Table IV: continued

No.	Chemical (COLIPA number)	*In vivo* toxicity	Run	Laboratory 1 MPE	Laboratory 1 Mean	Laboratory 1 Prediction	Laboratory 2 MPE	Laboratory 2 Mean	Laboratory 2 Prediction	Laboratory 3 MPE	Laboratory 3 Mean	Laboratory 3 Prediction	Laboratory 4 MPE	Laboratory 4 Mean	Laboratory 4 Prediction
11.	Protoporphyrin IX, disodium	pt	1	0.993	0.959	pt	1.05	0.975	pt	0.869	0.7825	pt	0.835	0.819	pt
			2	0.925			0.9			0.696			0.803		
12.	Chlorpromazine hydrochloride	pt	1	0.708	0.723	pt	0.318	0.501	pt	0.518	0.5705	pt	0.588	0.599	pt
			2	0.738			0.684			0.623			0.61		
13.	Anthracene	pt	1	1.067	1.0315	pt	0.823	0.7805	pt	0.461	0.6345	pt	0.798	0.7795	pt
			2	0.996			0.738			0.808			0.761		
14.	Acridine hydrochloride	pt	1	0.595	0.711	pt	0.918	0.8065	pt	0.478	0.6325	pt	0.794	0.6195	pt
			2	0.827			0.695			0.787			0.445		
15.	Ketoprofen	pt	1	0.887	0.9015	pt	0.792	0.8085	pt	0.565	0.6315	pt	0.78	0.665	pt
			2	0.916			0.825			0.698			0.55		
16.	Promethazine hydrochloride	pt	1	0.698	0.547	pt	0.852	0.8655	pt	0.719	0.6415	pt	0.525	0.5645	pt
			2	0.396			0.879			0.564			0.604		
17.	Amiodarone hydrochloride	pt	1	0.695	0.5995	pt	0.785	0.7455	pt	0.22	0.27	pt	0.408	0.5135	pt
			2	0.504			0.706			0.32			0.619		
18.	Demeclocycline hydrochloride	pt	1	0.097	0.3395	pt	0.946	0.932	pt	0.01	0.154	pt	0.75	0.4885	pt
			2	0.582			0.918			0.298			0.227		
19.	Bithionol	pt	1	0.501	0.4175	pt	0.572	0.641	pt	0.472	0.539	pt	0.506	0.542	pt
			2	0.334			0.71			0.606			0.578		
20.	Musk ambrette	pt	1	0.497	0.5245	pt	0.918	0.9125	pt	0.577	0.5255	pt	0.636	0.6205	pt
			2	0.552			0.907			0.474			0.605		

npt = non-phototoxic; pt = phototoxic; nd = not determined. Inaccurate predictions are shown in bold type.

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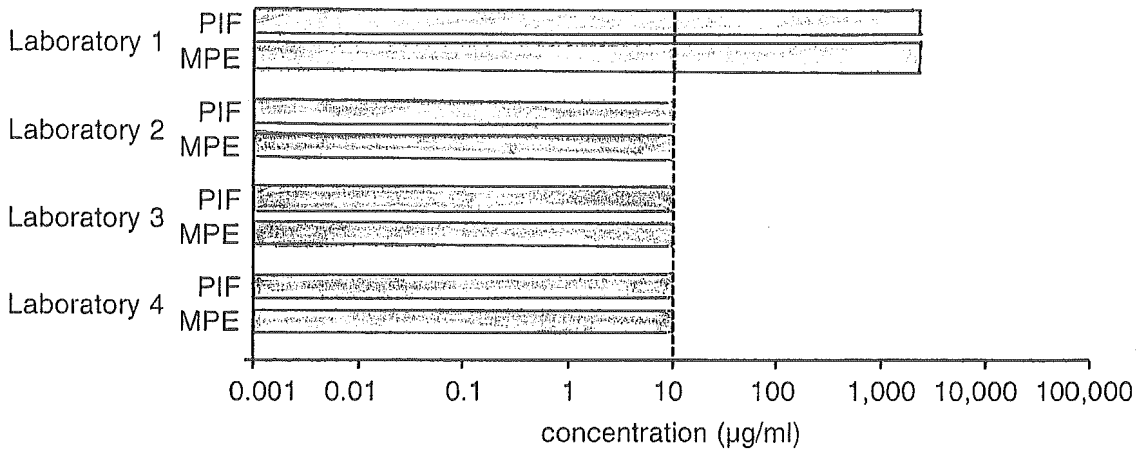
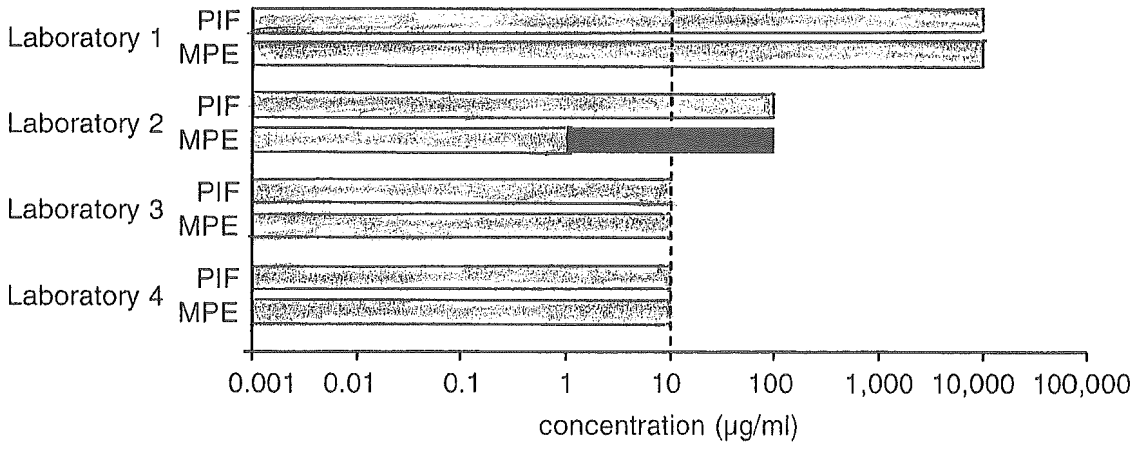
Figure 2: Effect of test concentration ranges on interlaboratory variability of Photoirritation Factor (PIF) and Mean Photo Effect (MPE) for 20 test chemicals

Chemical 1: Octyl salicylate (S13)



Chemical 2: Octyl methoxycinnamate (S28)



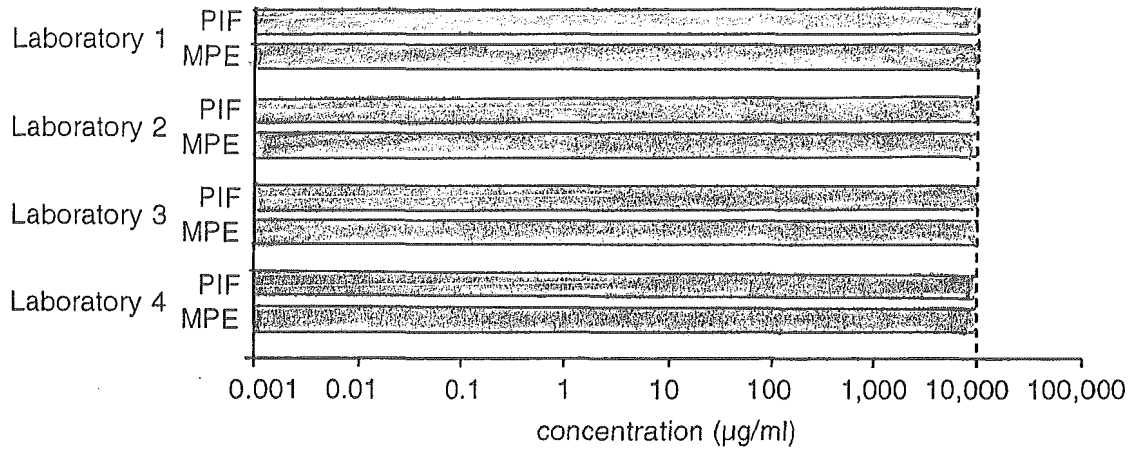
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▩).

