

Assessment of the sensitization potential of persulfate salts used for bleaching hair

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Background: Persulfate salts have been associated with both allergic contact dermatitis and bronchial asthma. Because there is currently no experimental data available on the sensitizing properties of persulfate salts (ammonium, sodium, and potassium persulfates), we determined their dermal sensitizing capacity, using the murine local lymph node assay (LLNA).

Material and Methods: For three consecutive days, BALB/c mice were dermally treated with ammonium, sodium, or potassium persulfate or with the vehicle alone (dimethyl sulfoxide) on each ear ($2 \times 25 \mu\text{l}$). On D6, mice were injected intravenously with [³H]-methyl thymidine. The draining auricular lymph nodes were removed, and the incorporation of [³H]-methyl thymidine was compared with that of vehicle-treated control mice. A stimulation index (SI) relative to the vehicle-treated control value was derived. The sensitizing potency of the chemicals tested was determined by estimating the concentration of chemical required to induce a SI of 3 (EC3).

Results: All three chemicals provoked positive responses in the LLNA, with dose-dependent increases in proliferation. Maximal SIs recorded were 6.8 ± 1.8 , 6.5 ± 1.2 , and 5 ± 1.0 at 5% for ammonium, sodium or potassium persulfate, respectively. The EC3 values were 1.9%, 0.9%, and 2.4% for ammonium, sodium, and potassium persulfates, respectively.

Conclusions: All three persulfate salts need to be considered strong-to-moderate sensitizers according to the murine LLNA.

Key words: ammonium persulfate; BALB/c mice; local lymph node assay; potassium persulfate; sodium persulfate. © Blackwell Munksgaard, 2009.

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Persulfate salts are reactive low-molecular-weight chemical compounds widely used in different manufacturing processes in the chemical, pharmaceutical, metallurgic, textile, photographic, food, and, particularly cosmetic industries (1). These salts may be present in hair lightening products at concentrations up to 60% (2).

Persulfates are capable of causing immunological sensitization and subsequent allergic disease, such as contact dermatitis (3–5) and bronchial asthma (6–9). Currently, there is a lack of infor-

mation about the skin sensitizing properties of persulfates. Only the sensitization potential of ammonium persulfate has been determined in a commercial setting (10). Ammonium persulfate was considered a sensitizer according to the guinea-pig maximization test, an Organization for Economic Cooperation and Development (OECD)-approved predictive test using guinea-pigs for establishing dermal sensitization potential (guideline no. 406).

The local lymph node assay (LLNA) is a validated and accepted hazard identification method that is currently widely used as a predictive test for the identification of dermal sensitizers. The

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method is based on the immunological events that occur during the induction phase of contact sensitization. Contact allergens are identified as a function of lymphocyte proliferative responses provoked in draining lymph nodes following open, epicutaneous application of the test material (11, 12), with the degree of lymphocyte proliferation correlating closely with the skin sensitizing potency of the chemical (13). One of the main advantages of this technique is that it permits accurate measurement of relative potency, thus allowing an effective risk assessment and risk management strategy. Furthermore, using the LLNA, it is possible to distinguish prevalence (overall frequency of contact dermatitis caused by a chemical) from potency (determination of the allergic potential of a chemical, applying a certain concentration on the skin). This is important because a relatively high prevalence is not necessarily attributable to a high potency (14).

In a first step to try to identify the mechanisms involved in the sensitization to persulfate salts, we have compared, using the murine LLNA, the sensitization potential of three persulfate salts: ammonium persulfate, sodium persulfate, and potassium persulfate. To our knowledge, these three salts have not been studied using the LLNA.

Materials and Methods

Mice

Male BALB/c mice (~ 20 g, 6 weeks old) were obtained from Harlan (Horst, the Netherlands). The mice were housed in a conventional animal house with 12:12-hr dark–light cycles. They received lightly acidified water and pelleted food (Trouw Nutrition, Ghent, Belgium) *ad libitum*. All experimental procedures were approved by the local Ethical Committee for Animal Experiments.

