

Allergenicity evaluation of Bioban CS-1135 in experimental animals

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An industrial preservative, Bioban CS-1135, was evaluated for its contact allergenicity by means of multiple-dose guinea-pig maximization test and non-radioactive murine local lymph node assay. In the guinea-pig test, an induction dose of 0.5% Bioban CS-1135 sensitized all animals of the group. The dose–response study of the elicitation phase determined a minimum elicitation dose of 5% for positive skin reactions. In the murine assay, Bioban CS-1135 at doses of 10% and more exerted significant effects on lymphoid cell proliferation. Although the data clearly designated Bioban CS-1135 as a skin sensitizer, its relative potency was ranked lowest among skin-sensitizing biocides previously evaluated in this laboratory.

Key words: 5-bromo-2'-deoxyuridine; allergic contact dermatitis; biocides; guinea-pig maximization test; murine local lymph node assay; risk assessment. © Blackwell Munksgaard, 2004.

Accepted for publication 6 April 2004

Bioban CS-1135 is a broad-spectrum bactericide based on oxazolidine (4,4-dimethyloxazolidine) chemistry. It has been widely used as a preservative in products and applications such as metal-working fluids, oilfield water systems and mineral slurries. Most biocides exert their toxic effects on microorganisms through reactivity with proteins, and this, in turn, suggests a higher risk of biocides as skin sensitizers, because a low molecular weight chemical has to bind with endogenous proteins/peptides to be recognized as an immunogen. In fact, numerous cases of allergic contact dermatitis from various kinds of biocides have been reported (1, 2). Among them, positive skin reactions to a series of Biobans are not rare, especially in metalworkers, with 3.4% of 408 workers being positive to some kinds of Biobans (3). However, the skin reactions are often weak and not reproducible. Furthermore, in many cases, the clinical relevance of skin reactions with the use of Biobans is not ascertained. These data suggest that, although Biobans have intrinsic potencies for skin sensitization, the potencies are relatively weak compared with other biocides. Thus, in this study, we decided to evaluate the sensitization potency of Bioban CS-1135 using 2 different animal tests: multiple-dose guinea-pig

maximization test (GPMT) and non-radioactive local lymph node assay (LLNA).

Materials and Methods

Chemicals

Bioban CS-1135 (active ingredient: 78% 4,4-dimethyl-1,3-oxazolidine) was obtained from Nagase & Co., Ltd. (Tokyo, Japan). All other reagents used were of superior grade.

Guinea-pig maximization test

5- to 6-week-old female Hartley guinea pigs from Japan SLC, Inc. (Shizuoka, Japan) were used. The GPMT (4) was performed with some modifications in that dose–response profiles were evaluated for both induction and elicitation phases, and challenge test was done openly (5). 5 or 10 animals were used for each sensitization group. The concentrations for the first (intradermal) and the second (topical) inductions are summarized in Tables 1 and 2. 2 weeks after topical induction, 0.1 ml aliquots of various concentrations of Bioban CS-1135 in acetone or an undiluted solution were applied all at once to a shaved area of the flank for challenge. 48 h after the

Table 1. Results of first guinea-pig maximization test for Bioban CS-1135

Induction (%)		Elicitation (%)*	SR (%)	n
Intradermal	Topical			
0	0	100	0	5
0	10	100	60	5
0.5	10	100	100	5

SR = sensitization rate.

*Animals were challenged 2 weeks after 2nd induction with 100% Bioban CS-1135, and skin reactions were evaluated 48 h thereafter.

challenge, each site was scored for erythema (0–4) and oedema (0–3) according to the criteria of Sato et al. (6). For each challenge concentration group, the percentage of animals showing a positive reaction was taken as the sensitization rate.

Non-radioactive murine LLNA

6- to 8-week-old female BALB/c mice from CLEA Japan, Inc. (Tokyo, Japan) were used. The assay was performed according to the method of Takeyoshi et al. (7) with some modifications (8). Briefly, groups of mice ($n=4$) were exposed to 25 μ l of various concentrations of Bioban CS-1135 in vehicle (acetone/olive oil, 4:1) or as an undiluted solution, through application to the dorsum of both ears on 3 consecutive days (days 0–2). On day 4, 5-bromo-2'-deoxyuridine (BrdU, 150 mg/kg/15 ml saline) was administered intraperitoneally to each mouse. The next day, a pair of auricular lymph nodes from each mouse was excised, and single-cell suspensions were prepared by gentle mechanical disaggregation in saline. The total cell count in each suspension was measured, and the suspensions were then diluted with saline to a concentration of 4×10^5 cells/ml and dispensed (50 μ l) into the wells of a flat-bottom microplate. BrdU concentrations in the wells were measured by enzyme-linked immunosorbent assay (ELISA). Total lymph node cell

count in a chemical-treated animal divided by mean lymph node cell count in control group was designated as a cellularity index, whereas BrdU incorporation (OD) per well of cells from a chemical-treated animal divided by mean BrdU incorporation (OD) per well of cells from a control group was used as a BrdU incorporation index. A stimulation index (SI) was calculated by multiplying the cellularity index by the BrdU incorporation index.