Chemicals 1–10 = non-phototoxic chemicals; chemicals 11–20 = phototoxic chemicals.

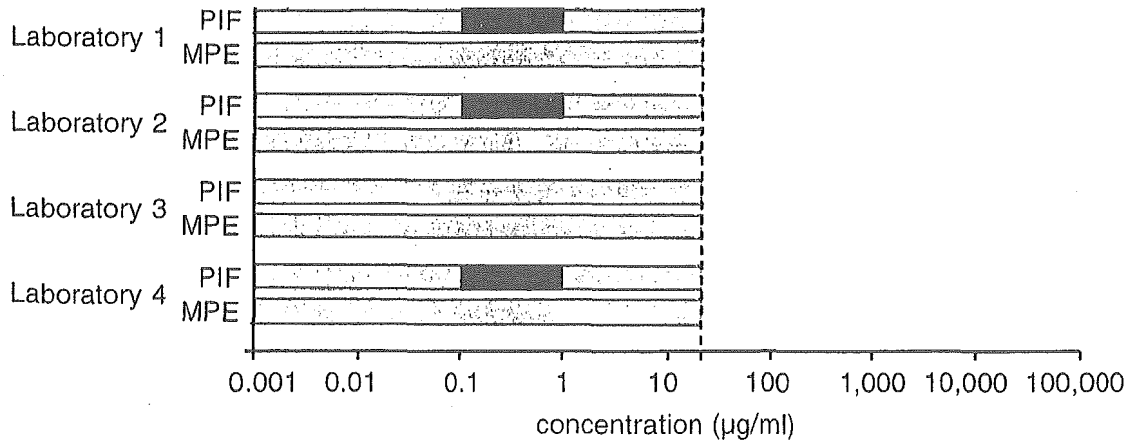
- - - - = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 3: Benzylidene camphor sulphonic acid (S59)



Chemical 4: 4-Methylbenzylidene camphor (S60)



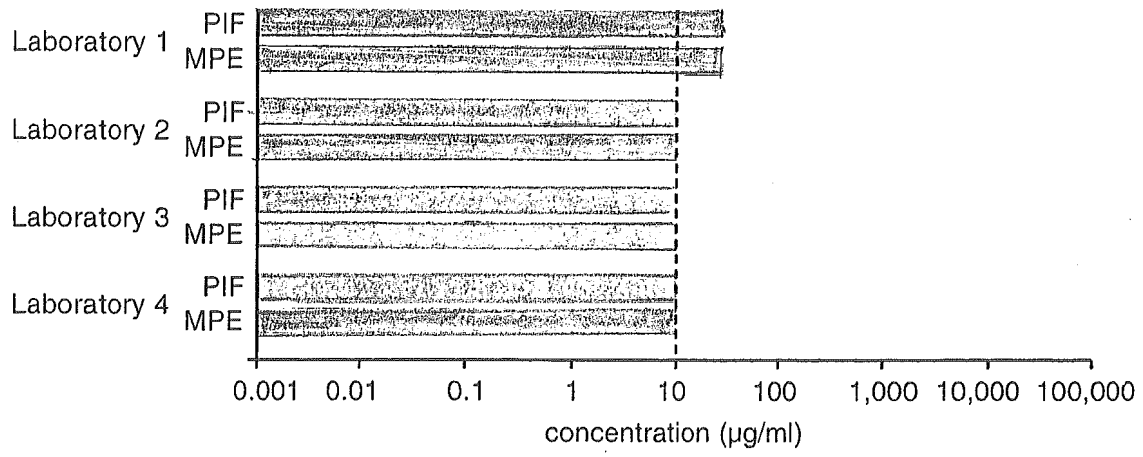
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▒).

Chemicals 1–10 = non-phototoxic chemicals; chemicals 11–20 = phototoxic chemicals.

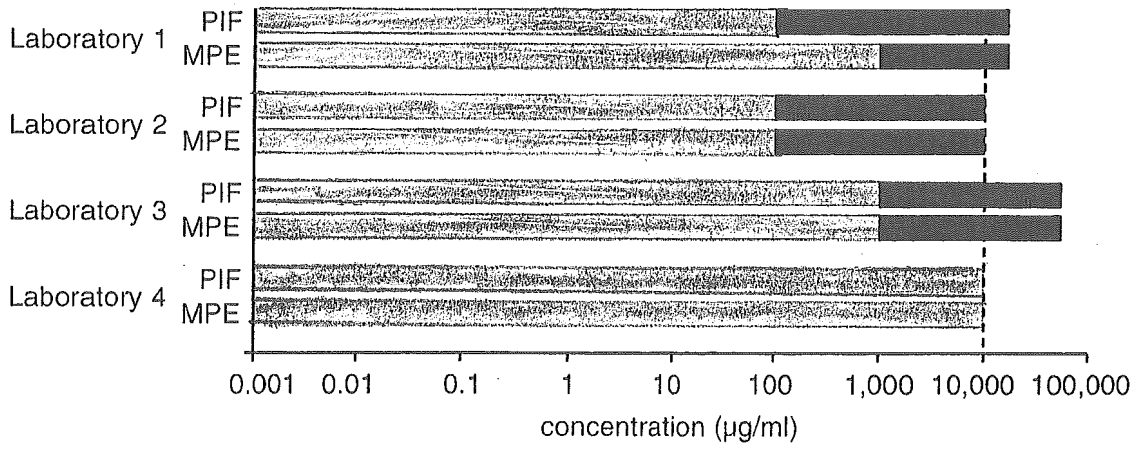
- - - - = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 5: 3-Benzylidene camphor (S61)



Chemical 6: Terephthalidene dicamphor sulphonic acid (S71)



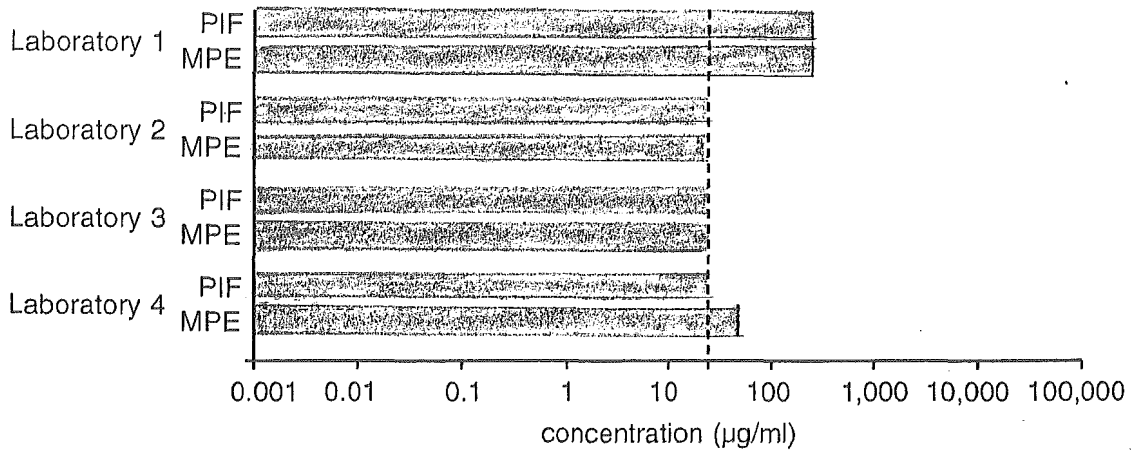
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▒).

Chemicals 1–10 = non-phototoxic chemicals; chemicals 11–20 = phototoxic chemicals.

