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To Design and Develop a Biogel Containing *Centella asiatica* Extract-Loaded Silver Nanoparticles and Assess Its *Invivo* Toxicity Profile

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ABSTRACT

Aim: To perform a phytochemical analysis of a biogel containing Centella Asiatica extract-loaded silver nanoparticles and evaluate its *invivo* toxicity profile through dermal toxicity and dermal irritation studies. **Methods:** The primary goal is to synthesize silver nanoparticles, incorporate them into a biogel formulation, and assess the biogel's phytochemical characteristics using various advanced techniques. The synthesized silver nanoparticles were recovered by centrifugation and characterized using UV-Vis spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD). For the *in vivo* toxicity evaluation, two key studies were performed like acute dermal toxicity in Sprague Dawley rats and Acute dermal irritation/corrosion study in New Zealand White rabbits. **Results:** Silver nanoparticles (AgNPs) have been effectively attained from bioreduction of silver nitrate solutions with *Centella asiatica* leaf extract. Silver nanoparticles have been characterized using UV-vis spectroscopy, SEM, TEM and XRD analysis. Results denoted *Centella asiatica* leaf extract to be a better reducing agent and exposed the efficient capping and stabilization properties of these AgNPs. Further, *in vivo* studies were performed and no evidence of acute dermal toxicity or significant irritation in the animals treated with biogel. **Conclusion:** The biogel containing Centella Asiatica extract-loaded silver nanoparticles demonstrated promising phytochemical properties, with no evidence of acute dermal toxicity or significant irritation. These results recommended that the biogel is safe for dermal application, supporting its potential use in topical therapeutic products. Further studies will be conducted to prove the long-term safety and efficacy of the wound healing effect.

Keywords: Phytochemical analysis, extraction, synthesis, silver nanoparticles, toxicity.

1. INTRODUCTION

Medicinal plants are the traditional source of pharmaceutically significant compounds which are established by the pharmaceutical companies for the preparation of several formulations. Currently, there is a notable rise in the global utilization of herbal products. The World Health Organization (WHO) has reported that over 80% of the global population depends on herbal medicines [1]. The significance of medicinal plants lies in their unique chemical combinations that produce physiological effects on the human body. These bioactive constituents contain saponins, flavonoids, alkaloids, sterols, tannins, and phenols. The country is home to approximately 426 biomes, each contributing to a diverse array of habitats that serve as some of the richest centers for plant genetic resources globally [2]. Among the 18,665 flowering species, only around 3,000 have been utilized in various formulations within traditional medical systems similar to Ayurveda, Siddha, and Unani [3].

Centella asiatica is one of the traditional therapeutic plant belongs to family Apiaceae (previously recognized as Umbelliferae) and generally known as ‘Gotu kola’, ‘Indian Pennywort’ or ‘Mandookaparni’ in India. Centella asiatica was used as a perennial medicinal herb found thousands of centuries before in the tropical and subtropical countries like India, China, Sri Lanka, Nepal, Bangladesh and Madagascar [4].

Centella asiatica had a glabrous stem and lengthy petiolated fleshy leaves rooting at nodes. It stands a softly perfumed plant that reaches height up to 15cm. Stem remains smooth and rooting occurs at the nodes. It propagates extensively in damp, marshy, and wet places and flowering happens during April to June with white to purple or pink flowers. In India, it grows up to an altitude of 600-1800 meters above the sea level on moist, clayey or sandy soils making a dense green carpet [5].

Centella asiatica develops well at monsoon in well drained substratum. The nodal sections of this creeping plant grow roots for stability, while long petiolate leaves arise from these nodes. The optimal temperature for its growing is approximately 25 ºC ± 3 ºC. The extreme demand for this plant in the market stances a risk of gradual depletion of natural resources in the future. Additionally, these plants are susceptible to common threats such as climate change and habitat destruction [6].

Centella asiatica is recognized for its numerous beneficial properties, including antileprotic, antifeedant, antistress, and anti-tuberculosis effects, as well as its wound-healing capabilities, antibacterial and fungicidal activities related to atherosclerosis [7].

