INTERNATIONAL JOURNAL OF SOCIAL HEALTH
https://ijsh.ph/index.php/rp
Volume 3 Number 9 September 2024 E-ISSN: 2984-7079

Dermal Acute Toxicity Test

Meizora Aripima Meza[1]*, Ema Hermawati[2]
Universitas Indonesia, Jawa Barat, Indonesia[1,2]
Email: meizora.bia@gmail.com

KEYWORDS
In Vivo, Toxicity Test, Acute Dermal

ABSTRACT
The aim of the dermal acute toxicity test is to identify the intrinsic toxicity of a product by assessing its hazard potential following acute skin exposure. This test also provides preliminary data that can be used to determine the appropriate dosage level and guide the design of subsequent toxicity studies, including determining the LD50 value and information about skin absorption. In this study, female rats (Rattus Nervegicus) are used as test subjects. The test material is prepared by diluting 15 ml of the sample to a final concentration of 994.9 mg/mL. The rats are exposed to the material for 24 hours. Post-exposure, microanatomical observations, and surgery in the abdominal and thoracic areas revealed no abnormalities.

DOI: 10.58860/ijsh.v3i9.240

Corresponding Author: Meizora Aripima Meza*
Email: meizora.bia@gmail.com

INTRODUCTION

The effect of exposure to a substance on humans can be understood by investigating its cumulative effects, toxic, carcinogenic, teratogenic, mutagenic, and other harmful impacts (Sun et al., 2021). Typically, this information is derived from experimental studies using animal models in various toxicity tests, one of which is the dermal acute toxicity test (Hamm et al., 2017). These animal-based toxicity tests provide critical evidence to evaluate the safety of the substance and to anticipate potential risks that human exposure might entail (Berggren et al., 2017).

Animal models are instrumental in assessing biochemical, physiological, and pathological reactions to a substance (McGonigle, 2014). The dermal acute toxicity test, specifically, evaluates toxic effects that may arise immediately after a single exposure via the skin. In this test, groups of animals, usually of one sex, are exposed to the substance at various dosages. The initial dose is chosen based on preliminary tests and is designed to elicit signs of toxicity without causing severe harm or death (Erkekoglu & Kocer-Gumusel, 2018).

The primary objectives of the dermal acute toxicity test are to identify the intrinsic toxicity of a substance, determine hazard information following acute skin exposure, and provide preliminary data that informs the dosage levels and designs of future toxicity studies (Zwickl et al., 2022). Additionally, the test helps establish the LD50 (lethal dose for 50% of the test subjects), classify the substance's toxicity, provide labeling information, and understand skin absorption characteristics.

In the case of Dugstrip Turbo, acute dermal toxicity testing using experimental animals is essential to detect toxic effects that manifest shortly after exposure. This test will not only provide a systemic overview of the product's safety but also generate important data regarding the LD50 value, hazard classification, and absorption through the skin (Strickland et al., 2018). This information is crucial for determining safe dosage levels and designing future toxicity assessments for Dugstrip Turbo.

In summary, previous studies focusing on acute dermal toxicity tests, including tests performed on similar substances, provide a foundational understanding of the procedures and outcomes (Tollefsen et al., 2014). However, this research aims to fill the gap in knowledge specific to Dugstrip Turbo's toxicological profile, particularly its acute dermal toxicity and systemic effects on humans. The study's

novelty lies in expanding current knowledge on the acute toxicity of Dugstrip Turbo, ensuring a more comprehensive risk assessment and providing new data for safety regulation and labeling.

METHOD

The test animal used was a rat (Rattus norvegicus) with 12 female Wistar strains. The animals are caged individually in a room with a temperature of ±24°C with a relative humidity of 30-70%. Lighting is set at 12 hours of light and 12 hours of darkness. Feed and drink are given ad libitum.

The test animals were anesthetized first using ketamine to minimize stress. Ketamine injection through the intra-peritoneal route (Deasy Andani, Lesmana, & Pratiwi, 2022). Animals are shaved in the dorsal area, which is not less than 20% of the surface of the body 24 hours before being given the treatment. Shaving starts from the shoulder blade area to the groin (lumbar bone) and the left and right side halves. The animal is weighed as the starting weight.

The Dugstrip Turbo test material was taken as much as 50mL and then diluted in 100mL so that the concentration of the solution was 50%. The test material was measured using a pycnometer. The specific period is used as the concentration of the sample in the calculation of the volume of the dose (Guérin et al., 2017).

