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Comparison of International Guidelines of Dermal Absorption Tests Used in Pesticides Exposure Assessment for Operators

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The number of farmers who have suffered from non-fatal acute pesticide poisoning has been reported to vary from 5.7% to 86.7% in South Korea since 1975. Absorption through the skin is the main route of exposure to pesticides for farmers who operate with them. Several *in vitro* tests using the skins of humans or animal and *in vivo* tests using laboratory animals are introduced for the assessment of human dermal absorption level of pesticides. The objective of this study is to evaluate and compare international guidelines and strategies of dermal absorption assessments and to propose unique approaches for applications into pesticide registration process in our situation. Until present in our situation, pesticide exposure level to operator is determined just using default value of 10 as for skin absorption ratio because of data shortage. Dermal absorption tests are requested to get exposure level of pesticides and to ultimately know the safety of pesticides for operators through the comparison with the value of AOEL. When the exposure level is higher than AOEL, the pesticide cannot be approved. We reviewed the skin absorption test guidelines recommended by OECD, EFSA and EPA. The EPA recommends assessment of skin absorption of pesticides for humans through the TPA which includes all the results of *in vitro* human and animal and animal *in vivo* skin absorption studies. OECD and EFSA, employ a tiered approach, which the requirement of further study depends on the results of the former stage study. OECD guidelines accept the analysis of pesticide level absorbed through skin without radioisotope when the recovery using the non-labeled method is within 80~120%. Various factors are reviewed in this study, including the origin of skin (gender, animal species and sites of skin), thickness, temperature and, etc., which can influence the integrity of results.

Key words: Dermal absorption, AOEL, Pesticides, Exposure assessment, Operator safety

INTRODUCTION

Pesticides are used as an important means for the control of disease of crops, pests and weeds. Although they have advantages of increasing the production of agricultural products and dramatically reducing the labor, they are becoming a serious risk factor to the health of the farmers who are directly spraying these pesticides.

Direct comparison of the international practices of occupational pesticide poisoning have limitations due to different methods of each country for defining a pesticide poisoning, investigation approach and scope but according to the report by the U.S, through pesticide poisoning surveillance system of the National Institute of Occupational Safety & Health, annual average of approximately 5~54 pesticide addict per 100,000 had occurred in the years of 1998~2005 (1,2). According to the results from a survey, 6.7% of Nicaraguan farmers (3), 6.5% Brazilian farmers, 8.8% of Chinese farmers, 31% of Vietnamese farmers and 83.6% of Indian farmers had experienced symptoms that fit the symptoms of acute pesticide poisoning in the last year. 209,512

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Abbreviations: AOEL, Acceptable-Operator-Exposure-Level; OECD, Organization for Economic Co-operation and Development; EFSA, The European Food Safety Authority; EPA, US Environmental Protection Agency; TPA, Triple pack approach.

farmers within the domestic male farmer reported occupational acute pesticide poisoning in 2012, estimating to be 24.7 people per 100 farmers (4).

In Korea, establishment of ADI (Acceptable Daily Intake) or MRL (Maximum Residue Limit) is required when registering a pesticide, so safety of food and consumers is relatively well managed but the system or device that can manage the impact of pesticide exposure in agricultural workers are relatively insufficient.

Despite the fact that the major exposure route of pesticide absorption of agricultural workers is through the skin, the risk due to exposure via the oral route has been prioritized (5). Thus, since a trustable toxicity study by dermal absorption lacks, risk factors through dermal exposure of pesticides have been evaluated by supplementing the uncertainty due to the difference of the route of administration. In other words, as for oral and dermal exposure, factors such as differences in the in vivo transformation first pass effects (response when first exposed in the body) and different absorption rate act as correction factors for differences of the exposure path (6).

The current pesticide registration standards in force in the country regulate pesticides with risk index, ratio of exposure amount and AOEL of more than 1 cannot be registered. When in vivo or in vitro dermal absorption test information is lacking, the basic skin absorption rate is set to be applied at 10%. However, validation of this 10%, assigned as default value for the absorption rate of the pesticide, is required and since the physical and chemical properties vary by pesticide, applying it by measuring the skin absorption rate through actual experiments will allow more accurate risk assessment for agricultural workers.

This study aims to introduce various scientific data and guidelines of international organizations on skin absorption testing and proposes an approach to evaluate skin absorption tests of the pesticide that fits the domestic conditions based on the results.

