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**Original** Article

# Multi-site study of an *in vivo* phototoxicity evaluation in Sprague-Dawley (SD) rats aimed at incorporating the phototoxicity assessments: effects of repeated administration and toxicokinetic blood collection on drug-induced phototoxicity

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**ABSTRACT** — The Sprague-Dawley (SD) rat has been widely used for general toxicity and toxicokinetic (TK) studies, and is also useful for phototoxicity assessments. We previously showed that phototoxicity assessments could be incorporated into general toxicity study. However, this research was performed at only one facility. Thus, the effects of repeated administration and TK blood collection were investigated in three facilities to explore the possibility of incorporating phototoxicity assessments into general toxicity study. Lomefloxacin and pirfenidone were tested as the phototoxic compounds. Six-week-old male and female SD rats were allocated to two groups for each compound: single-dose and repeated-dose. The single-dose group was irradiated after a single administration of the drug without blood collection for TK. The repeated-dose group was irradiated after 8 days of repeated administration of the drug with TK blood collection (total 0.72-0.84 mL) after the 1st and 7th administration. Phototoxic reactions on the ventral skin, dorsal skin, and auricle skin were observed macroscopically at 2, 24, 48, and 72 hr after irradiation, and skin reaction scores were evaluated. The phototoxic compounds produced skin reactions in rats at all facilities regardless of the presence or absence of repeated administration and TK blood collection. However, there were differences in the degree of skin reaction between the two groups and among the facilities. Although further studies are needed to standardize this new evaluation system, we expect that the incorporation of phototoxicity assessments will contribute to shortening the research and development period and support the 3R principle for animal experiments.

Key words: Phototoxicity, Sprague-Dawley (SD) rat, Repeated administration, Lomefloxacin, Pirfenidone, Toxicokinetic (TK)

# INTRODUCTION

Phototoxicity is defined as an acute light-induced tissue response to a photoreactive chemical in the Internation-

al Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guideline S10 (2013). During drug development, it is crucial to evaluate the phototoxic risks associated with compounds. Photo-

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toxic drugs are activated by sunlight and cause erythema and edema (Diffey *et al.*, 1998). Thus, patients who take phototoxic medicines cannot risk excess daylight exposure. Additionally, it was reported that ultraviolet treatment is beneficial for psoriasis (Stern *et al.*, 1983) and dermatitis (Reynolds *et al.*, 2001). However, ultraviolet treatment might produce elicit side-effects when patients use phototoxic drugs. Thus, phototoxic drug use restricts quality of life and treatment choices.

In a previous study (Yonezawa et al., 2015), we showed that phototoxicity assessments in Sprague-Dawley (SD) rats could detect phototoxic potential similarly to studies in guinea pigs, which are the typical model for phototoxicity (Morikawa et al., 1974). Another study (Kuga et al., 2017) also demonstrated the efficacy of using SD rats for phototoxicity assessments. The advantages of using SD rats are a shorter study period and a reduction in animal experiments. The ICH S10 guideline (2013) states that animals should be irradiated at approximately the time of maximum concentration (T<sub>max</sub>); therefore, phototoxicity studies require pharmacokinetic (PK) data. SD rats are widely used in experiments for drug development, which includes measuring PK profiles of drugs. Thus, the use of SD rats in phototoxicity studies obviates the need for an additional PK study, leading to a shorter study period and a reduction of animal experiments.

SD rats are also used in general toxicity studies, so there is a possibility to incorporate phototoxicity assessments into general toxicity studies in SD rats. The Guidance for Industry Photosafety Testing (2003) published by the Food and Drug Administration (FDA) specifies, "assessments of photoirritation may be incorporated into ongoing general toxicity studies in some circumstances." Additionally, the ICH guideline S2(R1) (2008) and ICH guideline M3(R2) (2009) recommends the incorporation of assessments within general toxicity studies.

We previously demonstrated that phototoxicity assessments could be incorporated into general toxicity studies (Yonezawa *et al.*, 2017). However, this research was performed in only one facility. Thus, to standardize procedures for phototoxicity assessments in SD rats, evaluation by multiple facilities should be performed.

