

Skin sensitization potency and cross-reactivity of *p*-phenylenediamine and its derivatives evaluated by non-radioactive murine local lymph node assay and guinea-pig maximization test

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Background: *p*-Phenylenediamine (PPD)-related chemicals have been used as antioxidants in rubber products, and many cases of contact dermatitis caused by these chemicals have been reported.

Objective: The aim of this study was to investigate relative sensitizing potency and cross-reactivity among PPD derivatives.

Methods: Five PPD derivatives, *p*-aminodiphenylamine (PADPA), *N,N'*-diphenyl-*p*-phenylenediamine (DPPD), *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine (IPPD), *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine (DMBPPD), *N*-(1-methylheptyl)-*N'*-phenyl-*p*-phenylenediamine (MHPPD), and the core chemical PPD were evaluated for their sensitizing potency and cross-reactivity using the non-radioactive murine local lymph node assay (LLNA) and the guinea-pig maximization test (GPMT).

Results: PPD and all the derivatives were identified as primary sensitizers in both tests. The order of potency in the LLNA was as follows: IPPD and PADPA > PPD > DMBPPD and MHPPD > DPPD. In the GPMT, all six groups of animals sensitized with one of these chemicals cross-reacted to four other derivatives. Specifically, the five groups that have a common basic PADPA structure, that is PADPA, DPPD, IPPD, DMBPPD, and MHPPD, all reacted to each other at almost the same scores, while none of them reacted to PPD.

Conclusions: The cross-reactivity profile found in the study was to some extent different from that in previous human data, where distinction between cross-reaction and concomitant primary sensitization is not always clear.

Key words: contact dermatitis; cross-reactivity; rubber chemicals. © Blackwell Munksgaard, 2009.

Conflicts of interest: The authors have declared no conflicts.

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During manufacture, various chemicals are added to rubber: vulcanizers, accelerators, retardants, and antioxidants (1). The residues of these chemicals in rubber products such as gloves, boots, and tyres may cause allergic contact dermatitis (2, 3). The responsible allergens in most cases are thiurams, dithiocarbamates, benzothiazoles, and *p*-phenylenediamine (PPD) derivatives. In the risk management process for skin sensitization, it is important to evaluate the sensitizing potency of individual chemicals. Quantitative evaluation

may allow the ranking of allergenic potency within a certain group of chemicals used for the same purpose and thereby offer the opportunity to select the less allergenic chemicals. De Jong et al. (4) evaluated the sensitizing potency of 15 rubber chemicals using a modified local lymph node assay (LLNA), ranked their potency, and identified the rubber additives of choice among thiurams, dithiocarbamates, and benzothiazoles. Cross-reactivity to related chemicals is another factor in risk assessment. It is often the case that

a patient who shows positive skin reaction to a sensitizer reacts at the same time to structurally related chemicals. Thiurams and dithiocarbamates have similar chemical structures, and cross-reactivity among them has been reported (5). With respect to PPD derivatives, Herve-Bazin et al. (6) reported that some tyre manufacturing workers sensitized with *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine (IPPD) also showed positive skin reactions to *N*-phenyl-*N'*-cyclohexyl-*p*-phenylenediamine (CPPD), *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine (DMBPPD), and/or PPD. It is, however, difficult to distinguish whether the skin reaction observed in the patch test comes from cross-reaction or concomitant primary sensitization to these chemicals. Animal testing is a useful tool in elucidating the cross-reaction patterns among structurally related sensitizers. In the present study, five PPD derivatives and the core chemical PPD, which is well known as a strong sensitizer, were quantitatively evaluated for their sensitizing potency using LLNA and the cross-reactivity among them evaluated using guinea-pig maximization test (GPMT).

Materials and Methods

Chemicals

The names, abbreviations, and CAS registry numbers of the PPD and its derivatives are listed in Table 1. Their chemical structures are shown in Fig. 1. The tested chemicals were of superior grade and purchased from Tokyo Kasei Co., Ltd. (Tokyo, Japan).