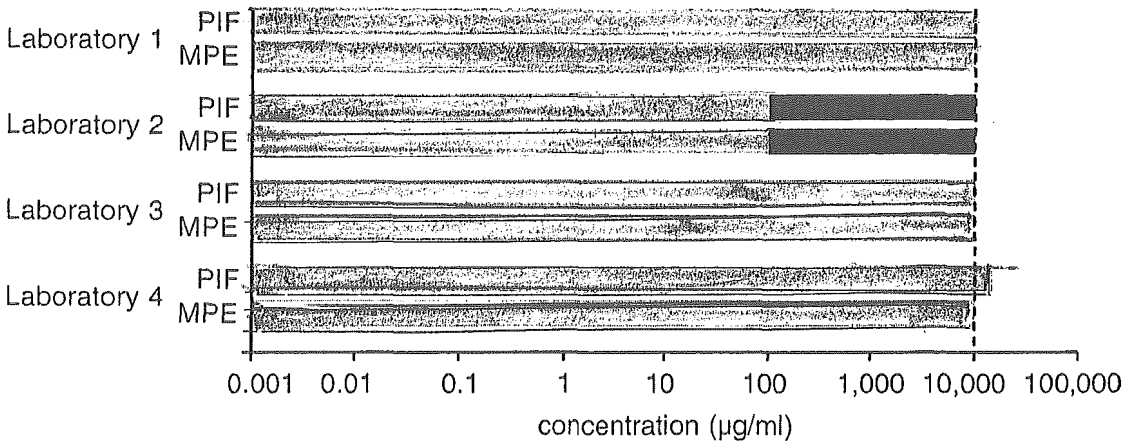
- - - - = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 7: Polyacrylamidomethyl benzylidene camphor (S72)



Chemical 8: Benzophenone-4 (S40)



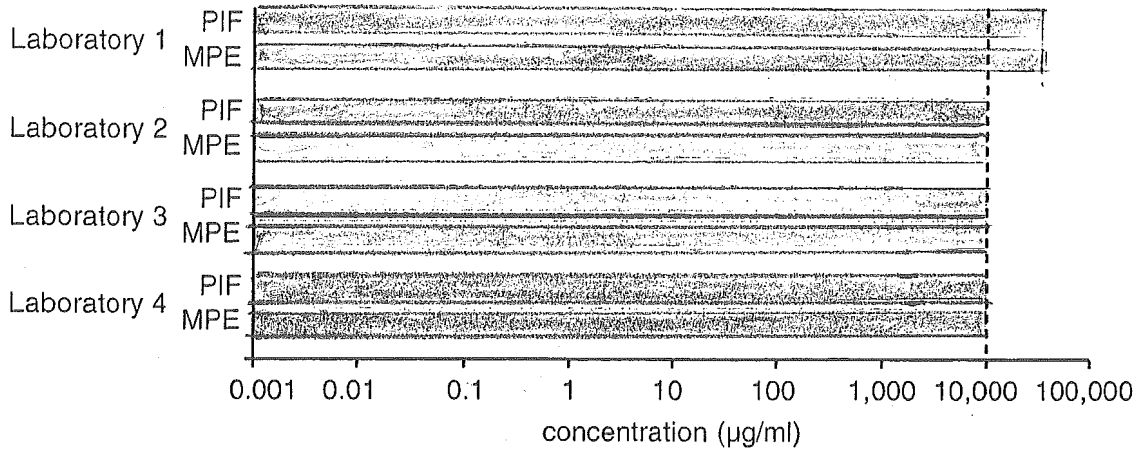
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▤).

Chemicals 1-10 = non-phototoxic chemicals; chemicals 11-20 = phototoxic chemicals.

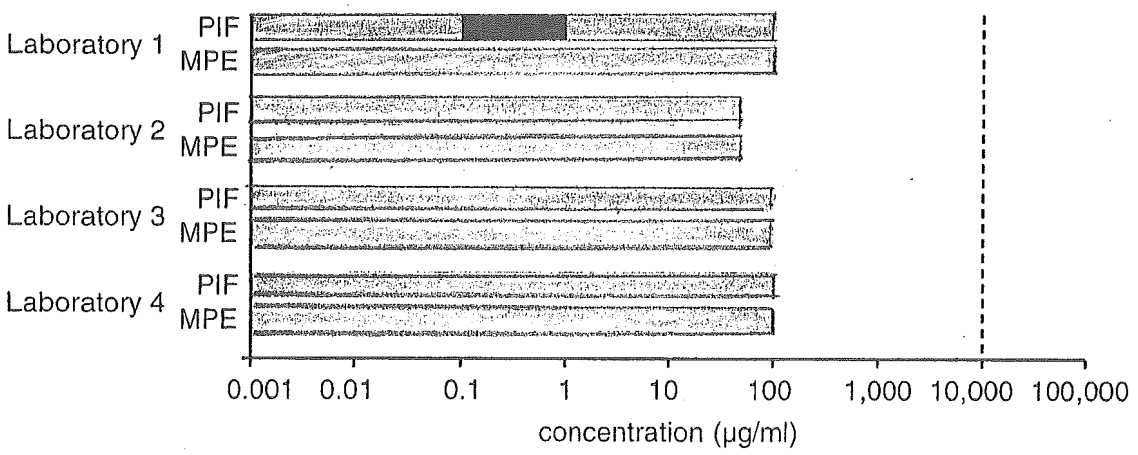
- - - - = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 9: L-Histidine free base



Chemical 10: Sodium lauryl sulphate



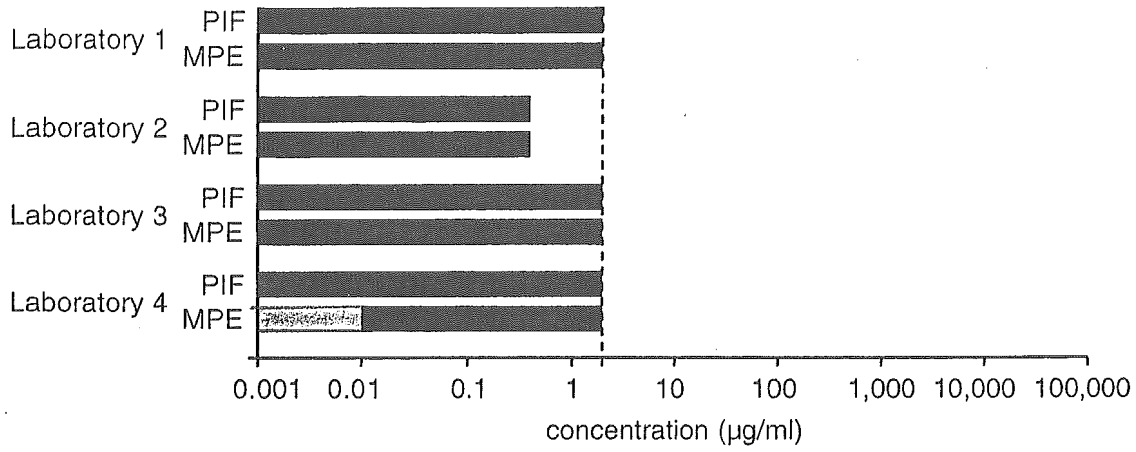
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▭).

Chemicals 1–10 = non-phototoxic chemicals; chemicals 11–20 = phototoxic chemicals.

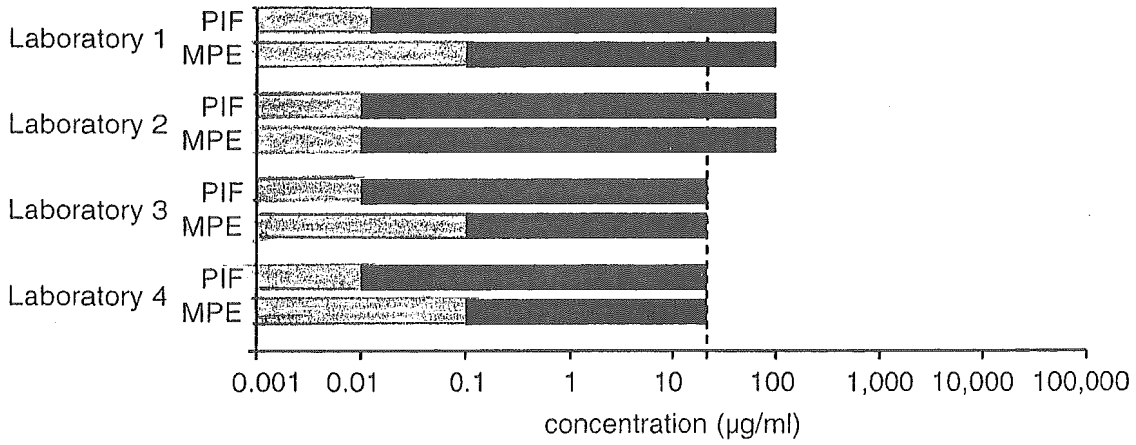
- - - - = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 11: Protoporphyrin IX, disodium