Figure 1: Centella Asiatica Plant

Due to its medicinal significance, Centella asiatica is similarly employed in the combination of silver nanoparticles. The presentation of nanoparticles is expanding fastly due to their unique features, including size, morphology, and distribution. These nanoparticles find utility across diverse sectors such as healthcare, biomedical applications, environmental health, cosmetics, food and feed industries, mechanics, optics, electronics, space technology, energy science, chemical industries and catalysis [8].

Naturally, nanoparticles remain synthesized through various physical and chemical methods, which may pose environmental hazards. The development of biologically inspired processes represents a significant area within nanotechnology. The advantages of synthesizing nanoparticles from biological sources, as opposed to chemical and physical methods, include cost-effectiveness, eco-friendliness, and the potential for large-scale production. Utilizing plant extracts for nanoparticle synthesis offers additional benefits by simplifying the process and eliminating the need for complex cell culture maintenance [9].

Table 1: Botanical Classification of Centella Asiatica		
Kingdom	:	Plantae
Division	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Apiales
Family	:	Apiaceae
Genus	:	Centella
Species	:	*Centella asiatica*

2. MATERIALS AND METHODS

2.1 Test Species

Species	:	Rat
Strain	:	Sprague Dawley Rats
Sex	:	Females
Age	:	~8-10 weeks (At the time of dosing)
Rational for selection	:	Rat is one of the suggested species for conducting acute toxicity study among rodents.
Number of Groups and Animals	:	Dose Range Finding Study – 1 animals. Main Study – 2 animals
Species	:	Rabbit
Strain	:	New Zealand White
Sex	:	Male
Rational for selection	:	As per OECD TG 404, rabbits are one of the recommended test species for conducting acute dermal irritation/corrosion test.
Age	:	~3-4 months (at the time of treatment)
Number of Animals	:	Three animals Initial Test - 1 animal Confirmatory Test - 2 animals

2.2 Preparation of Biogel:

Preparation of Plant Extract

The plant materials that had been powdered were used to make the extracts. 10 g of the powder were mixed with 100 mL of double-distilled water and taken for a boil in a water bath for 20 minutes. Following that, Whatman no. 1 filter paper was used to filter the extracted mixture. The materials were centrifuged for 20 minutes at 6000 rpm. After centrifugation, the samples were put into autoclaved vials and kept cold (4°C) for further examination.

Preparation of silver nanoparticle powder

Using refrigerated centrifuges, the Ag-NP solution was centrifuged. For 10 minutes, the pellet was processed at 8000 rpm. Afterward, it was taken out and given two washing with distilled water. Next, the pellet was dried at 60ºC after it had been cleaned. Ultimately, the powdered nanoparticles were collected and stored in an airtight Eppendorf tube.

Preparation of Aqueous Silver Nitrate

A solution of 1 mM silver nitrate was made and kept in an amber-colored bottle.

Optimization and Synthesis of Silver Nanoparticles

Different concentrations of Centella asiatica leaf extracts (2ml, 4ml, 6ml, 8ml, and 10ml) were prepared individually. To each extract, 1ml of 1mM silver nitrate solution was added, followed by continuous stirring and direct boiling. The solution's color was monitored at regular intervals. A transition in color from yellow to dark brown in the leaf extract signifies the successful synthesis of silver nanoparticles from the leaves. The Surface Plasmon Resonance characteristics of the silver nanoparticles were analyzed using UV-Visible absorption spectroscopy.

Production and Recovery of Silver Nanoparticles by Centrifugation

1ml of of Centella asiatica leaf extract demonstrates the presence of nanoparticles. Subsequently, this extract was selected for large-scale production. Following this, 10 mL of the leaf extract were combined with 100 mL of 1 mM silver nitrate. After the bioreduction process, the resulting solution containing hydrosols of silver nanoparticles was centrifugation at 10,000 rpm for 30 minutes. The resulting pellet was then dissolved in 0.1 mL of ethanol and allowed to air dry.

Preparation of Biogel

Incorporate AgNPs into a sodium alginate gel. Optimize the concentration of Centella Asiatica extract and AgNPs.

2.3 Phytochemical Analysis

The qualitative analysis of Centella asiatica was performed to determine the presence of secondary metabolites.

