# EVALUATING THE ACUTE DERMAL TOXICITY AND SKIN IRRITATION OF THE TEMEPHOS IMPREGNATED CELLULOSE NANOFIBER INTENDED FOR MOSQUITO LARVICIDE

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***Abstract***

Cellulose nanofiber (CNF) derived from kenaf bast fiber has the potential to be used as a biodegradable and renewable larvicide nanocarrier due to its excellent physicochemical properties. However, acute dermal toxicity testing of CNF with high aspect ratios and fibrous morphology is limited even though its exposure to the skin is possible. Herein, this study aims to investigate the skin irritation effect and acute dermal toxicity of kenaf CNF (KCNF 2.3 % w/v) & kenaf CNF impregnated with temephos (KCNF+T 2.3 % w/v) following OECD test guideline 402. Female Sprague Dawley rats were exposed with KCNF 2.3 % w/v and KCNF+T 2.3 %w/v, respectively, at a dose level of 2000 mg (kg body weight)$^{-1}$ for 24 hours followed by 14 days of observation for skin irritation effect, mortality, abnormal behaviours, and clinical signs of toxicity. Our result indicated that no skin irritation effect, treatment-related mortality, and abnormal behaviours in both exposed groups. However, KCNF+T 2.3 % w/v treated rats showed mild cholinergic signs of toxicity compared with the absence of clinical signs of toxicity in KCNF 2.3 % w/v treated rats. The KCNF 2.3 % w/v and KCNF+T 2.3 % w/v were non-skin irritants with the $LD_{50}$ value > 2000 mg (kg body weight)$^{-1}$ and therefore classified as non-hazardous chemicals of Category 5 according to the GHS system.

***Keywords:*** Cellulose Nanofiber; Temephos; Skin Irritation; Acute Dermal Toxicity; Lethal Dose

## *Introduction*

Cellulose is a basic component of the fibril structure from plant cell walls and has excellent swelling capability, high surface area, and flexibility (1). Nanocellulose is a term referring to cellulose materials from a plant (e.g kenaf), broken into smaller sizes at 1-100 nm in range and can be produced into two types, namely cellulose nanofiber (CNF) and cellulose nanocrystal (CNC), depending on the synthesis methods (2-4). Nanocellulose has been widely recognised as an environmentally friendly carrier in the biomedicine field (5-8), and recently, it has been reported to be used as a larvicide carrier (9, 10).

As a larvicide nanocarrier, the nanocellulose has been found to has high pesticide loading capacity, improve pesticide dissolution rate and enhanced the bioavailability of the active ingredient to target organisms due to its nano physicochemical properties (9, 10). In addition, United Nations has recommended that larvicide formulation to be environmental-friendly whereby it should be from renewable sources and low toxicity to non-target organisms in moving towards sustainable development through health and well-being goal (11). Therefore, nanocellulose has been recommended as a suitable candidate for renewable larvicide carriers (9, 10). While cellulose nanomaterials (CNM) are emerging in their field and provide an alternative material replacement for sustainable development, there is a

potential human health risk from the exposure to nanocellulose either from raw materials during processing or as end product regardless of any route of entry to the human body (12).

In terms of CNF toxicity, there is still limited information to conclude its safety, whereby various toxic effects were reported, ranging from no significant toxic effect to inflammatory response (13-15). Although exposure of CNF to the skin is likely to occur since the skin is the largest organ of mammals, no study on the acute dermal toxicity of CNF has been reported to date. Skin serves as a protective barrier for the underlying organs, and thus it is directly exposed to toxicants from the environment, including kenaf CNF (KCNF) and kenaf CNF impregnated with temephos KCNF+T (16). Therefore, the acute dermal toxicity was carried out based on the potential application of KCNF+T to be used in water storage containers for mosquito control. The public may use the water for domestic purposes such as bathing and washing where dermal exposure against the KCNF and KCNF+T likely to occur and probably resulted in adverse effects.

In the present study, the skin irritation and acute dermal toxicity of KCNF and KCNF+T were investigated to determine the skin irritation category, median lethal dose ($LD_{50}$) value and acute toxicity hazard class category. The study was carried out using OECD test guideline 402 Acute Dermal Toxicity-Fixed Dose Procedure (17). Subsequently, the acute toxicity class category of the skin irritation and acute dermal toxicity of the KCNF and KNCF+T were determined using Globally Harmonized System (GHS) for chemical classification (18).

