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# Effects of active sunscreen ingredient combinations on the topical penetration of the herbicide 2,4-dichlorophenoxyacetic acid

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Sunscreen use can reduce the incidence of certain skin cancers. However, a number of commercially available formulations have been shown to enhance the transdermal penetration of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D). Most of the active ingredients used in these compounds can individually act as penetration enhancers. Commercial sunscreens frequently contain multiple active ingredients in order to provide broad sunscreen protection. The purpose of this study was therefore to examine the effect of these active ingredient combinations on the transdermal absorption of 2,4-D *in vitro*. All six of the combinations tested resulted in increased cumulative penetration ($P < 0.01$) and faster lag times ($P < 0.05$). The 2,4-D cumulative penetration in the presence of the OFF! Deepwoods combination was significantly greater than the absorption with either the individual ingredients or their average ($P < 0.05$). A systematic study designed to isolate the chemicals responsible for this enhancement demonstrated that with UV absorbers DEET synergistically increased the 2,4-D penetration and that DEET's cumulative enhancement properties correlate with its concentration. By contrast, octocrylene significantly slowed the lag time when used in combinations and was the only active ingredient that showed any antagonistic effects on 2,4-D penetration. Because none of the active ingredient combinations were able to inhibit dermal uptake of 2,4-D, it seems that proper selection of inert ingredients may be the most feasible solution for reducing penetration enhancement. *Toxicology and Industrial Health* 2003; **19:** 1–8.

**Key words:** *2,4-D, DEET; sunscreens; transdermal; UV absorbers*

## Introduction

Many chemicals and solvents have been shown to be dermal penetration enhancers. Such chemicals may increase penetration by causing physical damage to the skin's barrier, by modifying the stratum corneum or underlying layers, or by promoting partitioning of the co-penetrating chemicals into the skin (Barry, 1991; Moser *et al*., 2001). While some enhancement effects serve useful purposes, such as to deliver pharmaceuticals transdermally (Kanikkannan *et al*., 2000), penetration enhancers can also increase transdermal transport of harmful chemicals (Baynes *et al*., 2002; Sartorelli, 2002).

While many individual enhancers have been characterized, few studies have quantified the effects of using multiple penetration enhancers simultaneously. The three enhancer combinations

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diethylene glycol monoethyl ether and propylene glycol monolaurate, diethylene glycol monoethyl ether and propylene glycol monolaurate, and propylene glycol and oleyl alcohol all significantly enhanced the permeability of tenoxicam through hairless mouse skin (Gwak and Chun, 2002). A combination of pyridostigmine bromide and diisopropylfluorophosphate enhanced N,N-diethyl-m-toluamide (DEET) absorption through isolated perfused porcine skin flaps (Riviere *et al*., 2003). In addition, penetration enhancement effects of multichemical formulations of which all ingredients are not necessarily penetration enhancers have been tested (Baynes and Riviere, 1998).

Penetration enhancement can have undesirable consequences. Previous research showed that at least six commercial sunscreen formulations significantly increased the transdermal penetration of the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) *in vitro* (Brand *et al*., 2002). Subsequent research demonstrated that eight of the individual active ingredients present in those commercial formulations also significantly enhanced penetration of 2,4-D *in vitro* (Pont *et al*., 2004). Commercially available sunscreens, however, usually contain multiple UV absorbers in order to provide broad spectrum protection. Therefore, the purpose of this work is to extend our previous studies by systematically determining how multiple sunscreen ingredients interact to enhance the dermal penetration of 2,4-D through hairless mouse skin *in vitro*.

## Materials and methods

### Chemicals and reagents

The following sunscreen ingredients were used to form the combinations tested: octyl methoxycinnamate (RONA, EM Industries, Hawthorne, NY), octyl salicylate (Aldrich, Milwaukee, WI), oxybenzone (BASF, Wyandotte, MI), homosalate (RONA, EM Industries, Hawthorne, NY), octocrylene (BASF, Wyandotte, MI), padimate-o (Aldrich, Milwaukee, WI) and DEET (9.5% and 19.0%, v/v) (Supelco, Bellefonte, PA). The initial formulations used were based on earlier studies (Brand *et al*., 2002) and are listed in Table 1. The one exception was the Banana Boat Formulation; our initial studies were performed on 'Groovy Grape' which contained the physical absorber titanium dioxide. Since the purpose of this study was to examine chemical absorbers, we switched the Banana Boat formulation to Banana Boat Sunblock Lotion SPF 15. Follow-up studies were later added to fill in missing combinations.