This study was performed in three facilities, namely, Takeda Pharmaceutical Company Limited (Takeda), Kaken Pharmaceutical Company Limited (Kaken), and LSI Medience Corporation (LSIM). This study was conducted using a single protocol under different conditions at these three facilities. We compared the phototoxic reactions in two groups—single-dose and repeated-dose —to determine whether repeated-dosing and toxicokinetic (TK) evaluations had any influence on the phototoxic response.

# MATERIALS AND METHODS

#### Chemicals

Lomefloxacin hydrochloride (LMFX) and pirfenidone (PFD) were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan). Sterilized 0.5 w/v% methylcellulose (MC) solution was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan) or Shin-Etsu Chemical Co., Ltd (Tokyo, Japan).

#### Animals

Male or female Crl:CD(SD) rats aged 5 weeks were purchased from Charles River Laboratories Japan, Inc. (Kanagawa, Japan) and acclimated to the laboratory environmental conditions for 5-7 days. On the day of dosing, rats were aged 6 weeks. The animals were housed in an animal room, at a temperature maintained between 20°C and 26°C. The animals were individually placed in rat cages with free access to feed and water. This study was approved by the Institutional Animal Care and Use Committees of Kaken Pharmaceutical Company, Kumamoto Laboratory (LSI Medience Corporation), and Shonan Research Center (Takeda Pharmaceutical Company Limited). The study also conforms to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

# **Experimental design**

The experimental design is shown in Fig. 1. For each phototoxic compound, SD rats were selected and divided into two groups: a single-dose group (n = 5 or 6), and a repeated-dose group (n = 5 or 6). The single-dose group was irradiated after a single administration of the drug. The repeated-dose group was irradiated after 8 consecutive days of drug administration. In addition, blood samples were collected from the repeated-dose group on days 1 and 7.

#### Drug administration and light irradiation

LMFX and PFD were suspended in 0.5 w/v% MC. Rats were weighed and orally administered 5 mL/kg suspension once. The hair on the backs and abdomens of the animals was removed using an electric clipper in Takeda and LSIM, and an electric shaver in Kaken. At 1 hr after administration of LMFX or 0.5 hr after administration of PFD, rats were irradiated under anesthesia. A mixture of medetomidine (0.15 mg/kg), midazolam (2.0 mg/kg), and butorphanol (2.5 mg/kg) was injected intramuscularly 15 min prior to light irradiation at Takeda and LSIM. At Effects of repeated administration and TK on phototoxicity in SD rats



Fig. 1. Experimental design of this study. For each phototoxic compound, 10 or 12 SD rats were selected and divided into two groups: single-dose (n = 5 or 6) and repeated-dose (n = 5 or 6). The single-dose group was irradiated after a single administration of the drug. The repeated-dose group was irradiated after 8 days of repeated administration of the drug. In addition, blood samples were collected from the repeated-dose group on days 1 and 7.

Table 1. Differences in study conditions among testing facilities.

	Kaken	LSIM	Takeda
Test compound	lomefloxacin, pirfenidone	lomefloxacin, pirfenidone	lomefloxacin
Light gourge	UV irradiation device	UV irradiation device	Solar simulator
Light source	(FL20S BL/DMR and TL20W/12RS)	(FL20S BL/DMR)	(SXL-5009V)
UVA irradiation	10 J/cm <sup>2</sup>	$10 \text{ J/cm}^2$	18 J/cm <sup>2</sup>
UVB irradiation	0.031 J/cm <sup>2</sup>	None	0.36 J/cm <sup>2</sup>
Visible light	None	None	Available
Sex	Male	Male	Female
Hair remover	Clipper and shaver	Clipper	Clipper
Irradiation site	Dorsal	Dorsal, Auricle	Ventral
Anesthetics	Pentobarbital (i.p.)	MMB (i.m.)	MMB (i.m.)

MMB: Mixture of medetomidine, midazolam, and butorphanol.

Kaken, pentobarbital (50 mg/kg) was injected intraperitoneally immediately before irradiation. Animals were covered with aluminum foil to delineate irradiated and nonirradiated sites. Then, the animals were irradiated using a solar simulator (SXL-5009V, Seric., Ltd, Japan) or a UV irradiation device with a UV-A light source (FL20S BL/DMR; Toshiba Medical Systems Corporation, Tokyo, Japan) and a UV-B light source (TL20W/12RS; Philips, Amsterdam, Netherlands). The study design and conditions, including light and anesthesia in each facility, are summarized in Table 1. The irradiation dose of UVB was set to be less than a half of the mean erythema

dose which had been determined previously at Takeda or Kaken. After light exposure, atipamezole (0.06 mg/kg) was injected intraperitoneally for awakening from anesthesia in Takeda and LSIM. Skin reactions were observed at 2, 24, 48, and 72 hr after the end of light irradiation, according to the Draize method (Table 2) (Draize, 1959).