The test doses used were 50 mg/Kg Bb, 200 mg/Kg Bb, 1000 mg/Kg Bb, and 2000 mg/Kg Bb. The volume of test material given to rats assuming a rat weight of 200 grams and a solution concentration of 994.9 mg/mL was:

$$Volume\ Pemberian\ (mL) = \frac{Dosis\ Pemberian\ (\frac{mg}{Kg})}{Konsentrasi\ Sampel\ (\frac{mg}{mL})}\ x\ \frac{Bobot\ Badan\ (g)}{1000}$$

Provision of Test Materials

The test material is presented thinly and evenly on the shaved back area as much as the calculated volume of the give (De Santis, Carozzi, de Felice, & Poggi, 2017). After being presented, the test preparation is covered with porous gauze and non-iratan plaster for 24 hours. After 24 hours, the dressing is opened, and the exposure area is washed with aquaades. The animals were then observed for symptoms of toxicity, and their body weight was weighed daily (Yun et al., 2018). During the administration of the test material (24 hours), the animals are caged individually. After the wrapping is opened, the animals are caged in colonies. Observations were carried out after 24 hours, 48 hours, 72 hours, 7 days, and 14 days after the administration of test materials. After 14 days of observation, the animals were euthanized, and their internal organs were explored.

Categorization of Test Materials

The test material is evaluated based on the onset of toxicity symptoms. The test results in the form of Lethal Dose (LD) values of 50 or dose values that can cause death in 50% of the population are categorized according to GHS values (Development, 2015).

Table 1.
Criteria for Classification of Test Materials

Dosage (mg/Kg BB)	Population Parameters	Category
50	2 out of 3 dead	1
	1 in 3 dead	2
200	No deaths and symptoms of toxicity	
	1 in 3 dead	3
1000	2 out of 3 dead	
	1 in 3 dead	4

2000	2 out of 3 dead	
	1 in 3 dead	5
	No symptoms of toxicity	5/*Unclassified*

Calculation of doses between species

The table of human equivalent doses (HED) based on body surface area between species is as follows:

Table 1.
Human Equivalent Dose Calculation Based on Body Surface Area

Species	Reference Body Weight (kg)	Working Weight Range (kg)	Body Surface Area (m^2)	To Convert Dose in mg/kg to Dose in mg/m^2, Multiply by Km	To Convert Animal Dose in mg/kg to HED in mg/kg, Either: Divide Animal Dose by	Multiply Animal Dose by
Human	60	-	1.62	37	-	-
Mouse	0.02	0.011-0.034	0.007	3	12.3	0.081
Hamster	0.08	0.047-0.157	0.016	5	7.4	0.135
Rat	0.15	0.08-0.27	0.025	6	6.2	0.162
Ferret	0.30	0.16-0.54	0.043	7	5.3	0.189
Guinea Pig	0.40	0.208-0.700	0.05	8	4.6	0.216
Rabbit	1.8	0.90-3.0	0.15	12	3.1	0.324
Dog	10	5-17	0.50	20	1.8	0.541
Monkeys (rhesus)	3	1.4-4.9	0.25	12	3.1	0.324
Marmoset	0.35	0.14-0.72	0.06	6	6.2	0.162
Squirrel Monkey	0.60	0.29-0.97	0.09	7	5.3	0.189
Baboon	12	7-23	0.60	20	1.8	0.541
Micro Pig	20	10-33	0.74	27	1.4	0.730
Mini Pig	40	25-64	1.14	35	1.1	0.946

The calculation of the dose level of human equivalent test (HED) against other species based on the body surface area factor to convert animal and human doses into HED is determined by the Equation:

HED (mg/kg) = Animal Dose (mg/kg) x (Animal Km / Human Km).

RESULT AND DISCUSSION

Observation of Toxic Symptoms and Death

After 24 hours of exposure to the test material, no mice showed symptoms of toxicity and death at doses of 50 mg/Kg Bb, 200 mg/Kg Bb, 1000 mg/Kg Bb, and 2000 mg/Kg Bb, so there was no need for further testing. The skin exposed to the test material also did not change. Observation continued for 48 hours, 72 hours, day 7, and day 14. In follow-up observation, no mice were found to experience symptoms of toxicity and death at all doses tested. Documentation of the test material exposure area is attached to Appendix 2.