Chemicals

Ammonium persulfate $[(\text{NH}_4)_2\text{S}_2\text{O}_8]$, sodium persulfate ($\text{Na}_2\text{S}_2\text{O}_8$), and potassium persulfate ($\text{K}_2\text{S}_2\text{O}_8$) (Sigma-Aldrich Corporation, St Louis, MO, USA) were used. Dosing solutions (0.1%, 0.5%, 1%, and 5% w/v) were prepared in dimethyl sulphoxide (DMSO, vehicle) (Sigma-Aldrich Corporation). Dosing solutions were prepared freshly for each application.

Local lymph node assay

The LLNA was performed according to a standard protocol (12). Briefly, the mice (five animals per test group) received dermal applications of 25 μl of various concentrations of $(\text{NH}_4)_2\text{S}_2\text{O}_8$,

$\text{Na}_2\text{S}_2\text{O}_8$, or $\text{K}_2\text{S}_2\text{O}_8$ or of the same volume of vehicle alone on each ear, daily for three consecutive days. The area around the exposure site was monitored for any signs of irritancy. Six days after the initiation of exposure, the mice were injected intravenously through the tail vein with (methyl- ^3H)-thymidine [$^3\text{HTdR}$], 2 Ci/mmol from ICN Pharmaceuticals (Asse, Belgium) in 250 μl of phosphate-buffered saline (pH 7.2). Five hours later, the mice were killed by an overdose of pentobarbital (90 mg/kg) and the draining auricular lymph nodes were excised, pooled for each mouse, and weighed (lymph node weight, LNW). A single cell suspension of lymph node cells (LNC) was prepared, and the LNC were washed twice. $^3\text{HTdR}$ incorporation was measured by β -scintillation counting (Beckman LS 5000CE, Irvine, CA, USA) and expressed as disintegrations per minute (d.p.m.). The SI was calculated as the ratio of $^3\text{HTdR}$ incorporation by lymphocytes from treated animals relative to that from concurrent vehicle-treated controls.

Data treatment and statistical analysis

The mean value of the d.p.m.s found was calculated for each experimental group, thus allowing the derivation of a SI. As threshold level for significance a SI value >3 was adopted. An estimate of the sensitizing potency of the chemicals tested was determined by calculating an estimated concentration of chemical required to induce an SI of 3 (EC3). The EC3 was derived by linear interpolation of dose–response data as described previously (15). The EC3 value was calculated by interpolating between two points on the SI axis, one immediately above and the other immediately below, the SI value of 3. Where the data points lying immediately above and below of 3 have the co-ordinates (a,b) and (c,d), respectively, then the EC3 value may be calculated using the following equation:

$$\text{EC3} = c + [(3 - d)/(b - d)](a - c)$$

The relationship of EC3 value to relative potency is inverse, hence the lower the EC3 value, the higher the potency.

Data is shown as means and SD. All data were analysed using parametric analysis of variance with a Dunnett's *post hoc* test (GRAPHPAD PRISM 4.01 GRAPHPAD Software Inc., San Diego, CA, USA). A level of $P < 0.05$ (two tailed) was considered to be significant.

Results

The skin sensitizing potential of three persulfate salts was determined using the LLNA, a method

Table 1. Lymph node weights in response to treatment with vehicle (DMSO), ammonium persulfate $[(\text{NH}_4)_2\text{S}_2\text{O}_8]$, sodium persulfate $(\text{Na}_2\text{S}_2\text{O}_8)$, and potassium persulfate $(\text{K}_2\text{S}_2\text{O}_8)^a$

Concentration (% w/v)	$(\text{NH}_4)_2\text{S}_2\text{O}_8$ in DMSO (LNW mg)	$\text{Na}_2\text{S}_2\text{O}_8$ in DMSO (LNW mg)	$\text{K}_2\text{S}_2\text{O}_8$ in DMSO (LNW mg)
0	2.6 ± 0.9	3.7 ± 1.1	3.4 ± 1.7
0.1	3.2 ± 0.9	3.9 ± 1.3	4.1 ± 0.7
0.5	3.5 ± 0.8	4.8 ± 1.6	4.5 ± 1.2
1	$4.3 \pm 0.9^*$	$6.7 \pm 0.9^{**}$	$7.5 \pm 2.2^{**}$
5	$6.6 \pm 1.4^{**}$	$11.6 \pm 1.8^{**}$	$9.5 \pm 2.3^{**}$

DMSO, dimethyl sulphoxide; LNW, lymph node weight.