# Skin reaction evaluations

The skin reaction scores (erythema and edema formation) of individual animals were summed for each site and time point, and the mean score and total mean score was Y. Yonezawa et al.

Table 2.	Evaluation	of Skin	Reactions	(Draize's	Criteria:	1959).
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calculated according to the following equation:

Mean skin reaction score = total of erythema and edema scores / number of animals tested.

The sums of the mean skin reaction scores at all time points were defined as the "Total mean skin reaction score." Additionally, the mean skin reaction score for the UV-irradiated site was analyzed using Bartlett's test for homogeneity of variance. Student's t-test and the Wilcoxon rank sum test were performed to compare data between the single-dose and repeated-dose groups, when the variance was homogeneous and heterogeneous, respectively.

# Blood sampling and TK analysis

In all facilities, blood samples were collected from all the animals in the repeated-dose group after the first and seventh administrations. At Kaken, blood was collected at six or seven time points in total: before dosing (the seventh administration only), and 0.5, 1, 2, 4, 8, and 24 hr after dosing. Approximately 120 µL of blood was collected from the caudal vein of each animal (the total amount was 0.72 or 0.84 mL). The blood was then immediately transferred to a 0.5 mL polypropylene tube containing heparin sodium (Novo-Heparin 5000 units/5 mL for injection; Mochida Pharmaceutical Co., Ltd. Tokyo, Japan) and mixed. Plasma samples were obtained by centrifugation (4°C, 3000 rpm, 15 min). The samples were then kept under ice-cold conditions until use. At Takeda and LSIM, the same amount of blood was collected once after dosing from the jugular vein or subclavian vein; however, collected blood samples were not used for any analyses. The plasma concentrations of the drugs were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS), and the TK parameters ( $T_{max}$ , the maximum concentration  $(C_{max})$ , and area-under-the-curve  $(AUC_{0.24}))$  were calculated. The LC system consisted of a pump, a Nanospace SI-2 autosampler (Osaka soda, Osaka, Japan), and a C18 reversed-phase analytical column (Inertsil ODS-3, 4 µm,

 $2.1 \times 50$  mm; GL Science, Tokyo), and was connected to a TSQ Quantum Ultra Triple-Quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). The injection volume was 2 µL for lomefloxacin and 5 µL for pirfenidone. Gradient elution was performed with mobile phase A, which consisted of 0.1% formic acid (v/v) in water (98%), and mobile phase B, which consisted of 0.1% formic acid (v/v) in methanol (2%). The following gradient program was used at a flow rate of 0.4 mL/min: 0-0.1 min, linear gradient from 98% A to 0% A (v/v); 0.1-1.6 min, hold at 0% A; 1.6-1.7 min, linear gradient from 0% A to 98% A (v/v); 1.7-4.2 min, hold at 98% A. Quantification of the protonated precursor ion and the related product ion was performed in the MRM mode using an internal standard method with peak area ratios. The mass transitions used for quantification were m/z 352.1→236.9, m/z 186.0→92.0 for lomefloxacin and pirfenidone, respectively. The quantitative range of the calibration curve was 0.1~10 µg/mL and 0.05~5 µg/mL for lomefloxacin and pirfenidone, respectively. The Xcalibur<sup>TM</sup> and LCquan<sup>TM</sup> software (Thermo Fisher Scientific) was used for data acquisition and analysis. The linearity of the calibration curves was assessed using weighted (1/y) least-squares linear regression of the analyte: IS peak area ratio.

#### RESULTS

#### Skin phototoxicity assessment

The results of skin reaction testing for LMFX and PFD are shown in Figs. 2 and 3, and detailed data is shown in Figs. 4 and 5, respectively (data of the single-dose group in the LMFX-treated group were quoted from Kuga *et al.*, 2019).