Chemical 12: Chlorpromazine hydrochloride



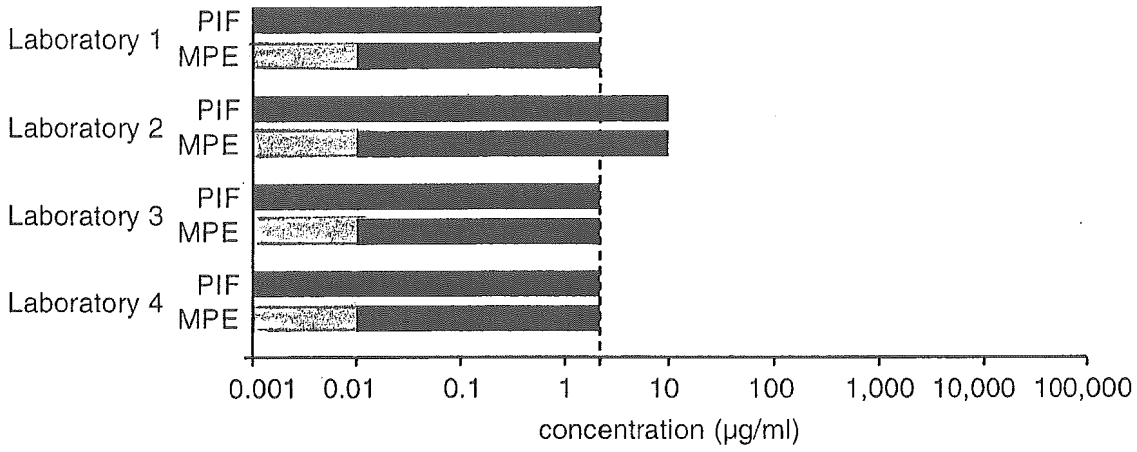
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▩).

Chemicals 1-10 = non-phototoxic chemicals; chemicals 11-20 = phototoxic chemicals.

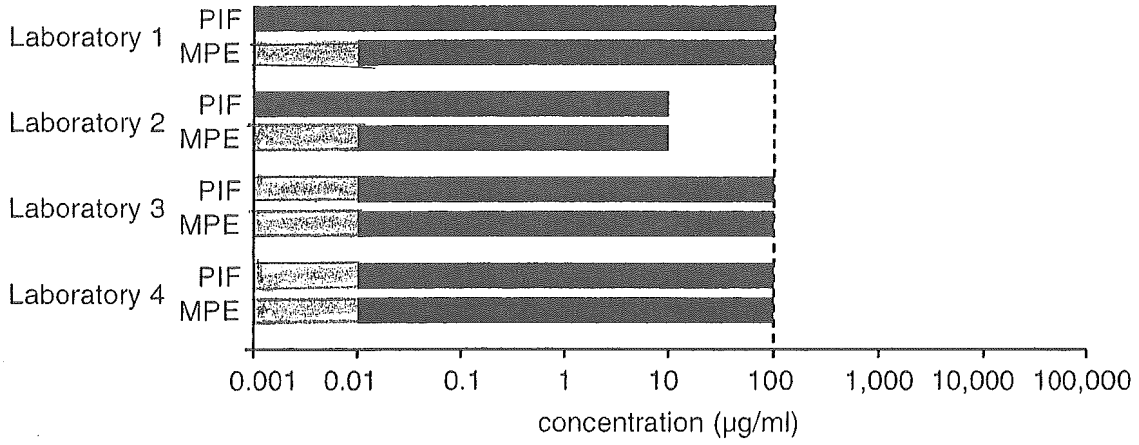
---- = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 13: Anthracene



Chemical 14: Acridine hydrochloride



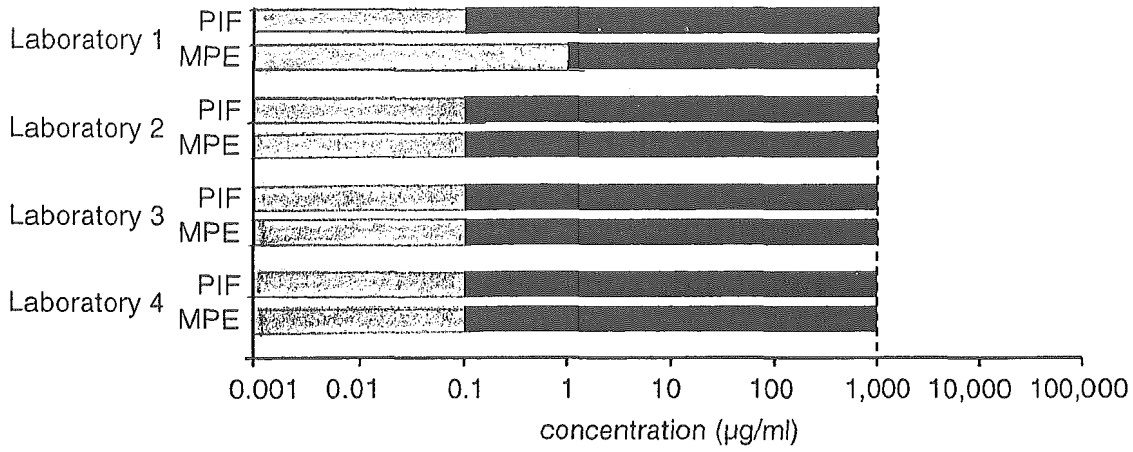
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▨).

Chemicals 1–10 = non-phototoxic chemicals; chemicals 11–20 = phototoxic chemicals.

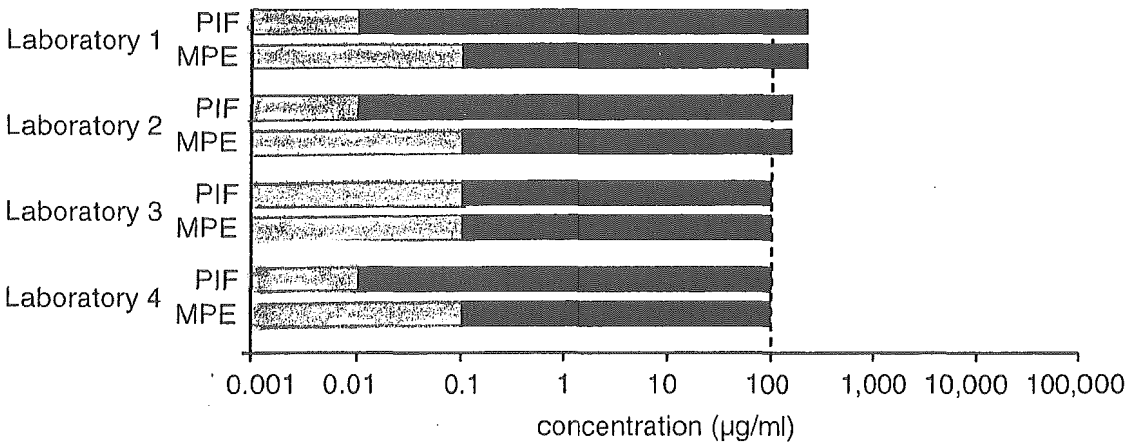
- - - - = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 15: Ketoprofen



Chemical 16: Promethazine hydrochloride



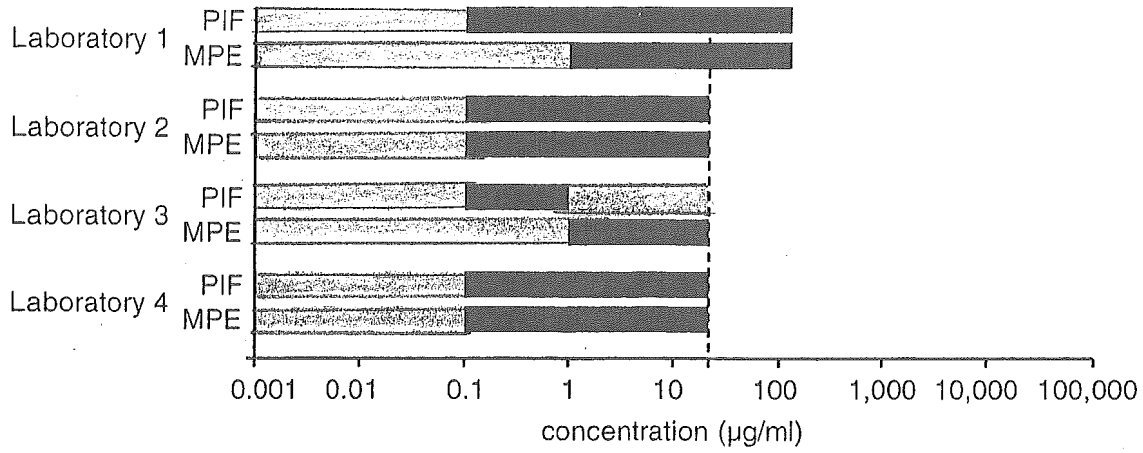
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▩).