^aIn the murine local lymph node assay, mice were dermally sensitized, on both ears, on D1, D2, and D3. On D6, they were intravenously injected with 20 μCi [methyl-³H]-thymidine (³HTdR), and 5 hr later, the auricular lymph nodes were removed, pooled, and weighed (lymph node weight). The two lymph nodes of each mouse were pooled. Numbers are means ($\pm$ SD) of 4–7 mice per group. * $P < 0.05$, ** $P < 0.01$ compared with the DMSO control.

in which the activity is measured as a function of induced proliferative responses in the auricular lymph nodes draining the site of topical application. In the LLNA, chemicals are defined as biologically relevant dermal sensitizers, when the test concentrations yield at least a threefold increase in SI compared with vehicle-treated controls (14). This is called the EC3. Both the LNW and the LNC count, measured by ³HTdR incorporation and presented in d.p.m., are presented.

The results of the LLNA for the persulfate salts are presented in Tables 1 and 2 and Fig. 1.

Exposure to $(\text{NH}_4)_2\text{S}_2\text{O}_8$ resulted in a maximal mean SI of 6.8 ± 1.8 at the highest concentration tested (5%) (Fig. 1a). Applying 5% solution of $(\text{NH}_4)_2\text{S}_2\text{O}_8$ caused the LNW to increase 2.5 times (Table 1) and a 6.5-fold increase in total LNC number (Table 2) was found when compared with the DMSO control. From the calculated SI values, the estimated EC3 of $(\text{NH}_4)_2\text{S}_2\text{O}_8$ is 1.9%.

After sensitization with $\text{Na}_2\text{S}_2\text{O}_8$, the maximal SI of 6.4 ± 1.2 at the highest concentration tested (5%) was calculated (Fig. 1b), while a threefold

increase in the LNW was measured (Table 1) and the LNC numbers increased almost 6.5-fold (Table 2). This resulted in an estimated EC3 of 0.9% for $\text{Na}_2\text{S}_2\text{O}_8$.

A slightly lower value was obtained after sensitization with $\text{K}_2\text{S}_2\text{O}_8$, which yielded a maximal mean SI of 5.0 ± 1.0 for the group exposed to the highest concentration (5%) (Fig. 1c). The LNW increased 2.8-fold, while the total LNC number was five times increased. This resulted in an estimated EC3 of 2.4% for $\text{K}_2\text{S}_2\text{O}_8$.

Discussion

The primary purpose of our study was to investigate the potential of three persulfate salts $[(\text{NH}_4)_2\text{S}_2\text{O}_8, \text{Na}_2\text{S}_2\text{O}_8, \text{or } \text{K}_2\text{S}_2\text{O}_8]$ to cause allergic contact dermatitis. This is necessary for further studying the potential of these salts to induce pulmonary effects and, moreover, to investigate the relation between dermal sensitization and respiratory responses (16–19). Kimber et al. (20) proposed a classification to categorize contact allergens according to potency, based on their EC3 value. In this approach, four categories are used. 1, extreme skin sensitizers are defined as agents with an EC3 value less than 0.1%; 2, strong sensitizers have an EC3 value between 0.1% and 1%; 3, moderate sensitizers have an EC3 value between 1% and 10%; and 4, weak sensitizers have an EC3 value above 10%. In this classification, $(\text{NH}_4)_2\text{S}_2\text{O}_8$ (EC3 = 1.9%) and $\text{K}_2\text{S}_2\text{O}_8$ (EC3 = 2.4%) are classified as moderate sensitizers and $\text{Na}_2\text{S}_2\text{O}_8$ (EC3 = 0.9%) is classified as a strong sensitizer.