Statistical analysis

In the LLNA, index values for each chemical-treated group and vehicle-treated control group were compared via Dunnett's or Steel's multiple comparison method using a StatLight software package (Yukms Co., Ltd, Tokyo, Japan).

Results

GPMT results for Bioban CS-1135 are summarized in Tables 1 and 2. An intradermal induction concentration of 0.5% and a topical induction dose of 10% were selected as the maximum tolerable doses for each step of the GPMT. All of 5 guinea pigs sensitized with maximum induction doses exhibited positive skin reactions when challenged with 100% Bioban CS-1135. Notably, Bioban CS-1135 could sensitize animals by the

Table 2. Results of second guinea-pig maximization test for Bioban CS-1135

Challenge dose (%)	Induction dose*			
	SR (%)†			
	0%‡	0.005%	0.05%	0.5%
0.05	0	0	0	0
0.5	0	0	0	0
5	0	0	0	70
25	0	0	0	100
50	0	0	0	100
100	0	0	0	100

SR = sensitization rate. Number of animals was 5 for 0% induction-dose group and 10 for the other 3 induction-dose groups.

*Each group of animals was sensitized using the same dose of Bioban CS-1135 for both intradermal and topical induction procedures.

†Skin reactions were evaluated 48 h after challenge.

‡Animals were treated with vehicle in both intradermal and topical induction procedures.

topical induction procedure alone, although the sensitization rate of the group was lower than that of the maximally sensitized group (Table 1). Because the topical induction procedure alone sensitized the animals, in the subsequent dose-response study, the topical induction concentrations were varied in line with intradermal induction concentrations. As summarized in Table 2, positive skin reactions were observed only at the highest induction dose of 0.5%. The minimum challenge dose of Bioban CS-1135 that elicited positive skin reaction in this group was 5%.

A dose-response profile for the allergenicity of Bioban CS-1135 in the induction phase was further evaluated by another animal test, the non-radioactive LLNA (Table 3). Statistically significant increases in the cellularity index, the BrdU index and SI, which is obtained by multiplying the cellularity and BrdU indices, were observed at doses of 10% and more of Bioban CS-1135.

We have thus far evaluated dose-response profiles for both induction and elicitation phases of allergic contact dermatitis with 20 different biocides using multiple-dose GPMT, and 15 of them have been designated as positive skin sensitizers. As summarized in Table 4, both the minimum induction dose and minimum elicitation dose of Bioban CS-1135 in the guinea-pig test ranked last place among the designated sensitizers.

Discussion

This study clearly designated Bioban CS-1135 as a contact sensitizer using 2 representative animal tests, GPMT and LLNA. Recently, Brinkmeier et al. (3) reported the patch test results on exposure to a series of Biobans among metalworkers, a high-risk group for this group of preservatives. The test showed that 3.4% out of 408 workers

reacted to at least 1 of the Biobans (2.2% P-1487, 2.0% CS-1246 and 1.5% CS-1135). Previous multicentre patch test data provided by the Information Network of Departments of Dermatology in Germany and Austria (2) showed a 1.1% positive reaction rate to Bioban CS-1135 in 1760 patients. In both reports, skin reaction was weak and most reactions were not reproducible during retesting.

In order to improve occupational hygiene and protect consumers from the risk of skin sensitization, it is essential to elucidate the relative sensitizing potencies of individual sensitizers. There have been several animal studies evaluating dose-response profiles of skin-sensitizing biocides (9–11). In our database of biocide-sensitizing potencies evaluated using multiple-dose GPMT, Bioban CS-1135 ranked last place in the list, especially for the minimum elicitation dose. Although the minimum induction dose of 0.5% Bioban CS-1135 in the multiple-dose GPMT also ranked last, this dose was within the range of those for other biocides. The minimum induction dose of 10% Bioban CS-1135 in the mouse test also seemed in the range of those for other chemical allergens. In our recent studies using non-radioactive LLNA, the minimum induction doses for 2 preservatives, *p*-chloro-*m*-cresol (PCMC) and *p*-chloro-*m*-xylenol (PCMX), were 10% and 25%, respectively (8). The results may suggest that the potency of Bioban CS-1135 to induce specific T-cell proliferation is comparable to other biocides, but that its intrinsic potency to cause positive skin reaction is relatively weak. This is in line with the weak skin reactions observed in patch testing in clinical studies, because it is the elicitation reaction that is observed in the patch test.