Non-radioactive murine LLNA

Six-week to eight-week-old female BALB/c mice from CLEA Japan (Tokyo, Japan) were used. The LLNA was performed as previously reported (7). Four animals were used for each sensitization group. Skin irritation was judged by measuring ear thickness at the time of auricular lymph node excision. Total lymph node cell count in a chemical-

Table 1. List of chemicals tested

Chemical name	Abbreviation	CAS no.
<i>p</i> -Phenylenediamine	PPD	106-50-3
<i>p</i> -Aminodiphenylamine	PADPA	101-54-2
<i>N,N'</i> -diphenyl- <i>p</i> -phenylenediamine	DPPD	74-31-7
<i>N</i> -isopropyl- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine	IPPD	101-72-4
<i>N</i> -(1,3-dimethylbutyl)- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine	DMBPPD	793-24-8
<i>N</i> -(1-methylheptyl)- <i>N'</i> -phenyl- <i>p</i> -phenylenediamine	MHPPD	15233-47-3

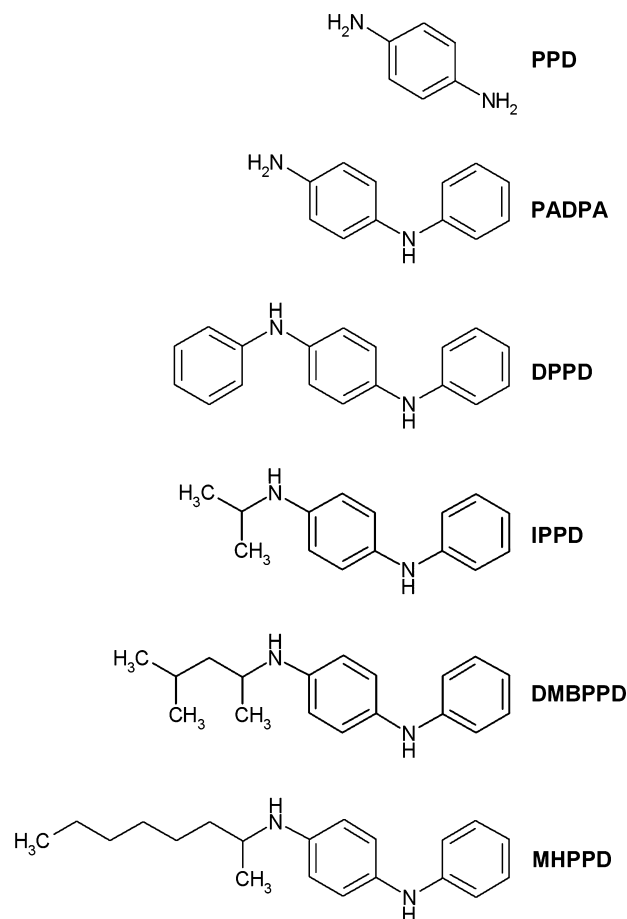


Fig. 1. Chemical structures of PPD and five derivatives. DPPD, *N,N'*-diphenyl-*p*-phenylenediamine; DMBPPD, *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine; IPPD, *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine; MHPPD, *N*-(1-methylheptyl)-*N'*-phenyl-*p*-phenylenediamine; PADPA, *p*-aminodiphenylamine; PPD, *p*-phenylenediamine.

treated animal divided by mean lymph node cell count in the control group was designated as the cellularity index, while 5-bromo-2'-deoxyuridine (BrdU) incorporation (expressed as optical density in enzyme-linked immunosorbent assay) per well of cells from a chemical-treated animal divided by mean BrdU incorporation per well of cells from the control group was used as the BrdU incorporation index. The stimulation index (SI) was calculated by multiplying the cellularity index by the BrdU incorporation index. The minimum dose at which statistically significant increase in the SI was detected compared with the control was designated as the minimum induction dose of the chemical.