The following concentrations were used in all relevant combinations: oxybenzone (0.6% w/v), homosalate (5% v/v) (Patel *et al*., 1992), octyl methoxycinnimate (7.5% v/v), octyl salicylate (5% v/v), and octocrylene (7% v/v) (Hayden *et al*., 1997), and padimate-o (8% v/v) (Finnin and Morgan, 1999). The concentrations for DEET were taken from the ingredient lists of OFF! Skintastic and OFF! Deepwoods commercial formulations, respectively (S.C. Johnson & Son, Racine, WI). All concentrations were within the regulatory limits set by the USFDA and were similar to amounts used in commercial sunscreens. The oxybenzone concentration was much lower than its USFDA limit due to the amount soluble in ethanol.

Eighty per cent ethanol (v/v) was selected as the solvent due to its presence in commercial spray and gel products such as Coppertone To Go Spray, Neutrogena Healthy Defense Oil Free Sunblock Spray, Coppertone Gel and Coppertone Sports Gel. In addition, the formulations used in commercially available lotions or oils are considered proprietary and are not readily available. The use of ethanol allowed direct comparison with previous studies (Pont *et al*., 2004).

The donor solution consisted of 2,4-D amine (Agriliance, St. Paul, MN) spiked with $^{14}$C-labelled 2,4-D (Sigma, St. Louis, MO). The receiver solution was Hanks balanced saline solution (HBSS) supplemented with 4% (w/v) bovine serum albumin (BSA) (Fisher Chemicals, Pittsburgh, PA). The BSA supplement increased the hydrophobicity of the receiver solution, thus increasing the partitioning of 2,4-D into the receiver compartment and helping maintain skin viability and metabolic function for more than 24 hours (Collier and Bronaugh, 1991).

**Table 1.** Penetration of 2,4-D following skin pretreatment with representative sunscreen combinations.

| Formulation tested | Active ingredients | Cumulative (% dose/24 hours) mean ± SEM | Lag time (hours) mean ± SEM |
|---|---|---|---|
| Coppertone to Go (CTG) | | **75.7 ± 5.5**[a] | **1.6 ± 0.1**[b] |
| | Octyl methoxycinnamate | 84.2 ± 1.8 | 1.7 ± 0.2 |
| | Octyl salicylate | 74.1 ± 3.1 | 1.1 ± 0.2 |
| | Homosalate | 72.5 ± 3.5 | 2.6 ± 0.4 |
| | Average | 76.5 ± 2.0 | 1.8 ± 0.2 |
| Coppertone Sport/No Ad (CS) | | **80.2 ± 4.0**[a] | **2.0 ± 0.3**[b] |
| | Oxybenzone | 74.9 ± 4.8 | 3.7 ± 0.4[c] |
| | Octyl methoxycinnamate | 84.2 ± 1.8 | 1.7 ± 0.2 |
| | Octyl salicylate | 74.1 ± 3.1 | 1.1 ± 0.2 |
| | Average | 77.6 ± 2.2 | 2.2 ± 0.3 |
| Neutrogena Oil Free (NOF) | | **81.3 ± 5.0**[a] | **2.2 ± 0.3**[b] |
| | Octyl methoxycinnamate | 84.2 ± 1.8 | 1.7 ± 0.2 |
| | Octyl salicylate | 74.1 ± 3.1 | 1.1 ± 0.2 |
| | Homosalate | 72.5 ± 3.5[c] | 2.6 ± 0.4 |
| | Oxybenzone | 74.9 ± 4.8 | 3.7 ± 0.4[c] |
| | Average | 76.3 ± 1.9 | 2.3 ± 0.2 |
| Banana Boat (BB) | | **80.9 ± 4.4**[a] | **1.7 ± 0.3**[b] |
| | Padimate-o | 86.9 ± 2.5 | 2.5 ± 0.4 |
| | Oxybenzone | 74.9 ± 4.8 | 3.7 ± 0.4[c] |
| | Octyl methoxycinnamate | 84.2 ± 1.8 | 1.7 ± 0.2 |
| | Average | 81.5 ± 2.2 | 2.6 ± 0.3 |
| OFF! Skintastic – DEET (OFFS) | | **83.7 ± 2.3**[a] | **1.8 ± 0.2**[b] |
| | Octocrylene | 65.6 ± 4.3[d] | 3.7 ± 0.3[c] |
| | Octyl methoxycinnamate | 84.2 ± 1.8 | 1.7 ± 0.2 |
| | Oxybenzone | 74.9 ± 4.8 | 3.7 ± 0.4[c] |
| | Deet – 9.5% | 83.3 ± 2.4 | 2.6 ± 0.2 |
| | Average | 77.8 ± 2.3 | 2.9 ± 0.2 |
| OFF! Deepwoods – DEET (DWOFF) | | **98.8 ± 0.6**[a] | **0.9 ± 0.1**[b] |
| | Octyl methoxycinnamate | 84.2 ± 1.8[c] | 1.7 ± 0.2 |
| | Oxybenzone | 74.9 ± 4.8[c] | 3.7 ± 0.4[c] |
| | Octyl salicylate | 74.1 ± 3.1[c] | 1.1 ± 0.2 |
| | Deet – 19.5% | 82.8 ± 3.4[c] | 2.4 ± 0.1 |
| | Average | 78.9 ± 1.9[c] | 2.2 ± 0.3 |
| No sunscreen control | | **54.9 ± 4.7** | **3.3 ± 0.3** |