Characterization of Silver Nanoparticles by UV-Visible Spectroscopy

UV-Visible spectrophotometer serves as an effective tool for observing the synthesis of silver nanoparticles. The process of Surface Plasmon Resonance results in quantized oscillations of surface charge within AgNPs, which produce a pronounced absorption peak within the 450-480 nm wavelength range. For this analysis, the UV-Visible spectrophotometer was employed. The absorption observed in this specific range provides a direct quantitative assessment of the concentration of the synthesized nanoparticles. To track the bio-reduction of Ag^+ ions, regular 1 ml aliquots of the sample were collected for analysis.

Characterization of Silver Nanoparticles by Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was performed to determine the size and shape of the silver nanoparticles that were biosynthesized from the sample. Thin films of the sample were created on a carbon-coated copper grid by depositing a minimal quantity of the sample onto the grid, followed by the removal of excess solution with blotting paper. Subsequently, the film on the SEM grid was permitted to dry, and images of the nanoparticles were captured. The images included annotations detailing the applied voltage, magnification, and the dimensions of the observed features.

Characterization of Silver Nanoparticles by Transmission Electron Microscopy

Transmission Electron Microscopy (TEM) is a technique utilized to create images of a sample by exposing it to electron radiation in a vacuum environment. Dried films of nanoparticles were prepared for analysis using TEM. In this process, a stream of electrons was directed through the sample, resulting in an image formed from the interaction between the sample and the electrons. This image was then enhanced and transmitted to an imaging device for additional examination. The image produced by the objective lens was further magnified through one or two additional stages using intermediate and projector lenses before being projected onto a photographic plate.

Characterization of Silver Nanoparticles by X-Ray Diffraction (XRD)

A completely dried powder of AgNPs was obtained by utilizing a hot air oven, and diffraction studies were conducted using a D8 Advanced Bruker X-ray diffractometer equipped with a Cu Kα (1.54 Å) source. This analytical technique is employed for phase identification and for ascertaining the dimensions of the unit cell.

2.4 Study Procedure

Dermal Toxicity: The study were executed in two stages that is dose range finding study then main study. The range finding study was conducted with one animal and the main study was conducted with two animals.

Phase of the Treatment	**Dose (mg/kg body weight)**	**No. of Animals**
Dose Range Finding Study	2000	1
Main Study	2000	2

Dermal Irritation: The study was conducted in two segments that is initial test and confirmatory test. Initial test was performed using one rabbit. Since no corrosive effect and severe irritant effect is detected in initial test, the confirmatory test was executed simultaneously and no corrosive effect or severe irritant result is detected in confirmatory test.

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Treatment	Quantity of biogel Applied (g)	Initial Test		Confirmatory Test			
		Left Trunk Region	Right Trunk Region	Left Trunk Region	Right Trunk Region	LeftTrunk Region	Right Trunk Region
		Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Untreated Control	NA	Yes(4hours)	No	Yes(4 hours)	No	Yes(4 hours)	No
CentellaAsiatica Biogel	0.5	No	Yes(4 hours)	No	Yes(4 hours)	No	Yes(4 hours)

Preparation of Animals

One day before biogel application, hair was removed (at least 10% of body surface area) from dorsal flank region of the randomized animals by carefully clipping using electrical clipper. Care was taken to elude the abrading of skin while clipping. The weight of the animal was taken into account for the removal of hair and on the dimensions of covering.

Frequency and Route of Administration:

The biogel was administered as a single dose. Route of administration was through dermal exposure as it is the possible route of exposure of biogel to humans.

2.5 Application of the Biogel Procedure

Dermal Toxicity: The biogel was applied in a step-wise manner to animals. The required quantity of biogel (based on the animal body weight) was applied as equally as conceivable over the exposed region of dorsal clipped skin. Biogel was allowed to held in interaction with the skin with the help of a absorbent gauze dressing and non-irritating adhesive tape throughout a 24-hour contact period. The application region was additional covered in a appropriate manner to maintain the gauze covering and biogel and make sure that the animals cannot swallow the biogel. Throughout the 24-hour contact period, the animals were housed in individual cages in order to evade oral ingestion of the biogel by additional animals in the cage. At the completion of the contact period, enduring biogel was removed by swabbing with excess water.