Dermal absorption rate test strategies and procedures. As for the skin absorption test methods of agricultural chemicals which have been developed and utilized so far, there are Test Guideline 428 Skin Absorption*: in vitro* Method, GUIDANCE DOCUMENT 28, GUIDANCE NOTES ON DERMAL ABSORPTION (7) and Panel on Plant Protection Products and their Residues (PPR) EFSA Journal 2012;10(4):2665 of EFSA, SCIENTIFIC REPORT OF EFSA SANCO/222/2000 rev. 7 EFSA Scientific Report (2009) 282 of OECD (8) and EPA/600/8-91/011B January 1992 Interim Report Dermal Exposure Assessment, Health Effects Test Guidelines OPPTS 870.7600 Dermal Penetration of U.S. EPA (9).

In case there is no information relevant to the skin absorption of a material, EFSA recommends, based on the physicochemical properties of active ingredients, if octanol/water partition coefficient (log Pow) is < -1 or > 4 and the molecular weight of > 500, the skin absorption rate can be applied at 10% by default (8). Other than the above case, if there is no information related to skin absorption of a material, the default value is set to be minimum of 10% - maximum of 75% (EFSA 2010) (Fig. 1) (10), or minimum of 10% - maximum of 100% (EFSA 2002) (11) of the amount exposed to the skin depending on the content of the active ingredient of the product.

Therefore, the absorption rate that was set seemed to have been set in a fairly conservative way but it is accepted as being essential in that it has made a huge impact on risk assessment for the pesticide.

When conducting a skin absorption experiment, a 'Triple Pack' approach method is used to most accurately estimate the skin absorption rate from a person (7).

$$\textit{In vivo}\ \text{human absorption} = \frac{\textit{In vivo}\ \text{rat absorption} \times \textit{In vitro}\ \text{human absorption}}{\textit{In vitro}\ \text{rat absorption}}$$

The Triple Pack method consists of a total of three types of tests, two types of in vitro test (in vitro rat skin test, body skin exam) and 1 type of in vivo rat test, and as a method to minimize the possibility of error that each of the test method possess, this method was selected on the basis that rats and humans will be the same and the absorption difference between humans and rats will show in the same proportion in both in vitro and in vivo tests. This method has been introduced as the most accurate method in all EFSA (8), OECD (7) and EPA test guidelines (9).

However, it is costly to proceed with all three experiments and there are difficulties in ethics in its use of experimental animals. Therefore, EFSA and the OECD have adopted a Tiered Approach (Fig. 1).

Diffusion cell as an apparatus for the measurement of dermal absorption rate. Although there are various types of skin absorption rate measurement equipment (Diffusion cell) and equipment used in the skin absorption rate measuring exists, by default, it is composed of a donor chamber that can process test materials and a receptor chamber where the tested material that penetrated the skin can remain, and there also should be a device that can securely fix the skin tissue between the two chambers (Fig. 2).

In addition to these conditions, in order to obtain more accurate results, measuring equipment is required that is easy to use when sampling and can generate as little bubbles as possible, which could affect the rate of absorption.

The donor chamber can be divided into two types depending on the treatment method of the test substance. One is a static scheme designed to treat the test substance of a limited capacity and the other is a flow-throw scheme donor chamber designed to enable the continuous process of the test substance. In all the guidelines, use of any chamber is