[a] $P < 0.01$ formulation versus control.
[b] $P < 0.05$ formulation versus control.
[c] $P < 0.01$ individual ingredient versus formulation.
[d] $P < 0.05$ individual ingredient versus formulation.
Results are presented as mean ± SEM.
Bolded values represent 2,4-D penetration after pretreatment with the ingredient combination in 80% ethanol.
Individual ingredient results come from Pont *et al*. (2004).
Average is the mean values of all of the individual ingredients combined.

## Diffusion studies

All diffusion studies were performed as described previously (Pont *et al*., 2004). Briefly, excised hairless mouse skin was placed with epidermal side facing the donor into a Bronaugh-style flow-through diffusion chamber maintained at 32°C (Permegear, Rieglesville, PA). Hairless mouse skin has been shown to be a reasonable model for human skin when examining 2,4-D penetration in the presence of UV absorbers (Pont *et al*., 2004). The skin was allowed to equilibrate for 1 hour after which it was exposed to a 30-minute pretreatment with the sunscreen formulations. The 2,4-D was then added to the donor chamber and the experiments continued for 24 hours. Fractions were collected in 90-minute intervals and were analysed via liquid scintillation counting (Tricarb Model 1600CA, Packard, Perkin–Elmer, Wellesley, MA).

### Analytical methods

Data were analysed by cumulative percent flux (% penetrated versus time). Additionally, the linear portion of this curve was isolated, a regression line determined and the $x$-intercept (i.e., the 'lag time') was calculated. The total amount of 2,4-D penetrated and the lag times for each sunscreen combination were then compared to the 80% ETOH control via ANOVA. These parameters were also compared to previously published data for the individual active ingredients (Pont *et al*., 2004). Finally, for each combination, both the individual lag times and cumulative penetration of each constituent ingredient were averaged to obtain a theoretical value which was then statistically compared to the measured cumulative penetration and lag times for the combination. All statistical analysis occurred via either ANOVA followed by a Dunnett's Multiple Comparison Test or an unpaired $t$-test, with significance for both set to $P < 0.05$.

## Results

Table 1 contains the cumulative dermal penetration and lag times of 2,4-D after skin pretreatment with either Coppertone to Go, Coppertone Sport/No AD, Banana Boat, Neutrogena Oil Free, OFF! Skintastic, or OFF! Deepwoods combinations. All six combinations significantly increased the total 2,4-D flux through the skin versus control ($P < 0.01$). In addition, all six combinations significantly decreased lag time versus control ($P < 0.05$).