In addition to evaluating relative potencies, cross-reactivity is another important factor in risk assessment for skin sensitizers. With respect

Table 3. Results of local lymph node assay for Bioban CS-1135

Induction dose (%)	Cellularity index	BrdU incorporation index	Stimulation index
0	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.0
1	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.3
3	1.0 ± 0.2	1.0 ± 0.2	1.1 ± 0.4
10	1.7 ± 0.3*	1.5 ± 0.3†	2.7 ± 0.6*
25	3.0 ± 0.3†	1.8 ± 0.2†	5.4 ± 1.0*
50	2.4 ± 0.4†	1.8 ± 0.2†	4.4 ± 1.0*
100	2.7 ± 0.5†	1.8 ± 0.3†	4.8 ± 0.9*

Values are means ± SD.

Cellularity index = $\frac{\text{total lymph node cell count in a chemical-treated animal}}{\text{mean lymph node cell count in control group}}$

BrdU incorporation index = $\frac{\text{BrdU incorporation (OD) per well of cells from a chemical-treated animal}}{\text{mean BrdU incorporation (OD) per well of cells from control group}}$

Stimulation index = Cellularity index × BrdU incorporation index.

*Significantly different from control, $P < 0.05$.

†Significantly different from control, $P < 0.01$.

Table 4. Results of guinea-pig maximization test for various biocides

Chemicals	Abbreviation	Minimum induction dose (p.p.m.)*	SR (%)†	Minimum elicitation dose (p.p.m.)‡	Reference
2,4,5,6-tetrachloroisophthalonitrile	TPN	0.5	100	0.5	§
2-(thiocyanomethylthio)benzothiazole	TCMTBT	10	100	1	(12)
<i>p</i> -chlorophenyl-3-iodopropargylformyl	CPIP	5	100	50	(13)
2,3,5,6-tetrachloro-4-(methylsulphonyl)pyridine	TCMSP	50	100	50	§
1,2-benzisothiazolin-3-one	BIT	50	50	50	(14)
3-iodo-2-propenyl butylcarbamate	IPBC	5	100	100	(13)
<i>N,N'</i> -tetramethylene-bis(4-carbamoyl-1-decylpyridinium bromide)	TMBCDPPB	100	100	1000	(15)
<i>N,N'</i> -hexamethylene-bis(4-carbamoyl-1-decylpyridinium bromide)	HMBCDPPB	10	100	1000	(15)
<i>p</i> -chloro- <i>m</i> -cresol	PCMC	5	100	500	(8)
hiba oil	HO	5000	50	5000	(16)
2-chloroacetamide	CAA	500	100	1600	(17)
<i>p</i> -chloro- <i>m</i> -xyleneol	PCMX	5	90	5000	(8)
<i>N</i> - <i>n</i> -butyl-1,2-benzisothiazolin-3-one	BBIT	5000	20	500	(18)
2-bromo-2-nitropropane-1,3-diol	BNPD	5000	10	5000	(17)
4,4-dimethyl-1,3-oxazolidine	Bioban CS-1135	5000	100	50000	

*Minimum first (intradermal) induction dose that sensitized at least one animal in the group.

†Sensitization rate observed in the maximally sensitized group in a dose-response study.

‡Minimum challenge dose that elicited allergic skin reaction at least one animal in the group.

§Noda et al. unpublished data.

to the 3 sensitizing Biobans, P-1487, CS-1135 and CS-1246, some patients reacted to 2 or 3 of them (3). Although both CS-1135 and CS-1246 have the structure to release a formaldehyde molecule, it is difficult to elucidate whether the cross-reaction was due to structural analogy or simply due to concomitant exposure to them. In this respect, the GPMT has an advantage in that it can precisely evaluate cross-reactivity among sensitizers under strictly controlled conditions. Further information including animal study data would appear to be necessary to assess the risk of a wide range of preservatives such as Biobans.