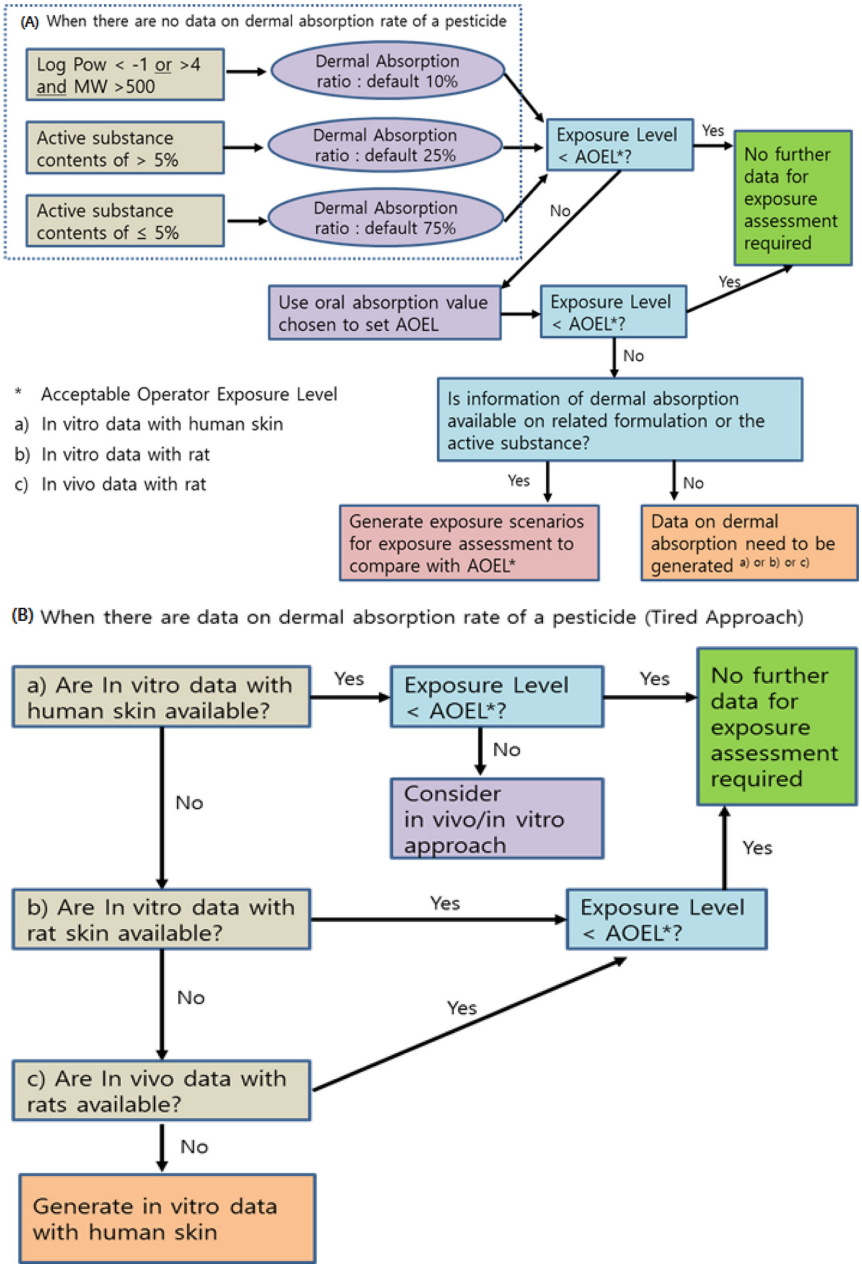
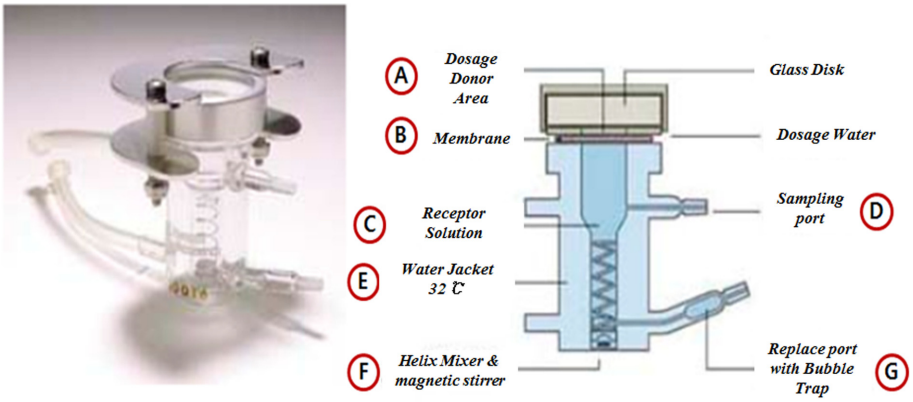


Fig. 1. Procedures for the decision of dermal absorption ratio based on the recommendation by EFSA (2012)[9]. (A) Upper figure presents a decision tree when there are no data on dermal absorption rate of a pesticide. In that case, starting points are dependent on the solubility of the compound (log Pow) and content of the active substance in a product for the determination of default absorption ratio of a pesticide into the skin of an operator. (B) Bottom figure presents a decision tree when data on dermal absorption rate of a pesticide. *In vitro* data with human skin, *in vitro* data with rat and *in vivo* data with rat are used with tiered approach for the decision of exposure level of a pesticide to an operator.

acceptable as long as a donor chamber is chosen that can reproduce a situation similar to the actual work condition in the field.

