards containing known amounts of T-2 toxin. In separate experiments it was shown that with this procedure it is possible to detect at least 0.2 µg of T-2 toxin, that is, about 0.5 % of the initial amounts used for the decontamination tests.

RESULTS AND DISCUSSION

In partial agreement with the results reported by Thompson & Wannemacher (1984) , we found that NaOCl alone was not very effective for the destruction of T-2 toxin, since as shown in Fig. 2, even with concentrations as high as 4 %, at least 48 hr were required for the removal of > 98 % of the toxin. When dilute NaOH was included in the reaction mixture, the rate of T-2 disappearance was greatly accelerated, but even so, we found that under the decontamination conditions proposed by the above-mentioned authors (0.25% NaOCl + 0.025 M NaOH, 4 hours), significant residues of intact T-2 could be detected: usually 3 - 5 % of the initial amount but up to 15 % in some individual experiments (data not shown). The reason for this disagreement is unknown, but it must be taken in consideration that different methods of evaluation were used, since Thompson & Wannemacher only determined toxicity in a protein synthesis inhibition bioassay, whereas in the present work actual concentrations of T-2 toxin were measured by TLC.

But there is another point which is perhaps of greater significance. The fact that small amounts of NaOH remarkably increase the rate of T-2 toxin disappearance indicates that this process may involve some degree of hydrolysis of the ester groups of T-2, such as the isovaleryl at C8, or the acetyl at C4 and C15. Our present experiments suggest that actually this might be the case, since the disappearance of T-2 toxin

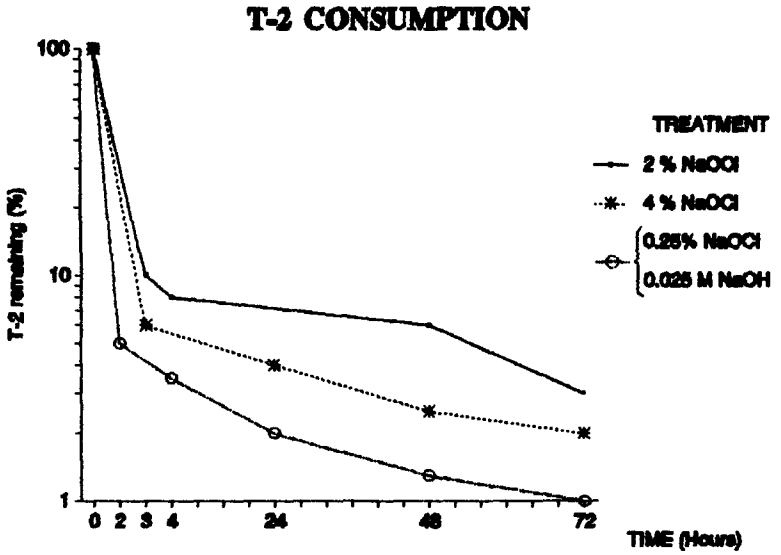

Figure 2. T-2 toxin remaining (% of initial amount) after incubation with different concentrations of NaOCl alone, or NaOCl/NaOH.

under the conditions mentioned above, is accompanied by the appearance of several breakdown products, which were given the trivial names A, B and C, with Rf's 0.60, 0.51 and 0.43, respectively, in the solvent system ethyl acetate/toluene 3:1. Unfortunately, due to the lack of authentic standards these products could not be unequivocally identified. However, the fact that these substances give a positive reaction with the NBP/TEPA reagent suggests that they may have an intact 12,13-epoxy trichothecene nucleus, as stated by Takitani et al (1979), indicating that they may be T-2 toxin hydrolysis products, such as, for instance, compounds II, III, IV or V (see Fig. 1). Further support for this hypothesis derives from the fact that a short incubation of T-2 with dilute NaOH gives way to breakdown products which have similar chromatographic properties.