Guinea-pig maximization test

Five-week to six-week-old female Hartley guinea-pigs from Japan SLC, Inc. (Shizuoka, Japan) were used. The GPMT was performed as described previously (8). The animals were divided into six

groups, and each group of four animals was sensitized with PPD or one of its derivatives. The first (intradermal) and second (topical and occluded) induction doses were set at 500 p.p.m. Two weeks after the second induction, 12 circles (16 mm in diameter) were marked on the dorsum of each animal, and the animals were challenged all at once with 50 p.p.m. and 5000 p.p.m. of the six sensitizers in 50 μ l acetone. Forty-eight hours after the challenge, each site was scored for erythema (0–4) and oedema (0–3) according to the criteria of Sato et al. (9). Total group scores (erythema plus oedema) based on the same challenge concentration and chemical in each group were added up and divided by the number of animals in the group to give the mean response value as an index of skin reaction to challenge.

Statistical analysis

In the LLNA, index values for each chemical-treated group and the 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).

Table 2. Results of LLNA for PPD and five derivatives

Chemicals	%	Ear thickness index	Cellularity index	BrdU incorporation index
PPD	0	1.00 $\pm$ 0.02	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
	0.03	1.01 $\pm$ 0.04	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
	0.1	1.02 $\pm$ 0.06	1.2 $\pm$ 0.0*	1.2 $\pm$ 0.1
	0.3	1.03 $\pm$ 0.04	1.8 $\pm$ 0.4*	1.7 $\pm$ 0.2**
	1	1.00 $\pm$ 0.02	2.3 $\pm$ 1.0*	2.2 $\pm$ 0.2**
PADPA	0	1.00 $\pm$ 0.03	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
	0.01	1.01 $\pm$ 0.04	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1
	0.03	1.03 $\pm$ 0.06	1.2 $\pm$ 0.1	1.4 $\pm$ 0.3**
	0.1	1.01 $\pm$ 0.05	1.3 $\pm$ 0.3*	1.4 $\pm$ 0.2*
	0.3	1.04 $\pm$ 0.11	1.5 $\pm$ 0.1**	1.5 $\pm$ 0.1**
DPPD	0	1.00 $\pm$ 0.04	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
	1	0.99 $\pm$ 0.04	1.0 $\pm$ 0.1	1.0 $\pm$ 0.2
	3	1.01 $\pm$ 0.04	1.0 $\pm$ 0.1	1.1 $\pm$ 0.1
	10	1.03 $\pm$ 0.05	1.1 $\pm$ 0.1	1.4 $\pm$ 0.3*
	30	1.01 $\pm$ 0.05	1.2 $\pm$ 0.2*	1.4 $\pm$ 0.3
IPPD	0	1.00 $\pm$ 0.03	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
	0.01	1.01 $\pm$ 0.04	1.0 $\pm$ 0.1	1.3 $\pm$ 0.1*
	0.03	1.00 $\pm$ 0.03	1.2 $\pm$ 0.1	1.4 $\pm$ 0.1**
	0.1	1.03 $\pm$ 0.06	1.7 $\pm$ 0.2**	1.9 $\pm$ 0.2**
	0.3	1.02 $\pm$ 0.04	1.9 $\pm$ 0.2**	2.1 $\pm$ 0.1**
DMBPPD	0	1.00 $\pm$ 0.03	1.0 $\pm$ 0.1	1.0 $\pm$ 0.2
	0.1	0.99 $\pm$ 0.06	0.9 $\pm$ 0.1	1.1 $\pm$ 0.1
	0.3	0.97 $\pm$ 0.03	0.9 $\pm$ 0.1	1.5 $\pm$ 0.2*
	1	1.05 $\pm$ 0.02	1.3 $\pm$ 0.1*	1.8 $\pm$ 0.3**
	3	1.02 $\pm$ 0.05	2.2 $\pm$ 0.2**	2.3 $\pm$ 0.3**
MHPPD	0	1.00 $\pm$ 0.02	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1
	0.3	1.00 $\pm$ 0.04	1.0 $\pm$ 0.1	1.0 $\pm$ 0.2
	1	1.05 $\pm$ 0.07	1.2 $\pm$ 0.2	1.5 $\pm$ 0.1**
	3	1.30 $\pm$ 0.05**	2.3 $\pm$ 0.7*	1.9 $\pm$ 0.2**
	10	1.41 $\pm$ 0.09**	3.3 $\pm$ 0.3*	2.6 $\pm$ 0.1**

*, ** Significantly different from control, $p < 0.05$ and 0.01, respectively.