Chemicals 1–10 = non-phototoxic chemicals; chemicals 11–20 = phototoxic chemicals.

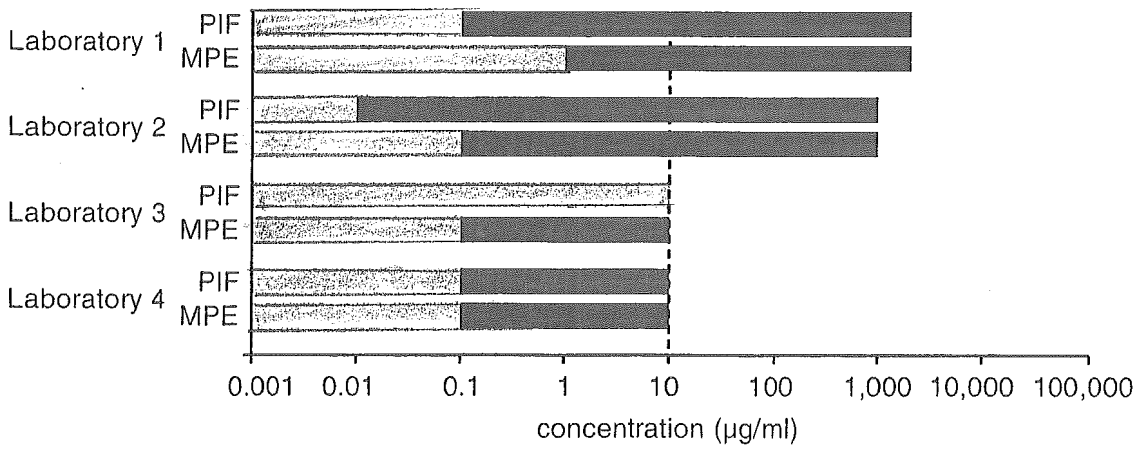
- - - - = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 17: Amiodarone hydrochloride



Chemical 18: Demeclocycline hydrochloride



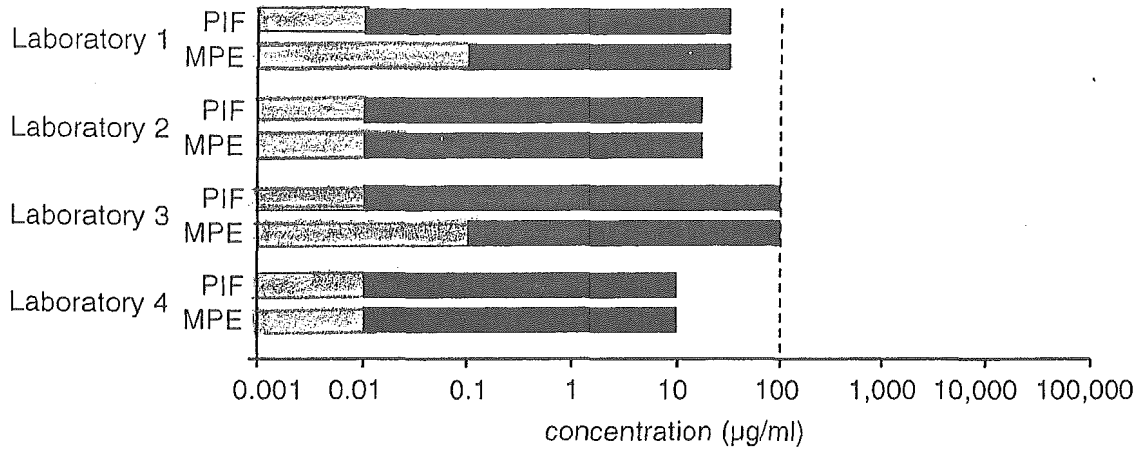
PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▒).

Chemicals 1–10 = non-phototoxic chemicals; chemicals 11–20 = phototoxic chemicals.

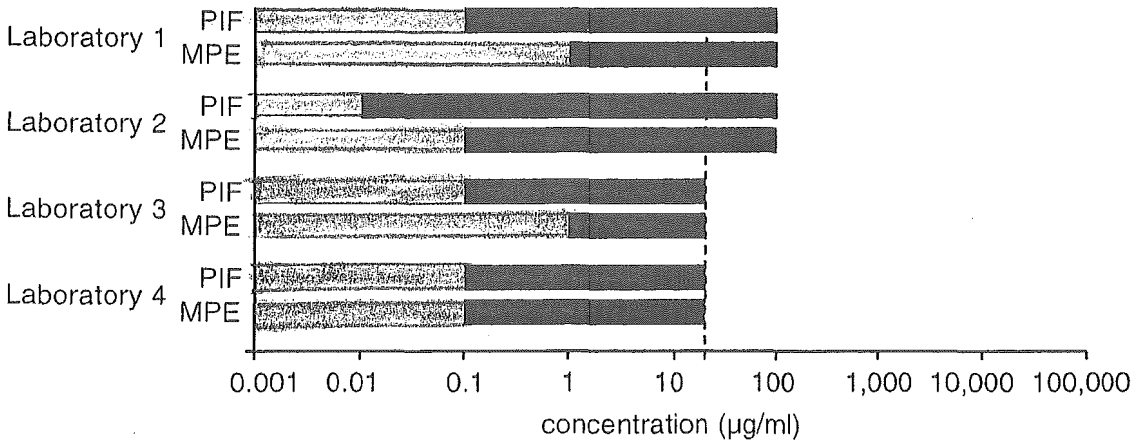
- - - - = highest test concentration recommended by ZEBET.

Figure 2: continued

Chemical 19: Bithionol



Chemical 20: Musk ambrette



PIF and MPE are given for the 20 chemicals tested in the study on UV filter chemicals, to illustrate the dependence of the determination of phototoxic potential on test concentration set used. Positive classification according to the prediction model for both PIF and MPE is indicated by a black bar (■), and negative prediction is indicated by a grey bar (▒).

Chemicals 1–10 = non-phototoxic chemicals; chemicals 11–20 = phototoxic chemicals.

- - - - = highest test concentration recommended by ZEBET.

rectly predicted to be phototoxic (Table IV).

Four false positive predictions were associated with the use of the PIF version of the model (Table III), and five with the MPE version (Table IV). Taking into account the balanced set of ten phototoxic and ten non-phototoxic test chemicals, the PIF and MPE versions of the prediction model provided a high accuracy of prediction that ranged from 85% to 100% for the individual laboratories. The MPE-based prediction provided 100% sensitivity and 100% negative predictivity in all four laboratories (Table V), so all the chemicals considered to be phototoxic *in vivo* were identified correctly. The almost perfect sensitivity of the MPE model is counterbalanced by a total of five false positive classifications, so specificity and posi-

Table V: Overall predictivity of Photoirritation Factor (PIF) and Mean Photo Effect (MPE) of all 20 chemicals

	***In vivo* classification**		
	Phototoxic	**Non-phototoxic**	**Total**
PIF			
***In vitro* classification**			
Phototoxic	38	4	42
Non-phototoxic	2	36	38
Total	40	40	80
Table statistics for the shadowed 2 × 2 table			
Sensitivity	95%		
Specificity	90%		
Positive prediction	90%		
Negative prediction	95%		
Accuracy	**93%**		
χ^2	**54.59**	**(>> 3.8)**	
MPE			
***In vitro* classification**			
Phototoxic	40	5	45
Non-phototoxic	0	35	35
Total	40	40	80
Table statistics for the shadowed 2 × 2 table			
Sensitivity	100%		
Specificity	88%		
Positive prediction	89%		
Negative prediction	100%		
Accuracy	**94%**		
χ^2	**58.72**	**(>> 3.8)**	

tive predictivity ranged from 75% to 100% in the individual laboratories, and was 89% overall (Table V).