A receptor solution filling the receptor chamber must be in contact with the lower surface of the skin, while the stirring device must be attached so that the test substance can melt well and a minimum temperature of 32 ± 1°C (EFSA, OECD, EU standard) or 37°C (EPA) must be maintained, as the skin absorption rate can be affected depending on the temperature.

The aqueous solution is to be selected in consideration of affinity to the skin tissue, solubility of the test substance and analysis status of the test substance. In detail, selection of the solution depends on whether the tissue used in the



A : Donor chamber : Test substance treatment position

B : Membrane : Position securing the skin samples

C : Receptor chamber : As a position used to store test materials that penetrated the skin, it is used by filling it with an aqueous solution

D : Sampling port : Passage to collect the test samples from test aqueous solution

E : Water jacket : Location where heated water circulates to adjust the temperature

F : Stirrer : Stirring device so that the test substance is well dissolved in the aqueous solution

G : Replace port : Passage that refills new aqueous solution after sampling

Fig. 2. Composition of Franz cell. It is an in vitro skin permeation assay apparatus that consists of two primary chambers separated by a membrane. A test material is applied to the membrane via the top chamber and the bottom chamber contains fluid from which samples are taken at regular intervals for analysis. The apparatus is designed to determine the amount of active substance that has permeated the membrane at each time point. Usually, the chamber is maintained at a constant temperature of 37°C.

experiment has living physiological activity or dead tissue without metabolic activity.

If the experiment is performed on a tissue with living physiological activity, a physiological solution, such as a cell culture medium must be selected to maintain the physiological activity and for experiments using tissues free of physiological activity, aqueous solutions should be selected in consideration of the solubility of the test substance. The most widely used aqueous solution, in case of evaluating water-soluble material, is saline solution with pH 7.4 and when evaluating a non-polar test substance, since test substances might not be dissolved in an aqueous solution, 6% polyethylene glycol 20 oleyl ether solutions or 5% bovine serum albumin mixed in saline can be used to increase solubility. In addition, if explainable, use of a different solution is possible (12).

Selection and preparation of skin tissue. According to the OECD guidance document 28 (7) that prescribes the measurement of skin absorption rate experiment targeting workers who are engaged in working among pesticides, chemicals or in biotechnology fields, although at times, experimental results utilizing monkey, pig and artificial and cultured skin are submitted, skin tissue that is used mainly in experiments are human or rat skin tissues. In addition to the selection of species to get a skin tissue for the experiment, the decision of whether to use a tissue with metabolic activity or without metabolic activity must be made.

Basically, although skin with metabolic activity is preferred, it is not always easy to find. Also, in case of when a change of the test substance by skin metabolism occurs, embodiment of the analysis to determine the absorption rate and estimation of the recovery rate must require more effort. Furthermore, since there are a lot of accumulated test results obtained by using skin with no metabolic activity for a long time, it is easier to prove integrity, so a method of using skin without metabolic activity has been recommended.

Skin used on the experiments with human skin is obtained from a cadaver or through surgery for cosmetic and urological surgery. Since the absorption rate of skin tissue of people differs depending on the collected area, it is recommended to use only the skin of the abdomen, chest or upper leg (Table 1) (13).

Reconstructed skin using skin tissue derived from people is used as an alternative of this. Each regulation has presented a different opinion on the possibility of use of reconstructed skin on the skin absorption rate experiment. In the guidelines of the OECD guidance document and EU, as long as it demonstrates that it shows similar results to the

Table 1. Comparison of dermal absorption at different anatomical parts of the body of human volunteers

Anatomical region	Parathion (4 μg/ml in acetone)	Malathion (4 μg/ml in acetone)
Abdomen	1.0	1.0
Forearm	0.5	0.7
Palm	0.6	0.6
Ball of foot	-	0.7
Back of hand	1.1	1.3
Inside elbow	1.5	-
Scalp	1.7	-
Jaw angle	1.8	-
Forehead	2.0	2.5
Armpit	3.5	3.1
Scrotum	5.5	

Data are normalized to abdomen = 1.0.

conventional results of representative reference substance such as caffeine, testosterone and benzoic acid, they are in the position of accepting it and even introduces some of the actual commercialized reconstructed skin (14).