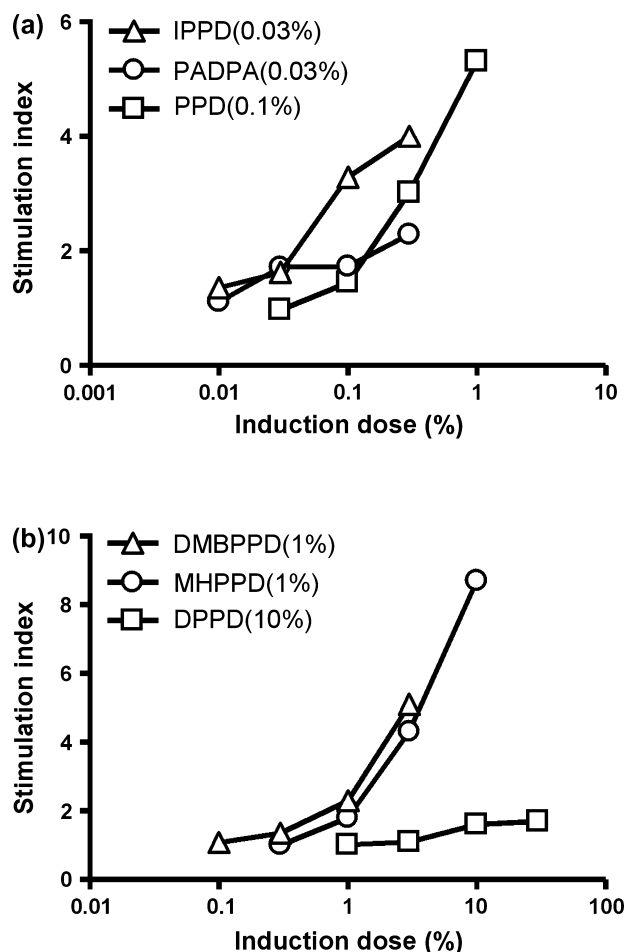


Fig. 2. Dose-response profiles for the induction phase of PPD and five derivatives in local lymph node assay. Each symbol represents dose-related stimulation index values as per Table 2: (a) group of chemicals with minimum induction dose of 0.1% or less; (b) group of chemicals with minimum induction dose of 1% or more. DMBPPD, *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine; DPPD, *N,N'*-diphenyl-*p*-phenylenediamine; IPPD, *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine; MHPPD, *N*-(1-methylheptyl)-*N'*-phenyl-*p*-phenylenediamine; PADPA, *p*-aminodiphenylamine; PPD, *p*-phenylenediamine.

Results

Dose-response analysis of induction phase with LLNA

The results of the LLNA for PPD and five derivatives are shown in Table 2. In our method, a chemical was designated positive when a statistically significant increase in the SI value was obtained using a non-irritant dose. Only MHPPD exhibited an irritant reaction in the dose range tested. All six chemicals including MHPPD, however, produced significant increase in the SI at a non-irritant dose and were designated as positive skin sensitizers. Dose-response profiles for the SI values are shown in Fig. 2. The most potent sensitizers in the LLNA were IPPD and *p*-aminodiphenylamine (PADPA),

with a minimum induction dose of 0.03%, followed by PPD (0.1%), DMBPPD and MHPPD (1%), and *N,N'*-diphenyl-*p*-phenylenediamine (DPPD) (10%).