When the PIF and MPE models are applied without taking into consideration the test concentration range, they show a tendency to overestimate the phototoxic potential. Thus, the overall analysis of predictions in this study must take into account the concentration range at which testing was conducted. This point is discussed in detail below.

Analysis of interlaboratory and intralaboratory data variability

The coefficients of variation (CV) were determined for inter-replicate variability, inter-experiment variability and intralaboratory variability for each laboratory and each chemical. Mean coefficients of variation for the three sources of variability were calculated by averaging across all the laboratories; these coefficients are represented by the bars in Figure 3. They show that, for both PIF and MPE, interlaboratory variability was generally higher than intralaboratory variability. As expected, according to the definition of the two measures of phototoxicity, for non-phototoxic chemicals the CV was smaller for PIF than for MPE, while for phototoxins the CV was smaller for MPE than for PIF. This result reflects the difference in the ranges of numerical values covered by the two measures of phototoxicity. By definition, MPE values are restricted to the interval $[-1,1]$, whereas PIF can attain arbitrary values $[0, \infty]$.

Figure 3 demonstrates that chemicals exhibiting the highest interlaboratory CV for PIF show a significantly smaller interlaboratory CV for MPE and vice versa. The highest variability of PIF values is seen at high values, which are by definition obtained with phototoxic chemicals. It is quite common for PIF values such as > 100 or > 10,000 to be determined for the same chemical by two different laboratories, due to differences in the concentrations used for testing. Moreover, high PIF values (i.e. those greater than 100), correspond to the maximum MPE values approaching unity, for example, 0.87 and 0.92, which exhibit a notably smaller variability than the corresponding PIF values. In general, however, the classification of phototoxicity derived from MPE and PIF values in the 3T3 NRU PT test was very robust, which suggests that variability of data in this *in vitro* test is small and that its effect on *in vitro* classification is negligible. No significant correlation could be detected between misclassifications and data variability for PIF and MPE.

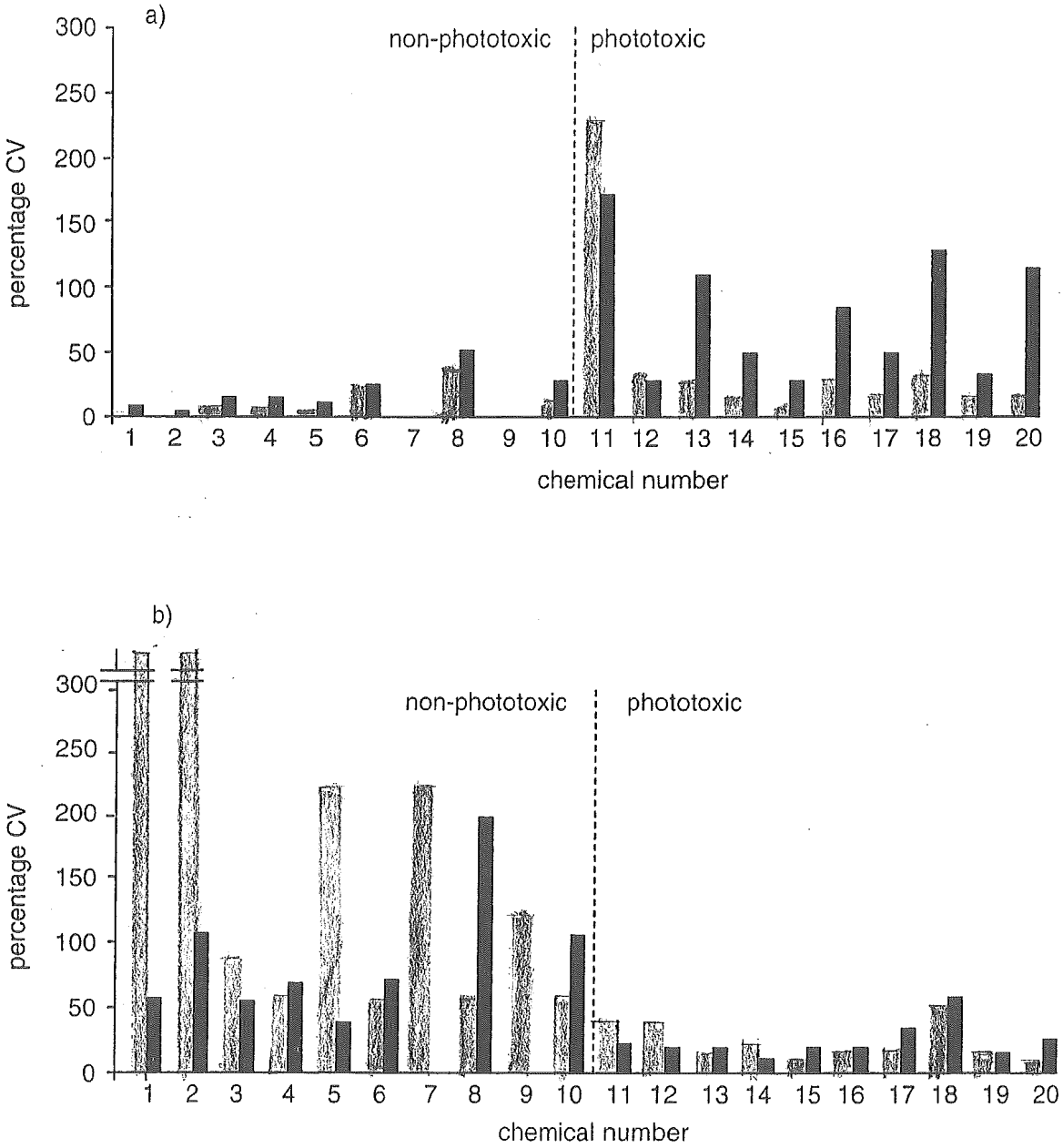
Effect of test concentration on the correct prediction of phototoxic potential

To investigate the influence of test concentrations on the prediction of phototoxic potential of chemicals in the 3T3 NRU PT test, the two versions of the method for predicting phototoxic potential were determined by comparing the +UV and –UV concentration–response curves over a restricted range (Figure 1). The upper limit of this concentration range at which PIF and MPE were determined, ranged from 0.01μg/ml up to 50,000μg/ml, the highest concentration achieved in the experiment and common to both the +UV and –UV concentration– response curves. The results are shown in Figure 2.

The results for the ten non-phototoxic test chemicals are shown in Figure 2. The bar graphs clearly indicate that the predicted phototoxic potential depends on the concentration range chosen. In a few instances, PIF provides a positive prediction for a single concentration interval, while a negative result is obtained at higher and lower concentrations, for example, for chemicals 4 and 10. As expected, at very high concentrations (i.e. those greater than 100μg/ml), positive predictions were obtained with a few UV filter chemicals which are not phototoxic *in vivo*, for example, for chemicals 6 and 8.

The data for the ten phototoxic chemicals are given in Figure 2. The bar graphs show that the concentration range over which the 3T3 NRU PT test provides positive results was very reproducible in all the participating laboratories. The concentration interval at which a negative result changes to a positive result generally covered only one order of magnitude in all laboratories. As shown in Figures 2 and 4, PIF generally seems to be a more sensitive indicator than MPE, except in the case of chemical 18, which provided a false negative prediction with PIF in laboratory 3 and a positive result with MPE. Figure 2 also shows that the ten phototoxic chemicals provide positive classification results at test concentrations of 1μg/ml, which is several orders of magnitude lower than the highest test concentration recommended in the original SOP for this test, as used in this study (10,000μg/ml).