Weight and Macroanatomical Observations

The body weight of the rats decreased in the first few days after the treatment (Figure 1). This can occur because the rats experience stress conditions due to the installation of porous gauze and non-irritating plaster as well as individual cages for 24 hours. After the stress period passed, the animal's weight increased again according to the growth phase of the rats (Demirci & Sahin, 2019).

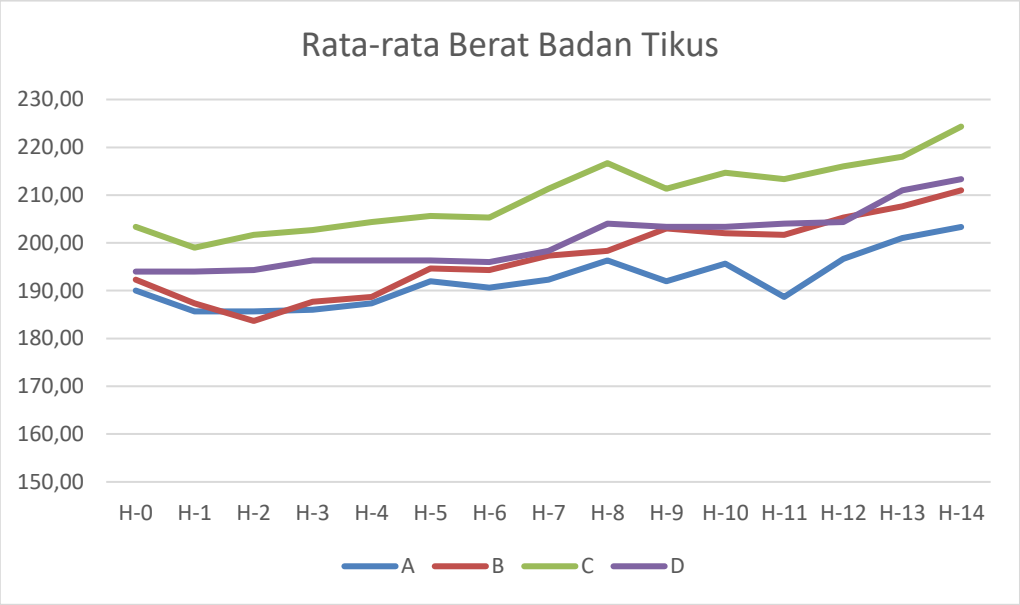

Figure 1. The average weight of the test rats was 14 days. A: Dose 50mg/kgBB, B: Dose 200 mg/kgBB, C: Dose 1000mg/kgBB, D: Dose 2000mg/kgBB.

After 14 days of observation, the animals were euthanized in accordance with animal welfare protocols, followed by surgical examination of the abdominal and thoracic areas. Organs in these regions were carefully observed for potential changes resulting from toxin exposure (Hernández-López et al., 2024). The microanatomical observations of the test mice revealed no abnormalities. These findings are consistent with previous studies by [Author, Year], which also reported no significant microanatomical changes under similar exposure conditions, suggesting that the tested toxin does not induce noticeable organ damage at the cellular level. A detailed microanatomical description is provided in Appendix 1.

CONCLUSION

The results of the acute toxicity test of dermal Dugstrip Turbo 50% on the skin of rats in the four dose groups, namely group A 50mg/kgBB, group B 200mg/kgBB, group C 1000mg/kgBB and group D 2000mg/kgBB did not show any harm due to direct exposure to the skin. The results of macroanatomical necropsy on the organ did not show any abnormalities; according to GHS in the OECD, The LD50 value of Dugstrip Turbo products is categorized as Unclassified/Category 5 where the parameters do not have symptoms of toxicity.

Based on the predetermined dose test group, it can be equated to the human equivalent dose (HED) namely, Group A 50mg/kg obtained HED is 8.1 mg/kg, Group B 200mg/kg obtained HED is 32 mg/kg, Group C 1000mg/kg obtained HED is 162 mg/kg, Group D 2000mg/kg obtained HED is 323 mg/kg. The results of the dermal acute toxicity test can be implicated in humans, this is based on the fact that the tests carried out on the selected test animals are indeed more sensitive than humans. It's just that in the claim for safety in humans, limited clinical trial steps in humans are needed.