Cross-reactivity of PPD derivatives in GPMT

Six groups of animals were sensitized with PPD or one of the five PPD derivatives at 500 p.p.m. and challenged with all six chemicals at once. Skin reaction scores 48 hr after the challenge are summarized in Table 3. When each group was challenged with the corresponding induction compound at 5000 p.p.m., all animals displayed a strong positive skin reaction and all groups were therefore designated positive in the GPMT. Radar charts of the cross-reactivity elicited by the six sensitizers at 5000 p.p.m. are shown in Fig. 3. Five induction groups, PADPA, DPPD, IPPD, DMBPPD, and MHPPD, exhibited approximately the same pattern in the radar charts, that is all the animals sensitized with any one of these five PPD derivatives showed a reaction of about the same potency to the other four, but no cross-reactivity to PPD. In the PPD-sensitized group, animals cross-reacted to the other PPD derivatives except DPPD, although the extent of skin reaction was much weaker than elicited by PPD itself.

Discussion

The PPD derivatives tested in the present study are added to rubber as antioxidants. PPD itself is used in hair dyes but is not used in rubbers.

Various cases of skin sensitization related to PPD derivatives have been reported. Patch tests conducted at St John's Hospital from 1971 to 1976 using a PPD rubber mix (IPPD, 0.1%; CPPD, 0.25%; and DPPD, 0.25%) showed that 0.6% of patients reacted to the mix, with IPPD the most important allergen, while most reacted to CPPD and/or DPPD at the same time (10). IPPD is indeed one of the main allergens associated with rubber products and many cases, especially related to tyres, have been reported (2, 6). In Japan, IPPD and DMBPPD in farm boots have been identified as causative sensitizers in allergic dermatitis (11). As for PPD, the principal structure of the derivatives tested, many cases of contact dermatitis have been reported among hair dye consumers and hairdressers (12). Although N-substitution of PPD may increase or decrease the sensitizing potency according to the length and steric constitutions of the substituents, quantitative evaluation has not been performed thoroughly.

Data from LLNA using PPD derivatives are rather scarce and are available only for PPD and IPPD. In one study, PPD was selected as a model compound to evaluate intra-laboratory and inter-laboratory variation of the standard LLNA (13). The results showed minimum induction doses (EC₃) of 0.06–0.09% and 0.09–0.20% in two laboratories. Ikarashi et al. (14) evaluated the sensitizing ability of four rubber additives including IPPD and reported a minimum induction dose of 0.14% for this chemical. Our results, with minimum induction doses of 0.1% and 0.03% for PPD and IPPD, respectively, were comparable

Table 3. Cross-reactivity of PPD and five derivatives in guinea-pig maximization test^a

Elicitation		Induction ^b					
Chemicals	p.p.m.	PPD	PADPA	DPPD	IPPD	DMBPPD	MHPPD
PPD	50	0.75 (2/4)^{c,d}	— ^c	—	—	—	—
	5000	6.25 (4/4)	—	—	—	—	—
PADPA	50	0.50 (1/4)	3.00 (4/4)	—	—	—	—
	5000	3.25 (3/4)	5.25 (4/4)	4.50 (4/4)	4.75 (4/4)	4.75 (4/4)	4.75 (4/4)
DPPD	50	—	—	2.25 (3/4)	—	0.25 (1/4)	—
	5000	—	0.75 (2/4)	5.50 (4/4)	2.00 (2/4)	3.50 (3/4)	2.25 (2/4)
IPPD	50	—	1.50 (3/4)	—	2.75 (4/4)	0.75 (2/4)	—
	5000	0.50 (1/4)	4.00 (4/4)	3.75 (4/4)	5.50 (4/4)	4.50 (4/4)	5.25 (4/4)
DMBPPD	50	—	1.25 (2/4)	—	—	3.50 (4/4)	1.50 (3/4)
	5000	0.50 (2/4)	3.25 (4/4)	3.75 (4/4)	4.75 (4/4)	6.25 (4/4)	5.75 (4/4)
MHPPD	50	—	1.75 (4/4)	—	1.00 (2/4)	3.00 (4/4)	4.00 (4/4)
	5000	0.50 (2/4)	4.00 (4/4)	4.25 (4/4)	5.00 (4/4)	6.25 (4/4)	6.75 (4/4)

DMBPPD, *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine; DPPD, *N,N'*-diphenyl-*p*-phenylenediamine; IPPD, *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine; MHPPD, *N*-(1-methylheptyl)-*N'*-phenyl-*p*-phenylenediamine; PPD, *p*-phenylenediamine; PADPA, *p*-aminodiphenylamine.