On the contrary, the EFSA does not recommend the use of this based on the result that the function of wall of the skin tissue cells produced by the culture does not clearly reflect the characteristics of this (15).

As for the skin tissue derived from animals, as mentioned earlier, the most widely used animal species are rats. Although rat's skin, when compared to human skin, indicates a significantly higher skin absorption rate showing many differences in the actual results, but these differences can be calibrated through the formula once results of in vivo experiments using rats and in vitro experiments using human are obtained, and it has an advantage in that it has many accumulated experiment results since it was used in skin absorption rate tests for a long time (16).

In skin absorption rate measurement tests of humans and animals, the full thickness of skin can be used but the use of skin tissue more than 1mm is not recommended and should be avoided except for a study that holds a special purpose such as experiments to determine the test substance distribution within the skin tissue. This is because, in the most of the skin tissue without physiological activity used in the rate experiment, absorption through the blood vessels does not occur, so the test substances have accumulated in the skin tissue to exist in a high concentration. In general, skin samples with a thickness of 200~500 μm has been recommended and tissue with only stratum corneum and epidermis after removing the dermis layer are mainly used.

Differences in absorption rate due to gender, in general, is reported to be absent in humans. However, these differences in animals have been reported and this difference is known to be caused by the differences in thickness of the stratum corneum and the thickness of the stratum corneum is affected by the thickness of the entire skin. Therefore, if the thickness of the stratum corneum and the entire skin are same, it is learned that the absorption rate does not differ due to gender (17). In case of rat, dorsal skin, according to gender, differ in thickness of stratum corneum or the entire skin but shows no difference in ventral skin thickness. Therefore, when dorsal skin is used, gender differentiation should not be necessary (Table 2) (18).

There are a number of ways to separate skin tissue depending on the animal species and the separation method (15,19,20). The most important thing when choosing a method of separating the skin tissue is, when one test substance is tested using several animal species, it must be analyzed using the same method, so even the same separation method should be used. Although the method is not contained in the guidelines, among published papers, there are cases of introducing the same separation method for human and rat skin (21). In addition, the method is known to use a dermatome that can be applied without considering the difference in species to produce split thickness skin and uses this cut skin tissue for the test.

The collected skin tissue can be used in the experiment immediately after collecting but can also be stored after freezing. A study on hypothesis, if appropriate freeze storage is made, does not affect the skin permeation rate, had been demonstrated by experiments using the skin of people and animals. The most suitable temperature to store the skin tissue is −20°C. The skin of humans or animals did not have a significant effect on the results when stored in 10°C for a maximum of 3 days and a penetration rate has shown to increase when stored in −80°C (22).

Table 2. Site- and gender-related differences in the skin thickness and absorption of Benzoic acid

Type of skin (n)	Skin thickness			% of Applied dose (after 5 hr)
	Stratum corneum	Epidermis (μm)	Whole skin (mm)	
Male				
Back (10)	34.7 ± 2.3	61.1 ± 3.0	2.80 ± 0.08	0.5 ± 0.07
Abdomen (13)	12.8 ± 0.08	30.4 ± 1.5	1.7 ± 0.06	5.1 ± 0.9
Female				
Back (10)	18.2 ± 1.0	31.2 ± 1.5	2.04 ± 0.05	2.5 ± 0.6
Abdomen (13)	13.7 ± 0.6	34.8 ± 1.8	0.93 ± 0.02	6.5 ± 0.9

On the other hand, a report has been published that skin tissue stored in −20°C showed no difference in absorption rate when stored up to a maximum of 466 days (23).

The most important thing in the process of preparing skin for the process or experiment is maintaining the integrity of the skin tissue, including the stratum corneum. The method for evaluating the integrity of the skin tissue varies. The first step is to verify the integrity of the skin by visual inspection and the second step is to hydrate the skin after alignment after mounting the skin specimen on the cell. Hydration closes small channels (hair channel, etc.) opened in the skin. Typical methods used to evaluate through the experiment are a method to check if the alternating current (AC) electric resistance value is more than 2 volts, a method to check whether or not trans-epidermal water loss (TEWL) measure value is within the normal range and a method to check the appropriateness of permeability of standard materials such as tritiated water.