The information on the sensitizing properties of persulfates is limited. One study describes the safety assessment of ammonium, potassium, and sodium persulfate (2). In that study, only ammonium persulfate was tested for its dermal sensitizing potential, using the Magnusson and Kligman guinea-pig maximization test (21), which is an OECD-approved guideline (no. 406, 1992) for

Table 2. Disintegrations per minute (d.p.m.) in the LLNA in response to treatment with vehicle (DMSO), ammonium persulfate $[(\text{NH}_4)_2\text{S}_2\text{O}_8]$, sodium persulfate $(\text{Na}_2\text{S}_2\text{O}_8)$, and potassium persulfate $(\text{K}_2\text{S}_2\text{O}_8)^a$

Concentration (% w/v)	$(\text{NH}_4)_2\text{S}_2\text{O}_8$ (in DMSO) d.p.m.	$\text{Na}_2\text{S}_2\text{O}_8$ (in DMSO) d.p.m.	$\text{K}_2\text{S}_2\text{O}_8$ (in DMSO) d.p.m.
0	1609 ± 681	1556 ± 870	1630 ± 284
0.1	1424 ± 720	1488 ± 547	1579 ± 240
0.5	2116 ± 250	2284 ± 357	2130 ± 323
1	$2942 \pm 475^*$	$5596 \pm 484^{**}$	$3145 \pm 649^{**}$
5	$10\,513 \pm 2739^{**}$	$10\,082 \pm 1936^{**}$	$8179 \pm 1568^{**}$

DMSO, dimethyl sulphoxide; LLNA, local lymph node assay.

^aIn the murine LLNA, mice were dermally sensitized, on both ears, on D1, D2, and D3. On D6, they were intravenously injected with 20 μCi [methyl-³H]-thymidine (³HTdR), and 5 hr later, the auricular lymph nodes were removed, pooled, and weighed (lymph node weight). ³HTdR incorporation was measured by β -scintillation counting expressed as d.p.m.. The two lymph nodes of each mouse were pooled. Numbers are means ($\pm$ SD) of 4–7 mice per group. * $P < 0.05$, ** $P < 0.01$ compared with the DMSO control.