In the LMFX-treated groups, the skin scores dose-dependently increased. At 30 mg/kg, the skin scores of the repeated group were significantly lower than the singleEffects of repeated administration and TK on phototoxicity in SD rats



**Fig. 2.** The total mean skin reaction scores of the UV-irradiated site following lomefloxacin (LMFX) treatment: (A) 30 mg/kg LMFX and (B) 100 mg/kg LMFX. The data for the non-irradiated site are not shown, because the scores were 0 at all facilities. The mean skin reaction score = total of erythema and edema scores / number of animals tested.



**Fig. 3.** The total mean skin reaction scores of the UV-irradiated site following pirfenidone (PFD) treatment: (A) 300 mg/kg PFD and (B) 750 mg/kg PFD. The data for the non-irradiated site are not shown, because the scores were 0 at all facilities. The mean skin reaction score = total of erythema and edema scores / number of animals tested.

dose group in Takeda at 2 and 24 hr after irradiation. At 100 mg/kg, the skin scores of the repeated-dose group were significantly higher than the single-dose group in the dorsal skin tested at LSIM (24 hr after irradiation). Additionally, the skin scores of the repeated-dose group were lower than the single-dose group at Kaken and Takeda (not significant). Otherwise, there were no clear differ-

ences in responses between the single-dose and repeated-dose groups in any other conditions.

In the PFD-treated groups, skin scores increased dosedependently except for the dorsal skin of LSIM, which produced no skin reactions over any observation period. At 300 mg/kg, there were no apparent differences between the single-dose and repeated-dose groups at

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Fig. 4. Plot of the skin scores for the UV-irradiated site following lomefloxacin (LMFX) treatment: (A) 30 mg/kg LMFX, dorsal skin, Kaken, (B) 30 mg/kg LMFX, dorsal skin, LSIM, (C) 30 mg/kg LMFX, auricle skin, LSIM, (D) 30 mg/kg LMFX, ventral skin, Takeda, (E)100 mg/kg LMFX, dorsal skin, Kaken, (F) 100 mg/kg LMFX, dorsal skin, LSIM, (G) 100 mg/kg LMFX, auricle skin, LSIM, (H) 100 mg/kg LMFX, ventral skin, Takeda. The skin scores of the repeated-dose group were significantly different from that of the single-dose group, \*P < 0.05 (Student's *t*-test). Each point represents the mean ± SD values of five (Kaken and LSIM) or six (Takeda) animals. The data for the non-irradiated site are not shown, because the scores were 0 for both compounds.

	Units	30 mg/kg lomefloxacin	Ν	100 mg/kg lomefloxacin	Ν
C <sub>max</sub>	ng/mL	2784 ± 791	3	9293 ± 1970	3
T <sub>max</sub>	h	$0.667 \pm 0.289$	3	$1.00 \pm 0.00$	3
AUC <sub>0-24</sub>	ng·h/mL	$11693 \pm 1210$	3	$42009 \pm 1379$	3
$(mean \pm SD)$					
(110011 = 5.2.)					
Day 7					
Day 7	Units	30 mg/kg lomefloxacin	N	100 mg/kg lomefloxacin	N
Day 7	Units ng/mL	$\frac{30 \text{ mg/kg lomefloxacin}}{3100 \pm 701}$	<u>N</u> 3	100 mg/kg lomefloxacin 8700 ± 499	<u>N</u> 3
Day 7 C <sub>max</sub> T <sub>max</sub>	Units ng/mL h	30 mg/kg lomefloxacin 3100 ± 701 0.667 ± 0.289	N 3 3	$\frac{100 \text{ mg/kg lomefloxacin}}{8700 \pm 499}$ $0.833 \pm 0.289$	N 3 3

Table 3.	Summary of	f TK parameters	for lomefloxacin
D (			

Blood samples were collected from all the animals (5 animals) in order to evaluate under the same conditions, and 3 out of 5 blood samples were used for TK analysis.

Kaken, but the skin scores of the repeated-group was significantly lower than the single-dose group in the auricle skin tested at LSIM (2 and 24 hr after irradiation). At 750 mg/kg, the skin scores of the repeated-group were significantly lower than the single-dose group in the auricle skin tested at LSIM (2 and 24 hr after irradiation).

In the control group or the non-irradiated area, no skin reactions were observed for any sites at any time points in any facilities (data not shown).