The foregoing considerations are in agreement with previous results from Burrows and Szafraniec (1986a,b), who indicated that verrucarol (VI), which was taken as a similar but simpler prototype for T-2 toxin, was relatively resistant to concentrated alkaline hypochlorite (8% NaOCl/0.4 M NaOH), since more than 20 hours were required for complete transformation into two stable breakdown products which lacked the 12,13-epoxy

group. In our present experiments we followed the disappearance of all the products capable of giving a positive NBP/TEPA reaction, as a function of time of incubation with 0.25 % NaOCl/0.025 M NaOH. As shown in Fig. 3, at least 48 hours were required for more than 98% destruction of all these substances (measured in terms of the initial amounts of T-2 toxin added to the system). Attempts to shorten the time required for this process by increasing NaOCl/NaOH concentration were relatively unsuccessful, since after 24-hr reaction with 1% NaOCl/0.05 M NaOH the residues of T-2 toxin plus A,B and C breakdown products were not significantly lower than those shown in Fig.3.
Although we agree that T-2 hydrolysis products, perhaps with the exception of HT-2 toxin (II), may have a low order of mammalian toxicity, we think that the presence of an intact 12,13-epoxy trichothecene nucleus might still entail a significant environmental hazard, since some living species might be sensitive to these substances. Furthermore, the possibility of biological esterifications, to reconstitute either the original toxin or other dangerous molecules cannot be excluded with certainty (Yoshizawa and Morooka 1975). Accordingly, it could be stated that, under the conditions described by Thompson & Wannemacher (1984), an actual decontamination of T-2 does not take place, but just a partial detoxification.

Thus it is recommended that, in order to "effectively" decontaminate T-2 toxin, a minimum incubation time of 48-72 hours with alkaline hypochlorite (0.25 % NaOCl/0.025 M NaOH or higher concentration) should be used as a routine procedure.

On the other hand, preliminary experiments with small amounts of deoxynivalenol (VII, DON) indicated that this toxin is rapidly destroyed by alkaline NaOCl (data not shown). This is in agreement with previous reports from Burrows and Szafraniec (1987), who found that only 2-hour incubation with concentrated alkaline NaOCl was required for complete transformation of DON into unstable derivatives, which on longer standing gave low yields of complex mixtures of degraded products. This behaviour may be due, at least in part, to the instability of the C8 keto group under alkaline conditions (Young et al. 1986).

Therefore, it would appear likely that the treatment proposed above might be effective for decontamination of, at least, group A and B trichothecenes. However, a definite statement can not be made until these substances are individually tested.

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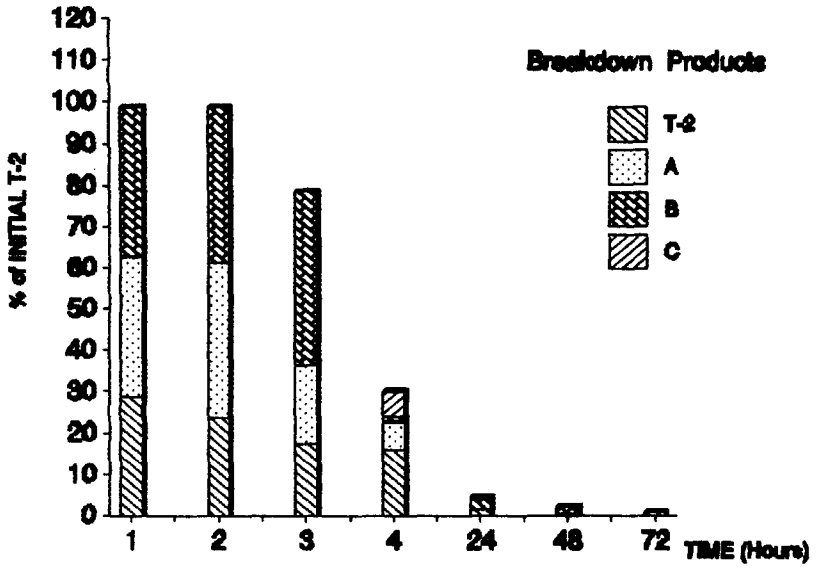

Figure 3. Destruction of T-2 toxin plus breakdown products A, B and C, by prolonged treatment with alkaline NaOCl.

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