^aValues are mean skin reaction scores 48 hr after challenge.

^bEach group of animals was sensitized with one of six chemicals at 500 p.p.m. and challenged with all of them at once with two dose levels.

^cNumber of animals with positive skin reaction in group.

^dBold type indicates skin reaction produced with same chemical as used in induction phase.

^eNo skin reaction observed in group.

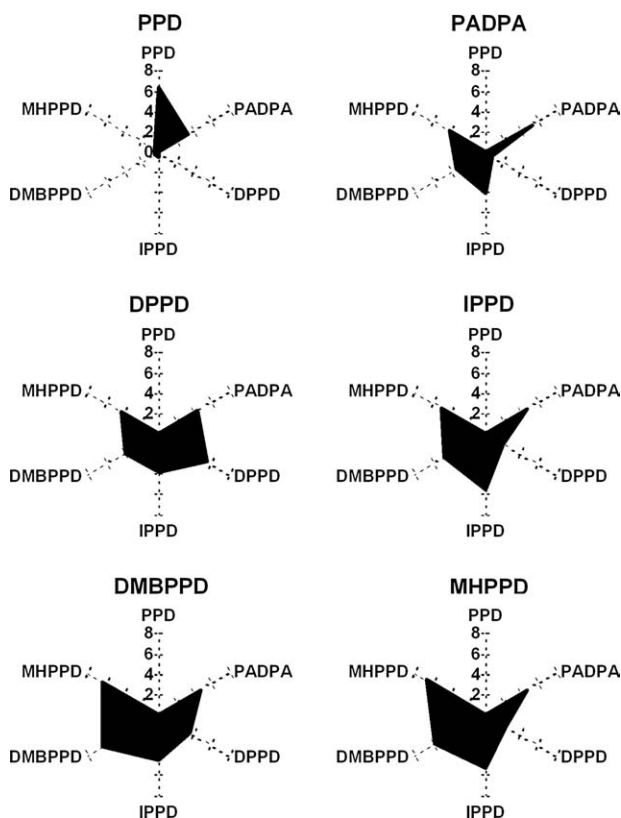


Fig. 3. Radar charts of cross-reactivity of PPD and five derivatives in guinea-pig maximization test. Each group of animals was sensitized with PPD or one of its derivatives at 500 p.p.m. and challenged with all chemicals at once. Values are mean skin reaction scores 48 hr after challenge at 5000 p.p.m. as per Table 3. PPD, *p*-phenylenediamine; DMBPPD, *N*-(1,3-dimethylbutyl)-*N'*-phenyl-*p*-phenylenediamine; DPPD, *N,N'*-diphenyl-*p*-phenylenediamine; IPPD, *N*-isopropyl-*N'*-phenyl-*p*-phenylenediamine; MHPPD, *N*-(1-methylheptyl)-*N'*-phenyl-*p*-phenylenediamine; PADPA, *p*-aminodiphenylamine; PPD, *p*-phenylenediamine.

with the previous data. The results of the LLNA confirmed that the PPD derivatives tested are all primary skin sensitizers. The order of sensitizing potency based on minimum induction concentration in the LLNA was as follows: IPPD and PADPA (0.03%) > PPD (0.1%) > DMBPPD and MHPPD (1%) > DPPD (10%). For chemicals of small molecular size like rubber additives to elicit allergic skin reaction, it is essential to penetrate the epidermis and conjugate with a macromolecule directly or after metabolic activation to create an immunogen. Theoretically, it is possible to predict qualitatively and quantitatively the skin-sensitizing activity of a chemical from its structure, and there have been many studies concerning the role of the structure-activity relationship in skin sensitization (15, 16). It has been postulated that PPD sensitization could result from metabolic activation of amino substituents to form an electrophile, suggesting that the location of amino substituents in the molecule may