Table 1 also contains previously published data on the ability of individual sunscreen ingredients to enhance the transdermal penetration of 2,4-D (Pont *et al*., 2004). For each combination, the theoretical average value for the cumulative penetration and lag time is presented. The values were calculated by combining the six replicates of each combination ingredient and then calculating the mean $\pm$ SEM. The combination data were then compared to the individual sunscreen ingredients via ANOVA followed by Dunnett's Test ($P < 0.05$) in order to determine whether significant interactions exist between the penetration enhancement characteristics of the individual UV absorbers.

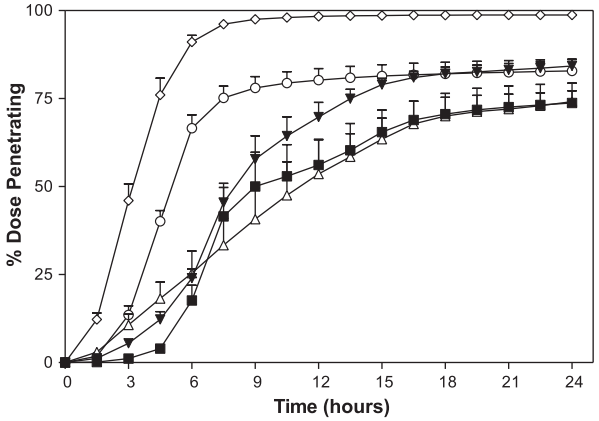


**Figure 1.** Cumulative dermal penetration of the active ingredients of OFF! Deepwoods, individually and as a combination ○ = 19% DEET, ▼ = 7.5% octyl methoxycinnimate, ■ = 0.6% oxybenzone, △ = 5% octyl salicylate, ◇ = combination, $n = 6$.

For cumulative 2,4-D penetration, only three combinations demonstrated significant differences from any of their individual ingredients. Neutrogena Oil Free enhanced penetration as compared to its weakest individual enhancer, homosalate ($P < 0.05$), as did OFF! Skintastic relative to octocrylene ($P < 0.01$). In both cases, however, the combination enhanced penetration at the average level of its ingredients and thus was not significantly influenced by the weaker enhancers. The Deep Woods OFF! combination, however, led to significantly greater 2,4-D penetration than any of the individual components taken alone or their average ($P < 0.05$), indicating a synergistic effect. Figure 1 demonstrates graphically the synergistic effect of OFF! Deep Woods' constituent ingredients.

Comparing the 2,4-D lag times after combination pretreatment with the 2,4-D lag times after individual ingredient pretreatment, demonstrates that oxybenzone and octocrylene caused significantly slower penetration individually than the combinations they were used in ($P < 0.05$). However, even with octocrylene and/or oxybenzone present, all of the combination lag times were similar to the average of the individual component's lag times ($P > 0.05$).

An additional systematic analysis was performed in order to isolate the effects on 2,4-D penetration enhancement of each of the individual ingredients when applied as part of a combination. Table 2 demonstrates the cumulative penetration and lag

**Table 2.** Penetration of 2,4-D after pretreatment with sunscreen combinations.

| Formulation tested | Active ingredients | Cumulative (% dose/24 hours) mean ± SEM | Lag time (hours) mean ± SEM |
|---|---|---|---|
| OFF! Skintastic – no DEET (OFFSNOD) | Octocrylene<br>Octyl methoxycinnamate<br>Oxybenzone | 76.3 ± 4.7# | 3.1 ± 0.4 |
| OM, OB | Octyl methoxycinnamate<br>Oxybenzone | 76.9 ± 4.2# | 1.6 ± 0.2# |
| OB, OS | Oxybenzone<br>Octyl salicylate | 76.9 ± 5.9# | 1.4 ± 0.3# |
| OM, OS | Octyl methoxycinnamate<br>Octyl salicylate | 78.4 ± 6.5# | 1.5 ± 0.2# |
| OB, H | Oxybenzone<br>Homosalate | 80.7 ± 2.7# | 2.5 ± 0.2 |
| OFF! Deepwoods – 9% DEET (9%DWOFF) | Octyl methoxycinnamate<br>Oxybenzone<br>Octyl salicylate<br>DEET – 9% | 83.8 ± 2.7# | 0.9 ± 0.1# |
| No sunscreen control | | 54.9 ± 4.7 | 3.3 ± 0.3 |