Selection of the test substance and condition of treatment. Using test substance during testing by labeling radioactive isotope ^{14}C on the most stable position in the structure of the material is considered as the most ideal method to be used (7,9,24). Isotopically labeled method has an advantage of being relatively easy in the determination of the distribution and recovering of the test substance and making more rapid, accurate and highly sensitive analysis possible. However, isotope labeling requires a lot of costs and in some cases, it is impossible depending on the nature of the test substance microcapsule, granular form, etc., and in some cases, isotopical labeling is not possible in accordance with the structure. In this case, the process should be performed by using an analysis method that passed an appropriate review test (12).

Test substances can obtain more useful information when the substance has same composition to the substance that is being used in the actual working condition or has been prepared in the most similar shape (in-use). The test substance in solid or powder form must be moistened with water to ensure good contact with the skin and should be applied in a quantitative and homogeneous state (25). The amount of water used for hydration must be determined in consideration of the humidity that can occur in the actual work site or influence of sweat (26).

Depending on the test materials applied in the experiment, it can be divided into infinite dose test and finite dose test. The infinite capacity test is mainly applied in household supplies such as bath salts and for chemicals tests such as agricultural chemicals, proceeding with limited capacity tests will more accurately reflect the actual environment.

The amount of test material used in the limited capacity experiment is at the maximum of 10 mg/cm^2, in case of solid test materials and 10 μl/cm^2, in case of liquid test materials and the reason for this is that any amount larger than this tends to spill before absorption. The basic principle for determining the test substance application method and the amount of time should be done in consideration of creating the most similar actual work environment of the agricultural worker.

The first thing to decide is whether to apply the test substance in a state of being closed or in a state of being open. When considering a typical work environment of agricultural workers, it is appropriate to apply in an open state but when considering situations where pesticides are seeping into clothes or flowing into gloves, applying in a closed state can better reflect the work environment.

The concentration of the test substance is usually defined as using two capacitor groups and at this time, when determining the concentration of test substance, it is advantageous to predict the toxicity of the pesticide exposure of workers when the dilution is determined in consideration of the concentration that the workers can be exposed to in the actual production environment.

Generally, since the skin absorption rate of a substance tends to show a higher absorption rate in the low concentration state rather than high concentration (6), it is recommended to proceed with the test in the diluted concentration that matches the actual lowest concentration used in the work environment, in addition to the undiluted pesticide products. If the dilution ratio is higher than normal, setting another concentration between the undiluted and the lowest dilution will be unique when testing the absorption rate of untested diluted concentration (7).

The applied time of the test substance, when considering the work time of agricultural workers, should at least be 6-10 hrs and it is usually defaulted to 8 hrs of exposure time. This is in consideration of the actual working conditions of the field that after 8 hrs of working time, the work would cease and workers would wash away the remaining substance such as pesticides rubbed on the skin.

However, not all pesticides are removed even when washing the skin after the job has ended and the possibility of residual pesticides on the skin being absorbed into the body is present. Therefore, the general method for measuring the absorption rate is to take an additional sample after 24 hrs of exposure after washing the skin after 8 hrs of exposure. Typical sampling times are before processing test substance (0hr) and at 1, 2, 4, 8 and 24 hrs after treatment with the test substance.

Since the absorption rate at the 4 and 8 hrs after the exposure becomes the basis for calculating the total absorption amount, it must be included.

In order to obtain more reliable results, it is necessary to repeat the test. Some of the differences within each relevant organization are present but it is generally recommended to undergo four repeat tests. Exceptionally, the OECD (7) typically requires four repeated test results but if

there is little variation between the test results, the results of 3 repeated tests are recognized.

Element affecting the result - skin temperature. Changes in the skin temperature have an effect on the absorption process. Therefore, the temperature of the receptor fluid that contacts the skin must be controlled (24). Regulations on the test temperature may differ according to the regulation mechanism. OECD (9), EFSA (7), EFSA, among others, regulate the need to maintain the temperature of human skin to $32 \pm 1^{o}C$, but NAFTA (24), assuming the worst case, regulates to maintain the temperature at $37^{o}C$, the same as the body temperature.

Because the temperature of the management is a factor that largely influences the results of the test, the temperature must be managed explicitly during the course of the experiment. Therefore, appropriate temperature control during the test process must be verified by measuring the skin or receptor fluid temperature by using the proper thermometer and, in addition, humidity does not have to be measured in all experiments, but in an experiment testing the standard, measuring it will increase the reliability of the result.