well affect sensitizing potency. DPPD was a weaker sensitizer than the other derivatives in the LLNA. The amino substituents of DPPD are located exclusively between benzenes, which may result in a different affinity for metabolic activation. The length of the alkyl substituents is another factor that influences sensitizing potency. Ikarashi et al. (17) quantitatively evaluated the contact sensitivity of thioureas using a modified LLNA and discovered that the sensitizing potency of dilauryl thiourea was greater than that of dibutyl thiourea and diethyl thiourea. Of the three antioxidants IPPD, MHPPD, and DMBPPD, which have the same basic PADPA structure, IPPD was the strongest followed by DMBPPD and MHPPD. It seems that, in the case of this group, the longer the length of the alkyl substituents, the weaker the sensitizing potency.

In the present study, although the precise dose-response profile was not evaluated in the GPMT, the order of sensitizing potency as determined by the latter was somewhat different from that indicated by the LLNA. van Och et al. (18) compared the allergenicity of three rubber additives using GPMT and LLNA and found that the two tests ranked the potencies of the three sensitizers differently. The inconsistency between the two tests seems natural considering the substantial differences in the procedures. Unlike the GPMT, which uses an injection of the test substance in addition to adjuvant as a vehicle, the LLNA uses only percutaneous exposure. Recent analytical studies have reported a close relationship between minimum induction dose in the LLNA and experimental human induction threshold values (19–21). Although various factors, such as the concentration of allergen in and leaching from products, the duration and frequency of exposure, and the condition of the skin, should be considered when evaluating risk, the LLNA seems to provide better quantitative information for the following risk assessment process. A drawback of the LLNA, however, is that it only evaluates the induction phase. In the GPMT, on the other hand, the allergic skin reaction is elicited and scored, which makes this test a useful tool in evaluating the cross-reactivity of sensitizers.

There have been a number of case reports on the cross-reactivity of PPD derivatives evaluated using patch testing. In Poland, 4.8% of 3224 dermatitis patients tested with PPD gave a positive result and 17.5% of them also reacted to IPPD (12). In France, all occupational dermatitis patients dealing with tyres who reacted to IPPD also cross-reacted to DMBPPD and 37% reacted to PPD (6). In the present study, all six groups of animals sensitized with PPD or one of the five

PPD derivatives cross-reacted to four other derivatives. Specifically, animals induced using one of the five derivatives that have a common basic PADPA structure, all reacted to each other with almost the same score, and none of them reacted to PPD. The cross-reactivity profile found in the study was to some extent different from that in previous human data. This inconsistency could be partly because the cross-reaction observed in the patch tests was actually based on concomitant primary sensitization to the chemicals. In the case of PPD sensitization, metabolic activation of amino substituents to benzoquinone or nitro substituents is thought to play an important role (22). Differences in the metabolism of PPD derivatives between animals and humans should, therefore, also be taken into consideration when extrapolating animal data.