$$\text{Quantity of biogel Applied (mg)} = \frac{\text{Body weight (g) x Dose (mg/kg B.w)}}{1000}$$

$$\text{Quantity of biogel Applied (mg)} = \frac{201.98 \times 2000}{1000} = 403.96 \text{ mg}$$

Dermal Irritation: The biogel was applied to a lesser area (~ 6 cm^2) of skin. For application, 0.5 gram of biogel was applied on the application site, covered with cotton gauze and detained in place with non-irritating tape. The patch was lightly held in contact with the skin by means of a appropriate gauze using adhesive tape for the period of the exposure. It was confirmed that the biogel was adhered to the skin in such a way that there remained good contact and uniform spreading of the biogel on the skin.

Grading of Skin Reactions

Table 2: Grading of Skin Reactions	
1. Erythema and Eschar Formation	**Score**
➢ No erythema	0
➢ Very slight erythema (barely perceptible)	1
➢ Well defined erythema	2
➢ Moderate to severe erythema	3
➢ Severe erythema (beet redness) to eschar formation preventing grading of erythema	4
2. Oedema Formation	**Score**
➢ No oedema	0
➢ Very slight oedema (barely perceptible)	1
➢ Slight oedema (edges of area well defined by definite rising)	2
➢ Moderate oedema (raised approximately 1 millimetre)	3
➢ Severe oedema (raised more than 1 millimetre and extending beyond area of exposure)	4

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3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The qualitative analysis of Centella asiatica illustrates the presence of bioactive compounds like alkaloids, carbohydrates, saponins, steroids, proteins and glycosides.

UV-Visible Absorption Spectroscopy

The initial formation of CANPs was evidenced by a color change in the plant sample upon the addition of a 2:1 ratio of AgNO3, resulting in a brownish-yellow solution that remained stable, suggesting that the nanoparticles did not aggregate and were well-dispersed in the solution. The phenomenon of Surface Plasmon Resonance, characterized by the oscillation of electrons, produced a peak at 430 nm during the bioreaction, thereby confirming the successful synthesis of silver nanoparticles, with the spectrophotometric investigation concluding after 8 hours.

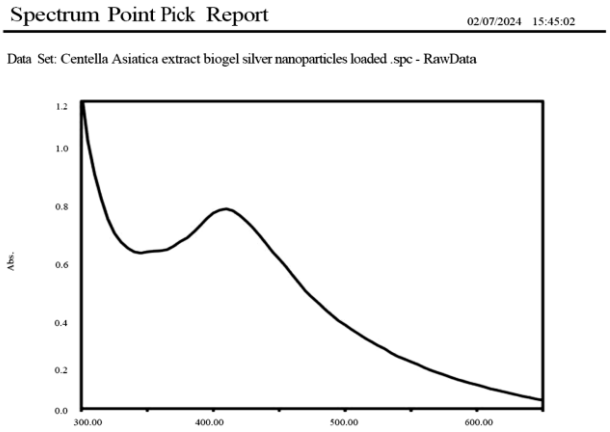

Figure 2: UV-Vis spectroscopy of silver nanoparticles

Scanning Electron Microscopy (SEM)

SEM analysis was conducted on the CANPs, and the resulting images distinctly indicate that they possess a spherical morphology at the room temperature during synthesis. The observed size range varied from 30 to 50 nm in diameter.

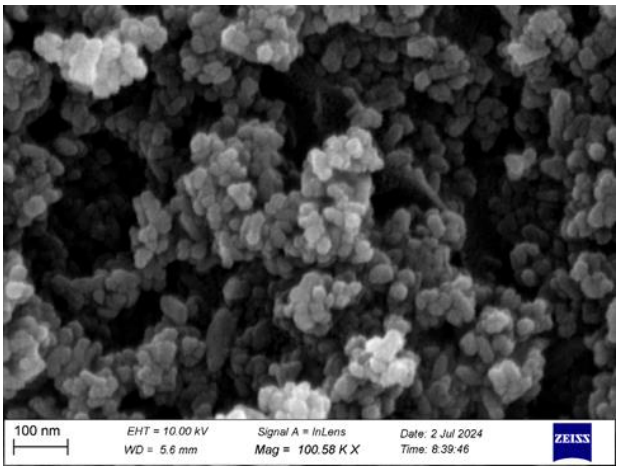

Figure 3: SEM analysis of silver nanoparticle

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Transmission Electron Microscopy (TEM)