End of the experiment and calculation of absorption rate. After the test is finished, test substances may still be present in the stratum corneum, epidermis, and dermis and receptor fluid of the skin. At this time, according to the judgment of whether to view the material absorbed in what layer, the absorption rate evaluation may vary widely.

When looking at the preceding studies (27), the amount of test substance present in the stratum corneum, the epidermal layer and the dermis is similar or more than the amount present in the receptor fluid, so it is necessary to provide a precise reference whether to view the amount within the skin tissue as the amount absorbed.

Regarding this, OECD (7) determines the amount of the test substance contained in the epidermal layer and the dermal layer as the absorbed amount but gives no clear guideline whether or not to include the amount in the stratum corneum and other and this is being debated. In some cases, determination is made based on the correlation to the accompanying experiment using human or animal skin. In EFSA (7), they regulate that until the second stripe of the tape strip (28), a method used to remove the stratum corneum, should be excluded since it will soon be eliminated due to the nature of the stratum corneum, and if 75% of the absorbed amount was absorbed within the first half of the visible absorption test period, all the tape strip results can be excluded. Currently, most authorized authorities calculate by "absorbed amount + amount remaining in the treated area tissue + (when necessary) amount remaining in the skin tissue after going through a washing process" when calculating the absorption amount.

Confirmation of the test method and representation of result values. In order to confirm the reliability of results, a process to demonstrate the stability of the test is required, in addition to the confirmation of the absorption rate. Various international standards, as a method to demonstrate the stability of the experiment, despite the scope of accreditation having some slight variation, requires a calculation of the mass balance.

In order to calculate the mass balance, on top of receptor fluid and skin tissue, an applicator that was used to apply the test substance, the recovered sample that was not absorbed, the cleaning solution used in the cleaning process after termination of the experiment and cleaning solution of the receptor chamber will also need to be analyzed. In addition, in order to calculate more exact mass balance, some consideration of analyzing the required sample is worthwhile. OECD (12) regulates that the sum of the analyzed test substance (mass balance) in the above-mentioned specimen must be within 90~110% of the applied amount in the isotope-labeled test and when the test substance is volatile or has no included isotope labeling, it recognizes up to 80~120%. In a recently amended EFSA guidance, when using an isotope-labeled test substance, the value of mass balance needs to be 95~105% of applied (7).

Standard deviation of the repeated test measurement result must be within 25% for its reliance to be recognized (12). When there is a test result that exceeds 25%, the exceeding result can be excluded but the number of re-tests must be increased. As such, when wanting to exclude some results, the reasons need to be clarified and even these excluded results should be included in the report and when the highest result is excluded, the same number must be excluded from the lowest results.

Therefore, in order to avoid re-examination, 2-4 additional repeated tests on top of the minimum number of repeated tests is necessary.

Since the result of skin absorption experiments vary widely according to what manner the result is rounded off to, it is necessary to round using certain criteria. Methods that uniformly apply to the guidelines of international organizations use two significant digits in the results of 10% or more and results from 1 to 9% are represented by 1 significant digit.

Table 3. Rounded value results of the dermal absorption

Result range	Example result	Rounded value	
		OECD	EFSA
Above 10%	10.4%	10%	10%
	15.6%	16%	16%
1 = 9%	1.43%	1%	1%
	2.65%	3%	3%
Below 1%	0.15%	/	0.2%

Table 4. Comparison of dermal absorption test guidelines of OECD, EFSA and EPA for exposure assessment

		OECD (Tiered approach)	EFSA (EU) (Tiered approach)	EPA (Triple pack, in vivo tests are mainly performed)
Animal	Species	Rat (Other species available)		
	Location of skin	Dorsal or ventral	Dorsal, ventral, Femoral	Dorsal or ventral
Human	Preparation of skin	Prefer most human breast, abdomen or leg		
	Reconstructed skin	available	not recommended	/
Common	Thickness of skin	200~400/500 μm		
	age	no influence		
	Test temperature	32.1 ± 1°C		37°C
	Tissue storage	–20°C (Maximum up to 466 days)		
	Dose	Finite		
	Concentration	Neat formulation and Ready-to-Use dilution		
	Volume of test substance	10 mg or 10 μl/cm^2		1 mg/cm^2
	Duration of application	6~8 hrs		Simulate actual environment
	Test duration	Maximum 24 hrs	Maximum 24 hrs	Reasonably adequate duration
	Analysis of stratum corneum	Determined according to the situation	Ready for decision-making procedures	Determined according to the situation
	Recovery	± 10% (or 20%)	± 5%	± 10% (or 20%)
	Washing effect	Evaluated in the absorbed amount		No clear decision