References

1. Cronin E. Rubber. In: *Contact Dermatitis*. New York, Churchill Livingstone, 1980: 714–770.
2. Conde-Salazar L, Del-Rio E, Guimaraens D, Gonzalez Domingo A. The type IV allergy to rubber additives: a ten-year study of 686 cases. *J Am Acad Dermatol* 1993; 29: 176–180.
3. Kaniwa M. Preventive measures against health damage due to chemicals in household products. *Bull Natl Inst Sci* 2006; 124: 1–20.
4. De Jong W H, van Och F M, Den Hartog Jager C F, Spiekstra S W, Slob W, Vandebriel R J, Van Loveren H. Ranking of allergenic potency of rubber chemicals in a modified local lymph node assay. *Toxicol Sci* 2002; 66: 226–232.
5. Knudsen B B, Menné T. Contact allergy and exposure patterns to thiurams and carbamates in consecutive patients. *Contact Dermatitis* 1996; 35: 97–99.
6. Herve-Bazin B, Gradiski D, Duprat P, Marignac B, Foussereau J, Cavelier C, Bieber P. Occupational eczema from N-isopropyl-N'-phenylparaphenylenediamine (IPPD) and N-dimethyl-1,3-butyl-N'-phenylparaphenylenediamine (DMPPD) in tyres. *Contact Dermatitis* 1977; 3: 1–15.
7. Yamano T, Shimizu S, Noda T. Allergenicity evaluation of p-chloro-m-cresol and p-chloro-m-xylene by non-radioactive murine local lymph-node assay and multiple-dose guinea pig maximization test. *Toxicology* 2003; 190: 259–266.
8. Nakamura A, Momma J, Sekiguchi H et al. A new protocol and criteria for quantitative determination of sensitization potencies of chemicals by guinea pig maximization test. *Contact Dermatitis* 1994; 31: 72–85.
9. Sato Y, Katsumura Y, Ichikawa H, Kobayashi T, Kozuka T, Morikawa F, Ohta S. A modified technique of guinea pig testing to identify delayed hypersensitivity allergens. *Contact Dermatitis* 1981; 7: 225–237.
10. Menné T, White I R, Bruynzeel D P, Dooms-Goossens A. Patch test reactivity to the PPD black-rubber-mix (industrial rubber chemicals) and individual ingredients. *Contact Dermatitis* 1992; 26: 354.
11. Nishioka K, Murata M, Ishikawa T, Kaniwa M. Contact dermatitis due to rubber boots worn by Japanese farmers, with special attention to 6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline (ETMDQ) sensitivity. *Contact Dermatitis* 1996; 35: 241–245.
12. Kiec-Swierczynska M, Krecisz B, Swierczynska-Machura D. Contact allergy to paraphenylenediamine: a 10-year observation held in the Nofer Institute of Occupational Medicine. *Med Pr* 2007; 58: 215–222.
13. Warbrick E V, Dearman R J, Lea L J, Basketter D A, Kimber I. Local lymph node assay responses to paraphenylenediamine: intra- and inter-laboratory evaluations. *J Appl Toxicol* 1999; 19: 255–260.
14. Ikarashi Y, Tsuchiya T, Nakamura A. Evaluation of contact sensitivity of rubber chemicals using the murine local lymph node assay. *Contact Dermatitis* 1993; 28: 77–80.
15. Karlberg A T, Bergstrom M A, Borje A, Luthman K, Nilsson J L. Allergic contact dermatitis – formation, structural requirements, and reactivity of skin sensitizers. *Chem Res Toxicol* 2008; 21: 53–69.
16. Patlewicz G, Roberts D W, Uriarte E. A comparison of reactivity schemes for the prediction skin sensitization potential. *Chem Res Toxicol* 2008; 21: 521–541.
17. Ikarashi Y, Ohno J, Momma J, Tsuchiya T, Nakamura A. Assessment of contact sensitivity of four thiourea rubber accelerators: comparison of two mouse lymph node assays with the guinea pig maximization test. *Food Chem Toxicol* 1994; 32: 1067–1072.
18. van Och F M, Vandebriel R J, Prinsen M K, De Jong W H, Slob W O, van Loveren H. Comparison of dose-responses of contact allergens using the guinea pig maximization test and the local lymph node assay. *Toxicology* 2001; 167: 207–215.
19. Schneider K, Akkan Z. Quantitative relationship between the local lymph node assay and human skin sensitization assays. *Regul Toxicol Pharmacol* 2004; 39: 245–255.
20. Basketter D A, Clapp C, Jefferies D, Safford B, Ryan C A, Gerberick F, Dearman R J, Kimber I. Predictive identification of human skin sensitization thresholds. *Contact Dermatitis* 2005; 53: 260–267.
21. Safford R J. The Dermal Sensitisation Threshold – a TTC approach for allergic contact dermatitis. *Regul Toxicol Pharmacol* 2008; 51: 195–200.
22. Smith C K, Hotchkiss S A M. *Allergic Contact Dermatitis*. London: Taylor and Francis, 2001.

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