TEM offered valuable information regarding the morphology and particle size distribution of the silver nanoparticles. The analysis confirmed the formation of spherical silver nanoparticles within the reaction mixture. The smaller nanoparticles demonstrated an ability to readily traverse the membrane. A TEM examination of the CANPs revealed a diverse size distribution, predominantly ranging from 8-15nm and 26-35nm. In instances where the nanoparticles exhibited a tapered shape, their dimensions were measured to be between 4.2-8.8nm in radial diameter.

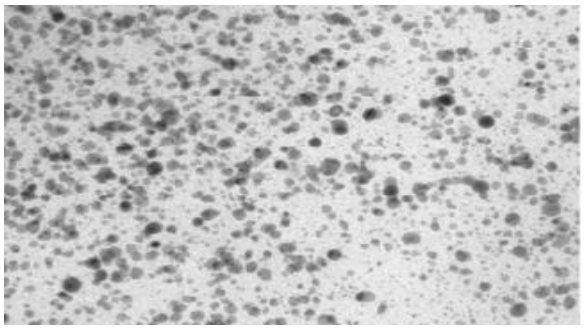

Figure 4: Transmission electron micrograph of silver nanoparticles C. Asiatica

X-Ray Diffraction (XRD)

This investigation identified the orthorhombic structure of silver nanoparticles. The crystalline characteristics of the purified solid CAgNPs were analyzed using Powder X-ray Diffraction (XRD). **Figure 5** illustrates the XRD pattern of CAgNPs synthesized with C. asiatica extracts. Several prominent diffraction peaks are observed at 2θ values of 27.84, 32.25, 46.26, 54.8, 57.52, 67.50, 74.51, and 76.80, which correspond to the (111), (200), (220), (311), (322), (400), (313), and (402) interplanar reflections of a cubic structure, respectively. The lattice parameters align with the standards provided by the Joint Committee on Powder Diffraction Standards, while the broadening of the diffraction peaks indicates the presence of small crystallite sizes. The average particle size was calculated using the Debye–Scherer formula [$D = k\lambda/\beta\cos(\theta)$], where D represents the average crystal size, k is the Scherer coefficient (0.891), λ is the X-ray wavelength (λ = 1.5406 Å), θ is Bragg's angle (2θ), and β is the full width at half maximum intensity (FWHM) in radians. According to the Scherer equation, the average particle size of CAgNPs is approximately 31 nm, which corroborates the findings from the Transmission Electron Microscopy (TEM) analysis.

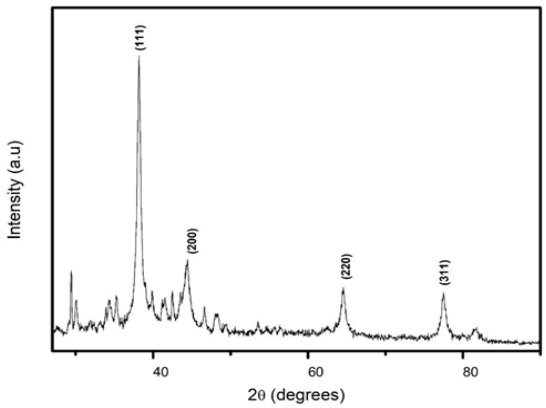

Figure 5: X-ray diffraction (XRD) pattern of silver nanoparticles

Dermal Toxicity Observations

In dermal toxicity experimental animals, no mortality/morbidity was detected throughout the experimental period. All the animals were found to be normal, and no clinical signs of toxicity were detected. No treatment related effect on body weight and body weight changes were observed in all the animals during the experimental period. No skin reactions were observed at 24, 48 and 72 hours observation after patch removal in both range finding study and main study animals. Gross pathological inspection of the animals conducted did not reveal any macroscopic lesions.