There is a slight difference for results of less than 1% and OECD (12) is currently discussing this, while EFSA (7) generally recommends 1 significant figure. When the results are consistent, it is possible to report as 'below 1%' but it is negative to report a result below 1% by rounding off as 1%. The end result should be performed on these roundings, in case of result value calculation using Triple pack.

Characteristics of each guideline of OECD, EFSA and US EPA. When looking at the contents of each provision from OECD, EU and EFSA related to skin absorption, adaptation of a similar method appears as a whole and differences appear only in details. For specific differences in OECD and EFSA, since they adopt a step-by-step test method, a rating evaluation of absorption rate is possible only with in vitro test results but EPA does not recognize results in this form.

Before conducting the tests on all the test criteria, they present default value that can predict the absorption rate only through looking at the chemical and physical characteristics of the test chemical substances, whereas EPA classifies into two criteria of 10% and 100% based on octanol partitioning coefficient and molecular weight. EFSA additionally adopts a concentration of active ingredient as the standard and even the default value is presented in 3 levels (10%, 25%, 75%), according this standard.

In addition, the difference in the temperature of the experiments is shown while the experiment is being conducted. Whereas OECD, EU and EFSA present 32 ± 1°C, the temperature of human skin, as the reference temperature but NAFTA has a difference in that they offer 37°C, based on the average human body temperature, as the reference temperature (Table 4).

As a result of confirming all guidelines, OECD, EU and EFSA have their own testing standards but are almost similar in contents, showing only the difference in time and through continuous revisions, they are arranging regulations even to detailed aspects of the research. Also, in order to minimize the actual experiment, at the same time of actively applying perdition programs such as QSAR, they offer test standards from in vitro test to in vivo tests so that step-by-step experiments are progressed depending on the results of each test.

EPA test standards, when compared to the standards of OECD, EU and EFSA, relatively applies the test criteria of the worst case and although they are using a prediction program, rather than a phased approach, they tend to show a trend of confirming the absorption rate by conducting all the possible Triple pack tests. Also, if a rationale for the detailed contents can be suggested, depending on the test substance and nature of exposed environment, they show a tendency of applying a method that suits the situation.

PERSPECTIVES

Considering the results of reviewing and comparison of

Table 5. Dermal absorption test methods recommendable and available

Category	Test condition
Device	Static Franze Cell
Skin	Animal : Rat-dorsal skin
	Human: Cadaver skin or those comes from cosmetic surgery or reconstructed human skin
	Non-viable, split thickness (dermatomed) skin (200~400/500 μm) Integrity evaluation: Histological examination, Electrical resistance
Test substance	Dose : Neat formulation and Ready-to-Use dilution, Finite dose
	Radiolabelled or non-Radiolabelled compound available
The duration of study period	Exposure time: at least 6 to 10 hrs
	Sampling time: 1 hr, 2 hr, 4 hr, 8 hr and 24 hr after application
Analysis compartment	Applicator, donor chamber dislodgeable from skin surface, *stratum corneum*, skin preparation, receptor fluid, receptor chamber
Mass balance	Radiolabelled test substance: 90~110% Non-radiolabelled test substance: 80~120%
Number of repeat tests	A minimum of 4 repeat tests per one concentration

each guideline of dermal absorption test for pesticides, it is found that a tiered testing approach is more efficient than the Triple pack approach.

There will be an advantage in terms of economic value to replace the method of analysis with raw material itself rather than radiolabelled substance. OECD guideline accepts analysis methods without radioisotopes when the analysis recovery is within 80~120%. However, in case materials of pesticides are registered for the first time in the country without foreign registration cases, it needs to be considered applying the Triple pack approach, which requires a calculation of the skin absorption ratio from both *in vitro* and *in vivo* studies and the final human absorption ratio is determined from the differences between *in vitro* and *in vivo* (Table 5).

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