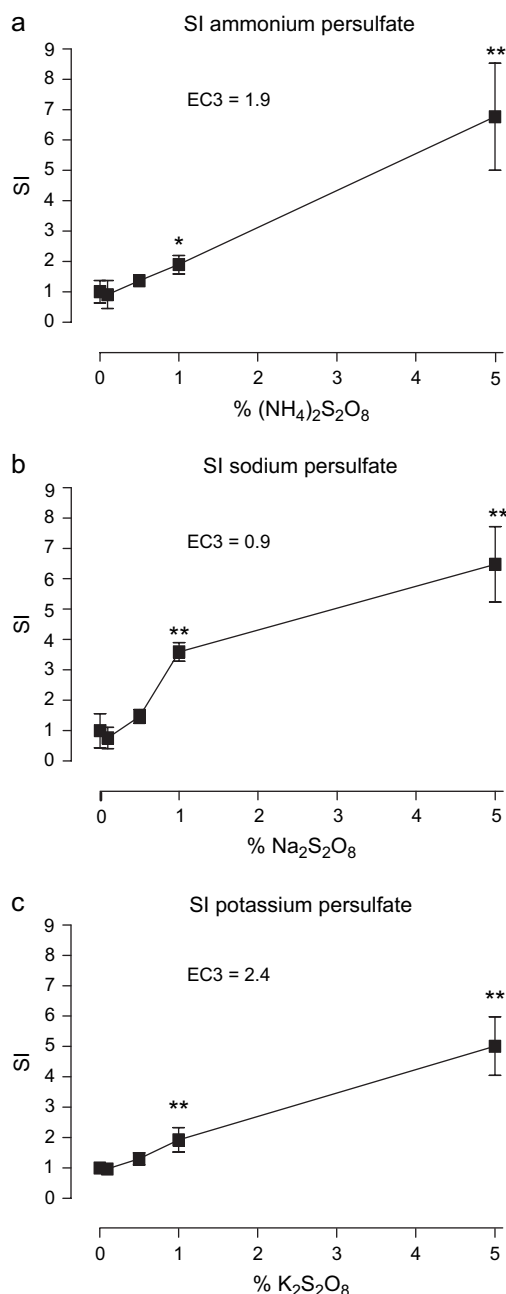


Fig. 1. Stimulation index (SI) of ammonium (a), sodium (b), and potassium (c) persulfate in the murine local lymph node assay. On D1, D2, and D3, mice were dermally sensitized on both ears. On D6, they were intravenously injected with 20 μCi [methyl- ^3H]-thymidine ($^3\text{HTdR}$), and 5 hr later, the auricular lymph nodes were removed, pooled, and weighed. The SI was calculated as the ratio of $^3\text{HTdR}$ incorporation by lymphocytes from treated animals relative to that from concurrent vehicle-treated controls. $(\text{NH}_4)_2\text{S}_2\text{O}_8$, $\text{Na}_2\text{S}_2\text{O}_8$, and $\text{K}_2\text{S}_2\text{O}_8$ persulfates were dissolved in dimethyl sulfoxide. The two lymph nodes of each mouse were pooled. Symbols represent means ($\pm\text{SD}$) of 4–7 mice per group. * $P < 0.05$, ** $P < 0.01$ compared with the DMSO control.

testing dermal sensitization. In that study, all the guinea-pigs reacted to an intradermal administration of a 0.1% solution in saline, whereas

80% of the animals reacted to epicutaneous application of a 1% solution. According to these results, $(\text{NH}_4)_2\text{S}_2\text{O}_8$ was considered a dermal sensitizer for the guinea-pig (2). Mensing et al. (22) demonstrated in a rabbit model that exposure to hair bleach containing persulfates increased airway hyper-responsiveness and that ammonium persulfate stimulated the release of nitric oxide in guinea-pigs (23).

Murine LLNA is an OECD-accepted method (OECD guideline no. 429, 2002) to identify contact sensitizers. The argument is that skin exposure to a sufficient amount of a relevant chemical allergen will induce an immune response of the quantity and quality necessary to cause systemic sensitization (24). In this present set-up, we slightly adjusted the murine LLNA. Instead of using CBA/Ca mice as described in OECD guideline 429, we used BALB/c mice. The purpose of this study was to investigate the dermal sensitizing potential of persulfate salts. This study will be elaborated with a second study in which the potential of these salts to induce respiratory sensitization and their ability to induce asthma are described. For studies of these pulmonary effects induced by a chemical, BALB/c mice are the preferred mouse strain (16–19).

According to the results obtained, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, $\text{K}_2\text{S}_2\text{O}_8$, and $\text{Na}_2\text{S}_2\text{O}_8$, which are known for their lung and skin sensitization potential in humans (1, 6–9), were positive in the murine LLNA. Ammonium and potassium persulfate can be categorized as moderate sensitizers, while sodium persulfate should be regarded as a strong sensitizer.

Differences between the persulfate salts have also been observed previously in several studies. Parsons et al. (25) reported that both ammonium and potassium persulfates caused histamine release from isolated rat peritoneal mast cells and from guinea-pig skin *in vitro* and *in vivo*. Histamine release induced by potassium persulfate was characterized by degranulation of the mast cells. However, with ammonium persulfate, alterations in the granules were observed but no apparent degranulation occurred. *In vitro* studies with slices of guinea-pig skin, potassium persulfate but not ammonium persulfate, appeared to release histamine selectively.

Several studies have already been conducted to relate LLNA EC3 values with skin sensitization based on clinical observations. These studies show a very close correlation between clinical observations and estimates of potency based on EC3 values (26, 27). The LLNA results obtained in the present study are consistent with the well-known ability of persulfates to cause sensitization among exposed human populations.

In humans, persulfates were reported to cause both delayed-type and immediate skin reactions, including irritant dermatitis, allergic eczematous dermatitis, localized contact urticaria, and generalized urticaria (1, 5). In addition to skin diseases, respiratory disorders have been frequently reported among hairdressers and chemical workers exposed to persulfates (6–9). Persulfate salts are frequently implicated agents in the origin of occupational asthma that may represent up to 4% of all occupational asthma cases (28).

In conclusion, we provided here a systematic comparative analysis of three persulfate salts of occupational significance using the LLNA. The study shows that all three persulfate salts, ammonium, sodium, and potassium, with a potential for sensitization in humans, were positive in the murine LLNA. Nevertheless, categorization according to EC3 values showed a (limited) distinction between ammonium and potassium persulfates, which are moderate sensitizers, and sodium persulfate, which is a strong sensitizer. These data will be of value in developing further accurate risk assessments.

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