REFERENCES

Berggren, E., White, A., Ouedraogo, G., Paini, A., Richarz, A.-N., Bois, F. Y., … Mahony, C. (2017). Ab initio chemical safety assessment: A workflow based on exposure considerations and non-animal methods. *Computational Toxicology*, 4, 31–44. Retrieved from https://doi.org/10.1016/j.comtox.2017.10.001

De Santis, S., Carozzi, F. G., de Felice, G., & Poggi, C. (2017). Test methods for Textile Reinforced Mortar systems. *Composites Part B: Engineering*, 127, 121–132. Retrieved from https://doi.org/10.1016/j.compositesb.2017.03.016

Deasy Andani, G. A. S., Lesmana, R., & Pratiwi, Y. S. (2022). The Role of Herbs Supplementation in Modulating Injury-induced Inflammatory Response in Skeletal Muscle: A Systematic Review. *The Natural Products Journal*, 12(7), 62–72.

Demirci, T., & Sahin, E. (2019). The effect of chronic stress and obesity on sperm quality and testis histology in male rats; a morphometric and immunohistochemical study.

Development, O. for E. C. and. (2015). *Test No. 404: acute dermal irritation/corrosion*. OECD Publishing.

Erkekoglu, P., & Kocer-Gumusel, B. (2018). Toxicity assessment of nanopharmaceuticals. In *Inorganic Frameworks as Smart Nanomedicines* (pp. 565–603). Elsevier. Retrieved from https://doi.org/10.1016/B978-0-12-813661-4.00013-4

Guérin, G., Christophe, C., Philippe, A., Murray, A. S., Thomsen, K. J., Tribolo, C., … Lahaye, C. (2017). Absorbed dose, equivalent dose, measured dose rates, and implications for OSL age estimates: Introducing the Average Dose Model. *Quaternary Geochronology*, 41, 163–173. Retrieved from https://doi.org/10.1016/j.quageo.2017.04.002

Hamm, J., Sullivan, K., Clippinger, A. J., Strickland, J., Bell, S., Bhhatarai, B., … Allen, D. (2017). Alternative approaches for identifying acute systemic toxicity: Moving from research to regulatory testing. *Toxicology in Vitro*, 41, 245–259. Retrieved from https://doi.org/10.1016/j.tiv.2017.01.004

Hernández-López, V., Reyes, R., García-Álvarez, N., Real, F., Díaz-Marrero, A. R., & Fernández, J. J. (2024). Changes at small intestine induced by food-fish contaminated with ciguatoxins. *Ecotoxicology and Environmental Safety*, 282, 116741. Retrieved from https://doi.org/10.1016/j.ecoenv.2024.116741

McGonigle, P. (2014). Animal models of CNS disorders. *Biochemical Pharmacology*, 87(1), 140–149. Retrieved from https://doi.org/10.1016/j.bcp.2013.06.016

Strickland, J., Clippinger, A. J., Brown, J., Allen, D., Jacobs, A., Matheson, J., … Casey, W. (2018). Status of acute systemic toxicity testing requirements and data uses by U.S. regulatory agencies. *Regulatory Toxicology and Pharmacology*, 94, 183–196. Retrieved from https://doi.org/10.1016/j.yrtph.2018.01.022

Sun, K., Song, Y., He, F., Jing, M., Tang, J., & Liu, R. (2021). A review of human and animals exposure to polycyclic aromatic hydrocarbons: Health risk and adverse effects, photo-induced toxicity and regulating effect of microplastics. *Science of The Total Environment*, 773, 145403. Retrieved from https://doi.org/10.1016/j.scitotenv.2021.145403

Tollefsen, K. E., Scholz, S., Cronin, M. T., Edwards, S. W., de Knecht, J., Crofton, K., … Patlewicz, G. (2014). Applying Adverse Outcome Pathways (AOPs) to support Integrated Approaches to Testing and Assessment (IATA). *Regulatory Toxicology and Pharmacology*, 70(3), 629–640. Retrieved from https://doi.org/10.1016/j.yrtph.2014.09.009

Yun, J.-W., Kwon, E., Kim, Y.-S., Kim, S.-H., You, J.-R., Kim, H.-C., … Jang, J.-J. (2018). Assessment of acute, 14-day, and 13-week repeated oral dose toxicity of Tiglium seed extract in rats. *BMC Complementary and Alternative Medicine*, 18, 1–12.

Zwickl, C. M., Graham, J. C., Jolly, R. A., Bassan, A., Ahlberg, E., Amberg, A., … Myatt, G. J. (2022). Principles and procedures for assessment of acute toxicity incorporating in silico methods. *Computational Toxicology*, 24, 100237. Retrieved from https://doi.org/10.1016/j.comtox.2022.100237