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Day I					
	Units	300 mg/kg pirfenidone	Ν	750 mg/kg pirfenidone	Ν
C <sub>max</sub>	ng/mL	$43063 \pm 11120$	3	$55837 \pm 10088$	3
T <sub>max</sub>	h	$0.5 \pm 0$	3	$0.5 \pm 0$	3
AUC <sub>0-24</sub>	ng·h/mL	$156235 \pm 11822$	3	$370741 \pm 101004$	3
$(\text{mean} \pm \text{S.D.})$					
Day 7					
	Units	300 mg/kg pirfenidone	Ν	750 mg/kg pirfenidone	N
C <sub>max</sub>	ng/mL	$12827 \pm 1128$	3	$20070 \pm 7611$	3
T <sub>max</sub>	h	$0.5 \pm 0$	3	$1.67 \pm 2.02$	3

Table 4.	Summary of TK	parameters	for pirfenidone
Dov 1			

ng·h/mL

 $(\text{mean} \pm \text{S.D.})$ 

 $AUC_{0-24}$ 

Blood samples were collected from all the animals (5 animals) in order to evaluate under the same conditions, and 3 out of 5 blood samples were used for TK analysis.

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 $215354 \pm 121666$ 

 $64648 \pm 11945$ 



Fig. 5. Plot of the skin scores of the UV-irradiated site following pirfenidone (PFD) treatment: (A) 300 mg/kg PFD, dorsal skin, Kaken, (B) 300 mg/kg PFD, dorsal skin, LSIM, (C) 300 mg/kg PFD, auricle skin, LSIM, (D) 750 mg/kg PFD, dorsal skin, Kaken, (E) 750 mg/kg PFD, dorsal skin, LSIM, (F) 750 mg/kg PFD, auricle skin, LSIM. The skin scores of the repeated-dose group were significantly different from that of the single-dose group, \*P < 0.05, \*\*p < 0.01, \*\*\*p < 0.01 (Student's *t*-test). Each point represents the mean ± SD values of five animals. The data for the non-irradiated site are not shown, because the scores were 0 for both compounds.

#### **Toxicokinetics**

The TK parameters for each compound are summarized in Tables 3 and 4. The concentrations of each compound in the plasma after oral administration are summarized in Figs. 6 and 7.

For both the LMFX-treated group and the PFD-treated group, the mean  $C_{max}$  and  $AUC_{0.24}$  increased in a dosedependent manner. In the LMFX-treated group, there

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**Fig. 6.** Plasma drug concentrations of the lomefloxacin (LMFX) group after single or repeated administration to SD rats: (A) 30 mg/kg LMFX, (B) 100 mg/kg LMFX. Each point represents the mean ± SD of five animals.



**Fig. 7.** Plasma drug concentrations of the pirfenidone (PFD) group after single or repeated administration to SD rats: (A) 300 mg/kg PFD, (B) 750 mg/kg PFD. Each point represents the mean ± SD of five animals.

were no apparent changes in the  $C_{max}$ ,  $T_{max}$ , or AUC<sub>0-24</sub> values between the single-dose and repeated-dose groups.  $T_{max}$  values of the single-dose and repeated-dose groups were 0.667-1.00 hr and 0.667-0.833 hr after administration, respectively. In both groups, the time of UV irradiation was near the  $T_{max}$ .

In the PFD-treated group, repeated administration decreased  $C_{max}$  and  $AUC_{0.24}$  values.  $T_{max}$  values of the single-dose and repeated-dose groups were 0.5 hr and

0.5-1.67 hr after administration. In both groups, the time of UV irradiation was near the  $T_{max}$  except for the repeated-dose group at 750 mg/kg.

# DISCUSSION

Our study examined the effects of repeated administration and TK blood collection on drug-induced phototoxicity with regard to exploring the incorporation of phototoxicity assessments into general toxicity studies.

With the LMFX-treated groups, both single and repeated dosing produced phototoxic reactions. However, the skin scores of the repeated-dose group were lower than those of the single-dose groups at Kaken and Takeda. This attenuation of skin reaction was also previously reported (Yonezawa et al., 2017). No major differences in the T<sub>max</sub>,  $C_{max}$ , or AUC<sub>0-24</sub> values of LMFX were observed throughout the dosing period. Thus, the decreases in skin reaction scores were not considered to be due to changes in TK, even though skin drug concentrations were not measured in this study. Other possibilities to explain these differences are changes in skin structures with aging or individual differences. In fact, the average body weight gain was over 20% from day 1 to day 7 in the Kaken study (Supplemental Table). This sudden change in body weight suggests a remarkable growth spurt, which may cause changes in skin thickness or structure.