#$P < 0.01$ versus control.
Results are presented as mean ± SEM.
OM = Octyl Methoxycinnamate, OB = Oxybenzone, OS = Octyl Salicylate, H = Homosalate.

times for each of these additional combinations. Table 3 presents the results of the systematic comparisons. For example, the Neutrogena Oil Free combination is essentially Coppertone to Go plus oxybenzone. Therefore, comparing the cumulative penetration or lag times of the two combinations can isolate the effect of oxybenzone on the other UV absorbers. Comparing the OFF! Deep Woods combination to an identical combination but without DEET (Coppertone Sport) supports that DEET has synergistic effects on cumulative 2,4-D penetration. None of the other UV absorbers acted synergistically to enhance cumulative penetration. DEET also decreased the lag times. Comparing the OFF! Skintastic without DEET combination to the OM + OB formulation demonstrated that octocrylene can slow lag times; this was the only antagonistic effect on 2,4-D penetration found in the study.

To determine whether the differences between OFF! Deepwoods and OFF! Skintastic combinations pretreatment were due to the UV absorbers (octyl salicylate versus octocrylene) or to the differences in the DEET concentration, a new OFF! Deepwoods combination with only 9.5% DEET (as in the OFF! Skintastic commercial

**Table 3.** Chemical isolations to interpret effects of individual ingredients in the formulations on 2,4-D penetration.

| Comparison | Chemical isolated | Cumulative *P* value | Cumulative significant? | Lag time *P* value | Lag time significant? |
|---|---|---|---|---|---|
| OFFSNOD versus OFFS | D | 0.044 | **Yes** | 0.009 | **Yes** |
| OFFD versus CS | D | 0.010 | **Yes** | 0.023 | **Yes** |
| NOF versus CTG | OB | 0.131 | No | 0.122 | No |
| OM, OS versus CS | OB | 0.814 | No | 0.185 | No |
| NOF versus CS | H | 0.332 | No | 0.683 | No |
| OM, OS versus CTG | H | 0.769 | No | 0.585 | No |
| OM, OB versus CS | OS | 0.603 | No | 0.309 | No |
| OM, OB versus OFFSNOD | OC | 0.938 | No | 0.010 | **Yes** |
| OM, OB versus BB | P | 0.101 | No | 0.666 | No |
| OB, OS versus CS | OM | 0.865 | No | 0.152 | No |
| DWOFF 9.5% versus DWOFF | DEET Conc | 0.002 | **Yes** | 0.83 | No |

OM = Octyl Methoxycinnamate, OB = Oxybenzone, OS = Octyl Salicylate, H = Homosalate, OC = Octocrylene, P = Padimate-O, D = DEET.

formulation) was made. The 19% OFF! Deep-woods combination had a cumulative penetration of $98.8 \pm 0.6\%$ over 24 hours versus $83.8 \pm 2.7\%$ for the 9.5% mixture ($P < 0.05$) and the combinations caused lag times of $0.90 \pm 0.09$ and $0.94 \pm 0.13$ hours respectively ($P > 0.05$), indicating that there is a concentration effect of DEET on 2,4-D transdermal absorption (Tables 2 and 3).

## Discussion

Recent studies have shown that certain commercial sunscreen formulations act as penetration enhancers for 2,4-D (Brand *et al*., 2002) and benzene (Nakai *et al*., 1997). Furthermore, six common active sunscreen ingredients also act as individual penetration enhancers for 2,4-D (Pont *et al*., 2004). The previous studies suggest that further research towards developing safer sunscreen formulations is needed. Thus, the current work investigates the penetration enhancement characteristics of several active sunscreen ingredient combinations.

In our study, active sunscreen ingredient combinations increased the dermal penetration of the herbicide 2,4-D by increasing total 2,4-D flux and accelerating the penetration process. The OFF! Deep Woods combination showed the largest enhancement in total dermal penetration and the greatest acceleration in 2,4-D diffusion. None of the combinations tested significantly inhibited 2,4-D diffusion across skin or displayed remarkable antagonistic effects on penetration enhancement.