Figure 3: Coefficients of variation (CV) for intralaboratory and interlaboratory variability for Photoirritation Factor (PIF) and Mean Photo Effect (MPE)



= *CV (intralaboratory);* ■ = *CV (interlaboratory).*

a) PIF; b) MPE.

Determination of optimum test concentration for the 3T3 NRU PT test

From the results shown in Figure 2, the percentage of correct positive and correct negative predictions, and the overall correct predictions of phototoxic potential, were used to analyse whether the highest concentration should be restricted to an upper concentration limit. A computer simulation was performed to assess whether the recommendation of an optimum test concentration would improve the predicted results according to the PIF and MPE models. Figure 4 demonstrates that, if low concentrations (0.01–0.1μg/ml) are chosen for testing, the proportion of correct negative predictions is around 100%, while the percentage of correct positive predictions is less than 100%, since phototoxic properties do not become evident at concentrations lower than 1μg/ml. At higher concentrations, the percentage of overall correct predictions, both positive and negative, increases, since the percentage of correct positive predictions increases, while the proportion of correct negative predictions remains around 100%. At concentrations greater than 1μg/ml, a plateau is reached which is followed by a decline at concentrations greater than 100μg/ml (Figure 4). Consequently, the risk of incorrectly predicting a chemical to be phototoxic (i.e. a false positive) increases at concentrations greater than 100μg/ml with both versions of the prediction model, while the number of correct positive predictions remains constant. Therefore, chemicals should be tested in the 3T3 NRU PT test over a concentration range between 1μg/ml and 100μg/ml, and concentrations greater than 100μg/ml should not be used, in order to avoid false positive predictions.

Discussion

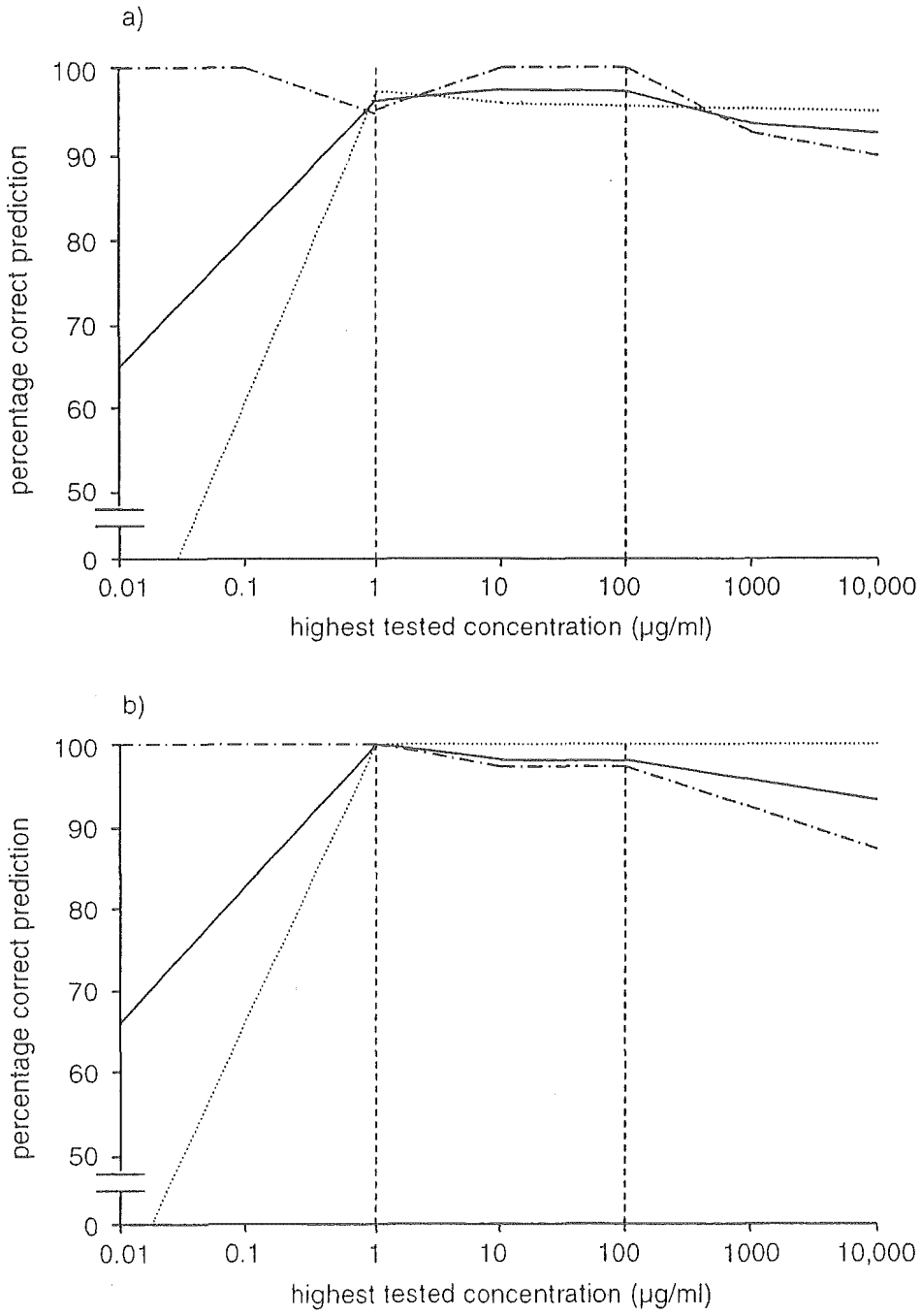
Overall biostatistical analysis

According to the results of this study on UV filter chemicals, summarised in Tables III–VI, the two versions of the prediction model proposed for the 3T3 NRU PT test, PIF and MPE, both provide an accurate prediction of *in vivo* phototoxic potential. The overall evaluation of the results reveals that seven out of eight UV filter chemicals tested were correctly predicted to have no phototoxic potential by the majority of the laboratories (three out of four) when the original prediction model was applied, which does not take into account the test concentration range. One of the UV filter chemicals (chemical 6) provided a positive result in three of the four laboratories at test concentrations greater than 100μg/ml. UV filter chemical 8 produced a false positive prediction in one laboratory at concentrations greater than 100μg/ml. These results suggest that, when the 3T3 NRU PT test is used, the concentration range at which a positive prediction is obtained has to be taken into account, as discussed below, and as now recommended in the draft of an OECD test guideline for phototoxicity testing, based on the *in vitro* 3T3 NRU PT test (10).

Determination of Photoirritation Factor versus Mean Photo Effect

In the formal validation study (1), it was shown that, from a biostatistical point of view, the use of the MPE is preferable to the use of PIF, since an MPE value can be determined for all of the chemicals, while exact, numerical PIF values cannot be determined for all chemicals. As discussed previously (1), the MPE cut-off value of 0.1 suggested by Holzhütter (2) is an arbitrary one chosen from a wider interval (~0.05–~0.2), which has to be confirmed by testing a larger number of chemicals in the 3T3 NRU PT test. Judging from the criteria generally used to assess the overall predictivity of an *in vitro* test (sensitivity, specificity, positive and negative predictivity, and accuracy), the MPE-based predictions were slightly more accurate than the PIF-based predictions (Tables V and VI). The MPE version of the prediction model provided a negative predictivity of 100% and a positive predictivity of 89%. Thus, all the phototoxic chemicals tested in the study on UV filter chemicals were correctly identified when the MPE method was applied to evaluate results obtained in the 3T3 NRU PT test. However, both MPE and PIF have a tendency toward overprediction at high concentrations. This study supports the conclusions drawn from the formal validation of the 3T3 NRU PT test that the MPE approach, as devised by Holzhütter (2), will prove to be useful in the development and validation of *in vitro* phototoxicity tests (1).

Figure 4: Effect of highest test concentration on correct prediction of phototoxic potential



The results were used to identify the optimum test concentration range for the 3T3 NRU PT test. ---- = limit of optimum test concentration.

—— = all chemicals; — · — = negative chemicals; ····· = positive chemicals.

a) Photoirritation Factor; b) Mean Photo Effect.