Dermal Irritation Observations

In dermal irritation experimental animals, no mortality/morbidity and abnormal clinical signs was observed during the experiment period. No treatment related adverse effects on body weight was observed throughout the experiment period. Application of the biogel to the intact skin of rabbits were not produced any skin reactions of erythema, and oedema formation in both initial and confirmatory test until 72 hours after patch removal.

4. CONCLUSION

This study highlights the potential of Centella asiatica as a ecological source for the green synthesis of silver nanoparticles (AgNPs). The bioreduction of silver nitrate by the Centella asiatica leaf extract delivers eco-friendly methods to produce AgNPs, with favorable applications in nanotechnology and biomedical fields. These nanoparticles were characterized through UV-vis spectroscopy, SEM, TEM, and XRD analyses. The findings indicated that Centella asiatica leaf extract is an efficient reducing agent, with strong capping and stabilization properties for the AgNPs. A key outcome of the study is the potential to develop value-added products from Centella asiatica for applications in biomedical and nanotechnology industries. Additionally, in vivo studies were conducted to provide scientific insights into the medicinal properties of the plant.

For dermal toxicity, the acute dermal LD50 value of the biogel containing Centella asiatica extract-loaded AgNPs in Sprague Dawley rats was found to be greater than 2000 mg/kg body weight, indicating low toxicity. Regarding dermal irritation, under the conditions and doses tested, the biogel was classified as non-irritant after a single topical application to the skin of male New Zealand White rabbits, with observations carried out over a 72-hour period.

5. REFERENCES

1. Jantan, I., Bukhari, S. N. A., Mohamed, M. a. S., Wai, L. K., & Mesaik, M. A. (2015). The Evolving Role of Natural Products from the Tropical Rainforests as a Replenishable Source of New Drug Leads.
2. Singh, J., & Yadav, A. N. (2020). *Natural Bioactive Products in Sustainable Agriculture*.
3. Schultes, R. E., & Von Reis, S. (2008). *Ethnobotany: Evolution of a Discipline*.
4. Rostagno, M. A., & Prado, J. M. (2015). *Natural Product Extraction: Principles and Applications*. Royal Society of Chemistry.
5. Rao, Sobana, S., & Alagumanian, S. (2021). *In Vitro Studies on Centella Asiatica (L.) Urban, An Ayurvedic Medicinal Herb*. JPS Scientific Publications, India.
6. Duke, J. A. (2017). *Handbook of Phytochemical Constituent Grass, Herbs and Other Economic Plants: Herbal Reference Library*. Routledge.
7. Verma, D., Macwan, D., Patel, J. D., Parmar, S. R., & Patel, H. V. (2022). Herbs that heal: Role of traditional herbal remedies as an immunity booster and effective against the infectious and systemic diseases. *Annals of Phytomedicine an International Journal*.
8. Karthik, L., Kirthi, A. V., Ranjan, S., & Srinivasan, V. M. (2019). *Biological Synthesis of Nanoparticles and Their Applications*. CRC Press.
9. Thomas, S., Thomas, R., Zachariah, A. K., & Mishra, R. K. (2017). *Spectroscopic Methods for Nanomaterials Characterization*. Elsevier.
10. Ayache, J., Beaunier, L., Boumendil, J., Ehret, G., & Laub, D. (2010). *Sample Preparation Handbook for Transmission Electron Microscopy: Methodology*. Springer Science & Business Media.
11. Echlin, P. (2011). *Handbook of Sample Preparation for Scanning Electron Microscopy and X-Ray Microanalysis*. Springer Science & Business Media.
12. Li, J., Zhang, M., Li, B., Monteiro, S. N., Ikhmayies, S., Kalay, Y. E., Hwang, J., Escobedo-Diaz, J. P., Carpenter, J. S., & Brown, A. D. (2020). *Characterization of Minerals, Metals, and Materials 2020*. Springer Nature.
13. Acute Dermal Irritation/Corrosion OECD Test Guideline No 404 Adopted: 28th July 2015.
14. CCSEA Guidelines for Laboratory Animal Facility.
15. OECD Guidelines for the Testing of Chemicals Test No. 402: Acute Dermal Toxicity, OECD Guidelines for the Testing of Chemicals, Section 4, Adopted: 09 October 2017.

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Conflict of Interest Statement: All authors have nothing else to disclose.