References

- Jacobs M C, White I R, Rycroft R J G, Taub N. Patch testing with preservatives at St John's from 1982 to 1993. *Contact Dermatitis* 1985; 33: 247–254.
- Schnuch A, Geier J, Uter W, Frosch P J. Patch testing with preservatives, antimicrobials and industrial biocides. Results from a multicentre study. *Br J Dermatol* 1998; 138: 467–476.
- Brinkmeier T, Geier J, Lepoittevin J P, Frosch P J. Patch test reactions to Biobans in metalworkers are often weak and not reproducible. *Contact Dermatitis* 2002; 47: 27–31.
- Magnusson B, Kligman A M. The identification of contact allergens by animal assay. The guinea pig maximization test. *J Invest Dermatol* 1969; 52: 268–276.
- Nakamura A, Momma J, Sekiguchi H. A new protocol and criteria for quantitative determination of sensitization potencies of chemicals by guinea pig maximization test. *Contact Dermatitis* 1994; 31: 72–85.
- Sato Y, Katsumura Y, Ichikawa H. A modified technique of guinea pig testing to identify delayed hypersensitivity allergens. *Contact Dermatitis* 1981; 7: 225–237.
- Takeyoshi M, Yamasaki K, Yakabe Y, Takatsuki M, Kimber I. Development of non-radio isotopic endpoint of murine local lymph node assay based on 5-bromo-2'-deoxyuridine (BrdU) incorporation. *Toxicol Lett* 2001; 119: 203–208.
- Yamano T, Shimizu S, Noda T. Allergenicity evaluation of *p*-chloro-*m*-cresol and *p*-chloro-*m*-xylenol by non-radioactive murine local lymph-node assay and multiple-dose guinea pig maximization test. *Toxicology* 2003; 190: 259–266.
- Jayjock M A, Lewis P G. Low-applied-dose extrapolation of induction and elicitation of contact allergy in the evaluation and management of sensitization risk from Kathon CG isothiazolone in products. *Am J Contact Dermat* 1992; 3: 86–91.
- Andersen K E, Vølund A, Frankild S. The guinea pig maximization test with a multiple dose design. *Acta Derm Venerol* 1995; 75: 463–469.
- Basketter D A, Rodford R, Kimber I, Smith I, Wahlberg J E. Skin sensitization risk assessment: a comparative evaluation of 3 isothiazoline biocides. *Contact Dermatitis* 1999; 40: 150–154.
- Yamano T, Shimizu S, Noda T. Allergenicity evaluation of *N*-(1-methylheptyl)-*N'*-phenyl-*p*-phenylenediamine and 2-(thiocyanomethylthio) benzothiazole by the guinea pig maximization test. *J Health Sci* 2001; 47: 331–338.
- Shimizu M, Yamano T, Noda T. Allergenicity evaluation of chemicals for use in household products IV: contact allergenicity of three halide bactericides, 3-iodo-2-propynyl butylcarbamate (IPBC), *p*-chlorophenyl-3-iodopropargylformyl (CPIP) and 1-bromo-3-ethoxycarbonyloxy-1,2-diiodo-1-propene (BECDIP) in guinea pigs. *Seikatsu Eisei* 2000; 44: 129–138.
- Noda T, Yamano T, Shimizu S. Allergenicity evaluation of chemicals for use in household products III: contact allergenicity of 2,4,5,6-tetrachloroisophthalonitrile (TPN), 1,2-benzisothiazolin-3-one (BIT), 2-hydroxy-4-isopropyl-2,4,6-cycloheptatrien-1-one (HICHO) and 2,5-di-tert-amylhydroquinone (DAHQ) in guinea pigs. *Annu Rep Osaka City Inst Public Health Environ Sci* 1998; 60: 24–34.
- Shimizu M, Yamano T, Noda T. Allergenicity evaluation of biocides for use in household products V: contact allergenicity of 4,4'-(tetramethylenedicarbonyldiamino) bis(1-decylpyridinium bromide) and *N,N'*-hexamethylene bis(4-carbamoyl-1-decylpyridinium bromide) in guinea pigs. *Annu Rep Osaka City Inst Public Health Environ Sci* 2002; 64: 1–5.
- Noda T, Shimizu S. Toxicity study of hiba oil. 1. Contact allergenicity of hiba oil in guinea pigs. *Seikatsu Eisei* 2000; 44: 13–19.
- Noda T, Yamano T, Shimizu S. Allergenicity evaluation of chemicals for use in household products VI: contact antimicrobial agents 2-chloroacetamide, 2-bromo-2-nitropropane-1,3-diol and zinc bis(2-pyridylthio-1-oxide) in guinea pigs. *Seikatsu Eisei* 2004; 48: 396–406.
- Noda T, Yamano T, Shimizu S. Toxicity study of *N*-n-butyl-1,2-benzisothiazolin-3-one. 1. Contact allergenicity of *N*-n-butyl-1,2-benzisothiazolin-3-one in guinea pigs. *Seikatsu Eisei* 2001; 45: 137–142.

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