Regarding PFD treatment, the skin score of the repeated-dose group was significantly lower than that of the single-dose group (the auricle skin of the rats was tested at LSIM). TK measurements demonstrated that repeated administration decreased  $C_{max}$  and AUC<sub>0-24</sub> values compared with single administration. Additionally,  $T_{max}$  was prolonged and differed from the irradiation timing. Based on these results, it was concluded that the decreased skin reactivity occurred via altered TK parameters besides changes in skin structure. Thus, the impact of changes in TK parameters on skin reactions should be carefully considered when evaluating phototoxicity after repeat dosing.

As mentioned above, the repeat-dosed group showed lower skin sensitivity to phototoxicity than the singledose group in several cases in this study. On the contrary, 100 mg/kg LMFX treatments on the dorsal skin at LSIM produced significantly higher skin reaction scores in the repeated-dose group than in the single-dose group. There were no major differences in the TK parameters as mentioned above, although fluoroquinolones is known to accumulate in the rat skin (Tanaka et al., 2004). This is why the accumulation occurs only in pigmented skin and not in albino skin, because fluoroquinolones have a strong affinity for melanin (Ono and Tanaka, 2003; Tanaka et al., 2004). This result suggested that skin reactions might occur due to reasons not related to drug concentration. Differences in the study conditions among these facilities may also be responsible for differences between the single-dose and repeated-dose groups. Therefore, further studies employing a larger number of animals, test compounds, and test facilities should be performed.

Regarding the effects of blood sampling, we considered that blood sampling had a very small influence on the phototoxicity assessment as we demonstrated in the previous study (Yonezawa *et al.*, 2017). Additionally, sampling of less than 0.9 mL of blood per day is known as the maximum volume that can be drawn from rats without causing changes in hematological parameters (Kurata *et al.*, 1997). In this study, the total volume of blood sampled was 0.72-0.84 mL, which is less than the 0.9 mL volume cited above. This fact also supports our hypothesis that blood sampling had a very small influence on the phototoxicity assessment.

There are a number of potential advantages to the incorporation of phototoxicity assessments and general toxicity evaluations into a single study. For example, a skin score was evaluated using one gender and one dose level in the single-dose phototoxicity study performed at Kaken. Conversely, both genders and multiple-dose are generally evaluated in the repeat-dose general toxicity study. Thus, the incorporated phototoxicity assessments will enable evaluation of the differences between the sexes and the dose-response. This evaluation meets the ICH guideline S10 (2013) statement that "If a negative result is obtained at the maximum dose, testing of lower doses is usually not warranted. However, if a positive result is anticipated, additional dose groups can support a NOAEL-based risk assessment." Skin scores increased with dose levels in both LMFX and PFD in this study. These observations indicate that LMFX and PFD can be evaluated by the NOAEL-based risk assessment through the incorporation of phototoxicity assessments into general toxicity studies. Additionally, we can evaluate the plasma drug concentration and skin reaction in the same animal, which will help us to confirm that irradiation was performed correctly, and evaluate the effects after repeated administration.

Finally, in this study, we discovered the pros and cons of incorporating phototoxicity assessments. Although incorporation model changed skin sensitivity in some case, both the single and repeated dose showed phototoxic reactions with LMFX and PFD. With some compounds such as PFD, whose exposure reduces by repeated administration, incorporating phototoxicity assessments should be carefully performed. However, the skin reaction could be adjusted by adjusting the type of light source or the irradiation site (Kuga *et al.*, 2017). Thus, this attenuation of skin reaction could be avoided to select the best method for each facility. On the other hand, there is a case that the skin reaction increases by repeated administration. In such cases, incorporating phototoxicity assessments could detect the phototoxicity which might be missed in the single-dose phototoxicity study.

Incorporating phototoxicity assessments provide useful information and reduces the number of studies and the number of animals required. This contributes to shortening the duration of research and development and upholds the 3R principle for animal experiments. We expect that this new evaluation system will allow a more accurate evaluation of phototoxic risks of compounds.

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**Conflict of interest----** The authors declare that there is no conflict of interest.

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