Overall, the data suggest that all combinations examined are penetration enhancers. Therefore, inert ingredients must be responsible for the lack of penetration enhancement observed in an earlier study that examined 2,4-D penetration after pretreatment with commercialized sunscreen formulations (Brand *et al*., 2002). This conclusion is consistent with the results of Baynes *et al*. who systematically demonstrated that inert ingredients were responsible for controlling the transdermal penetration of the carbamate insecticide carbaryl (Baynes and Riviere, 1998).

Formulation can also significantly alter the absorption of UV filters and DEET. Application of oxybenzone in petroleum jelly led to five times greater penetration through the skin than when applied in an emulsion gel (Treffel and Gabard, 1996). Studies have also demonstrated that varying the inert ingredients can reduce DEET transdermal absorption while maintaining its strength as an insect repellent (Proniuk *et al*., 2002; Stinecipher and Shah, 1997). In addition, a combination of 60% PEG400 and 30% PG was able to synergistically inhibit DEET penetration through skin at a level that was greater than when the compounds were applied singly (Ross and Shah, 2000).

All combinations tested significantly enhanced total 2,4-D penetration versus control and also significantly accelerated 2,4-D penetration. However, simply comparing combinations to the control data does not indicate how the individual ingredients interacted to produce the observed enhancement effects. Further analysis involving data from previous studies (Pont *et al*., 2004) was necessary to investigate ingredient interactions. A systematic study was therefore performed to isolate the effects of these ingredient interactions on 2,4-D penetration.

The penetration enhancement characteristics of DEET have been extensively studied, often with mixed results. It can act as a penetration enhancer (Windheuser *et al*., 1982), but has also been shown to have no effect (Baynes and Riviere, 1998; Moody *et al*., 1992) or to actually decrease absorption (Baynes *et al*., 1997), depending on the copenetrant. It improves the transdermal penetration of nifedipine when combined with either propylene glycol or diethyl sebacate; this enhancement occurs by increasing solubility and thereby maximizing the thermodynamic activity of the drug, not by influencing the stratum corneum directly (Kondo *et al*., 1988). Comparing the OFF! Skintastic combination with the OFF! Skintastic combination without DEET and the OFF! Deep Woods combination with its similar combination without DEET (Coppertone Sport) supports the idea that DEET had synergistic effects on both cumulative 2,4-D penetration and lag time.

A comparison between OFF! Skintastic without DEET and the OM, OB combination demonstrates that octocrylene can significantly decrease the 2,4-D lag time. This is not surprising given that the lag time after octocrylene-only pretreatment, 3.7 hours, was one of the slowest tested. Oxybenzone, however, also leads to a lag time of 3.7 hours but did not show a similar trend, indicating that lag

time alone is not enough to predict antagonistic effects. The difference between the effects of these two UV absorbers could be that, individually, octocrylene does not act as a penetration enhancer for 2,4-D while oxybenzone does (Pont *et al*., 2004).

Two of the UV absorbers examined have been established as penetration enhancers for chemicals other than 2,4-D. Padimate-o is a para-aminobenzoic acid ester and is a very potent UVB absorber (Patel *et al*., 1992). It has also been shown to significantly enhance skin penetration of testosterone, estradiol, progesterone, norethindrone acetate and NSAIDs *in vitro* as well as estradiol, testosterone and ibuprofen *in vivo* (Finnin and Morgan, 1999; Morgan *et al*., 1998). Octyl salicylate has been shown to cause a six-fold increase in the diffusion of testosterone across snake skin *in vitro* (Morgan *et al*., 1998), and significantly enhances the transdermal absorption of fentanyl across split thickness human skin. Topical studies examining the penetration of $^{3}H_2O$ indicate that padimate-o does not physically disrupt the stratum corneum, but that octyl salicylate does (Pont *et al*., 2004). The data presented in the current study indicate that neither of these chemicals interact synergistically with the other UV absorbers to further increase 2,4-D penetration.

Sunscreens provide a valuable service in reducing skin cancer and their use should be encouraged despite their potential for causing increased harmful chemical absorption. The results of this study, however, demonstrate a need for commercial sunscreen manufacturers to try and reduce this potential sunscreen usage side effect. Overall, the deleterious penetration enhancement of the active ingredients cannot be mitigated by using certain combinations of active ingredients. Instead, it must be lessened by using inert ingredients or solvents that antagonize or mask the enhancement effects of the active ingredients.

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