Intralaboratory and interlaboratory data variability

Analysis of the CV of the results obtained by the four laboratories in the 3T3 NRU PT test shows that, for PIF and MPE, interlaboratory variability was generally higher than intralaboratory variability (Figure 3). Differences in the intralaboratory and interlaboratory variability reflect the difference in the ranges of numerical values covered by the two measures of phototoxicity. This result, and the almost perfect correlation of the prediction of the phototoxic potential from *in vitro* data with the *in vivo* data for the test chemicals, demonstrate that data variability was not a problem in the study. This may be due to the implementation of well-defined testing conditions, including:

— strict adherence to an improved test protocol;

— improved software for data collection, which every laboratory had to use;

— use of the most appropriate solvent;

— provision of information on the highest test concentration for each coded test chemical; and

— participation of a reduced number of experienced laboratories.

Improving the prediction of the phototoxic potential by selecting the optimum test concentration range

Some of the UV filter chemicals, which have been shown to be non-phototoxic under use conditions in humans, provided false positive predictions at very high concentrations in the 3T3 NRU PT test (Tables III and IV). Analysis of the effects of increasing test concentrations on the classification in the 3T3 NRU PT test were carried out in a systematic manner for the first time in the present study. The results shown in Figures 2 and 4 clearly demonstrate that chemicals which have not shown any phototoxic potential *in vivo* and are classified as non-phototoxic at concentrations up to 100μg/ml, can generate false positive predictions of a phototoxic potential, if tested at very high test concentrations.

In order to put the results into perspective, the optimum test concentration range for the 3T3 NRU PT test was determined. An optimum concentration range of 1–100μg/ml was identified with the representative and balanced set of test chemicals used in this study. All the phototoxic test chemicals provided a positive result in the range of 1–100μg/ml, while the non-phototoxic chemicals were correctly predicted at test concentrations up to 100μg/ml. Taking this information into account, positive results obtained in this study at concentrations greater than 100μg/ml with some of the UV filter chemicals are, in fact, not correct (for example, the false positive predictions for chemicals 6 and 8).

Finally, an attempt was made to calculate the overall concordance of the predictions from *in vitro* data to the *in vivo* data, when taking into account the optimum test concentration derived in this present study (Figure 4) and recommended in the revised SOP (*INVITTOX* protocol 78). The results obtained by imposing an upper concentration limit greater than 100μg/ml are shown for PIF and MPE in Table VI. The data are the same as those shown in Figure 2. The overall prediction for PIF (Table VI) is 100% correct for the specificity and positive predictivity of chemicals providing a positive result in the 3T3 NRU PT test. The remaining parameters, namely, sensitivity, negative predictivity and accuracy, are almost perfect, with values greater than 95%. For MPE (Table VI), the overall prediction is 100% for negative predictivity and sensitivity, while the prediction of the remaining parameters is also excellent (98%). These results, which are derived from data obtained in a blind trial in four laboratories, confirm that, from a practical viewpoint, the improved SOP for the 3T3 NRU PT test, in which optimum test concentrations are recommended, provides data that are highly predictive of the phototoxic potential of test chemicals *in vivo* for both phototoxic and non-phototoxic chemicals.

Critical evaluation of results

Among the UV filter chemicals characterised by low solubility in water (< 100μg/ml), chemical 4 was cytotoxic but not phototoxic to 3T3 cells under defined test conditions. The negative result in the 3T3 NRU PT test can therefore be considered to be satisfactory. In contrast, UV filter chemicals 1 and 5 did not reveal any cytotoxic or phototoxic properties at the maximum test concentration recommended by ZEBET (10μg/ml), and

UV filter chemical 7 provided the same result at 32μg/ml. When this was tested at higher concentrations in some of the laboratories, no phototoxic effects could be detected.

Among the remaining UV filter chemicals, chemicals 3, 6 and 8, which are highly soluble in water and cytotoxic to 3T3 cells at high concentrations, did not show any phototoxic effects in the relevant test concentration range, i.e. up to 100μg/ml. For chemical 3, the biometrical analysis did not reveal any phototoxic potential. Chemical 6 produced a positive photo-cytotoxic effect according to the two versions of the prediction model in three laboratories at concentrations greater than 100μg/ml (1000–10,000μg/ml), which is above the concentration range recommended for testing as a

Table VI: Overall predictivity of Photoirritation Factor (PIF) and Mean Photo Effect (MPE) for test concentrations up to 100μg/ml

	In vivo classification		
	Phototoxic	**Non-phototoxic**	**Total**
PIF			
***In vitro* classification**			
Phototoxic	38	0	38
Non-phototoxic	2	40	42
Total	40	40	80
Table statistics for the shadowed 2 × 2 table			
Sensitivity	95%		
Specificity	100%		
Positive prediction	100%		
Negative prediction	95%		
Accuracy	**98%**		
χ^2	**68.62**	(>> 3.8)	
MPE			
***In vitro* classification**			
Phototoxic	40	1	41
Non-phototoxic	0	39	3539
Total	40	40	80
Table statistics for the shadowed 2 × 2 table			
Sensitivity	100%		
Specificity	98%		
Positive prediction	98%		
Negative prediction	100%		
Accuracy	**99%**		
χ^2	**72.25**	(>> 3.8)	

result of the present study, in order to avoid false positive results. The same evaluation holds for chemical 8, which revealed a positive phototoxic effect in one laboratory at concentrations greater than 100μg/ml.

The negative PIF predictions for two phototoxic chemicals (chemicals 17 and 18) by one laboratory (laboratory 3) in Table III, can be analysed more appropriately from Figure 2, which shows that PIF and MPE provided conflicting results. Experienced toxicologists would take into account the information provided by the pairs of dose–concentration curves that were used to calculate both PIF and MPE, and would try to identify technical problems that could have contributed to the inconclusive result.

Practical use of the 3T3 NRU PT test in the hazard assessment process

The information provided in Figure 2 can be used to illustrate the use of the 3T3 NRU PT test in hazard assessment. The results shown in Figure 2 support the validity of the 3T3 NRU PT test to study UV filter chemicals, since they all produced negative results at concentrations up to 100μg/ml. Significant phototoxic potential with these chemicals under conditions of use *in vivo* may therefore be considered unlikely, provided that the UV filter chemicals being tested are compatible with the 3T3 NRU PT test system. In contrast, the data in Figure 2 demonstrate that all of the phototoxic chemicals provided positive results at concentrations close to 1μg/ml. This result shows that the 3T3 NRU PT test was capable of detecting the phototoxic potential of both strong and weak phototoxins, irrespective of their aqueous solubility.

A negative result in the 3T3 NRU PT test, possibly due to low solubility, does not provide sufficient assurance that the chemical was compatible with the test system. Prior to any use in sunscreen products, further testing should be considered to confirm the negative result. Human photoirritation testing could be considered after appropriate ethical review, including an evaluation of other safety information, as well as toxicity data on photoallergy and photomutagenicity. The 3T3 NRU PT test is likely to provide data on phototoxic potential that is satisfactory for the hazard evaluation of most chemicals for most uses.

Conclusions and Recommendations

In a study initiated by ECVAM at the request of the SCC, eight UV filter chemicals which are regulated under Annex VII of EU *Directive 76/768/EEC*, were tested under blind conditions in four laboratories in the 3T3 NRU PT test. The study proved that the previously validated 3T3 NRU PT test is characterised by high specificity, sensitivity and predictivity. The eight UV filter chemicals and two additional non-phototoxic chemicals, and the ten phototoxic chemicals, were correctly classified according to the two variations of the prediction model for the test, PIF and MPE. The MPE provided no false negative classifications. This investigation revealed an optimum concentration range of 1–100μg/ml for testing in the 3T3 NRU PT test and, in addition, a tendency toward false positive classifications at concentrations greater than 100μg/ml. The management team and the participating laboratories in the present study conclude that the 3T3 NRU PT test can be used for regulatory purposes to assess the phototoxic potential of UV filter chemicals for concentrations up to 100μg/ml. Thus, the result of the study on UV filter chemicals supports the decision of the ESAC, ECVAM and DGXI of the European Commisison to accept the 3T3 NRU PT test as a scientifically validated test that is ready to be used for regulatory purposes (4). More recently, DGIII of the European Commission has also recommended acceptance of the 3T3 NRU PT test for regulatory purposes.

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