## *Materials and Methods*

### *Materials*

The KCNF was obtained from Nanotechnology and Catalysis Research Center (NANOCAT), Universiti Malaya (19). The physicochemical properties of the KCNF such as fibre morphology, particles size distribution, functional group, crystallinity index, specific surface area and particle stability was carried out using field emission scanning electron microscope (FESEM), transmission electron microscopy (TEM), Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), Brunauer–Emmett–Teller (BET), and Zeta potential, respectively, and has been described earlier (20). The KCNF+T preparation method also has been explained elsewhere (10).

### *Methods*

The study was carried out following OECD test guideline 402 Acute Dermal Toxicity-Fixed Dose Procedure, complied with Directive 2010/63/EU (17). The method comprised of two sequential steps which were the dose range-finding step (Step 1) and the main confirmation step (Step 2) with two endpoints namely skin irritation effect and acute dermal toxicity. Animal ethical approval was obtained from USM Animal Ethics Committee, approval number USM/IACUC/2019/(119)(1016) prior to initiation of the study.

#### *Sample preparation*

The KCNF and KCNF+T samples were prepared by dispersed 0.2 g of dry KCNF and KCNF+T in 8.88 ml distilled water, resulted in a 2.25 % w/v concentration (~2.3 % w/v). Subsequently, the samples were sonicated for 90 minutes at 50 % amplitude setting using a probe sonicator to ensure sample homogenization and remove the particle aggregation. The samples were prepared as described in article published by Pengiran et. al in 2021 (20). The impregnated temephos at this concentration is stable as reported in the previous study (10) while allows higher safety risk threshold evaluation of the KCNF+T because the commercial larvicide product contains 1% w/w temephos.

#### *Animal selection, dose preparation and administration*

A total of six healthy female young rats (Rattus norvegicus) of Sprague Dawley (SD) strain (8-10 weeks old) with intact skin, nulliparous, and non-pregnant was utilized in this study. The rats were supplied by Animal Research Centre, Advanced Medical and Dental Institute (AMDI), USM Penang. The weight variation of rats used was minimal and not exceeded ± 20 % of the total mean weight of the rats (15). The rats were acclimatized for 7 days at a temperature ranging from 19 to 25 °C, relative humidity (RH) 50 to 60 % and photoperiod cycle of 12h/12h light-dark. The rats were fed with conventional grain pellets with an unlimited supply of drinking water and were group-caging for welfare reasons in polypropylene cage with corn cob as bedding material. The rats were divided into two treatment groups (KCNF 2.3 % w/v and KCNF+T 2.3 % w/v) comprising three rats in each group. The control group was not carried out considering animal welfare reasons to reduce the number of animals used in acute toxicity testing, which is consistent with OECD test guideline 402 procedure (17, 22).

The dose was prepared according to the intended dose corresponding to the rats' body weight. A limit test with a dose of 2000 mg (kg body weight)$^{-1}$ was carried out based on the previous literature of acute oral toxicity of KCNF and temephos (17, 23-24). In addition, the 2000 mg (kg body weight)$^{-1}$ dose is the highest limit of the GHS acute dermal hazard categories (18). Rat's fur at dorsal/flank area was removed of at least 10 % from the total body surface area by closely clipping using a razor on the day before dose administration (17). The rat's total body surface area to be cleared was based on Meeh's formula that was 9.83 x rat's weight$^{2/3}$ (25).

The sample was applied uniformly over the clipped area, covered with dressing film, and fastened with a crepe bandage to retain the sample firmly for an exposure period of 24 hours (17). The dressing film and crepe bandage

were removed after 24 hours and sample residue on the skin was wiped with a 70 % alcohol swab. Step 2 of the test was carried out after 48 hours of the rat exposure in Step 1.

***Observation***

The observation for skin irritation was carried out upon removal of the dressing film at 24, 48, and 72-hour post-dosing using Draize criteria for skin reaction grading (17). The systemic effect of acute dermal toxicity was observed at 0.5, 1, 2, 4, 6, 12, and 24-hour after the dosing and thereafter once daily up to 14 days for mortality, moribund, and severe pain/distress including changes in physiological state and behavioral pattern for 14 days onwards (17, 26). The body weight of the rats was recorded on days 0, $7^{th}$ and $14^{th}$ to analyse changes in body weight upon exposure with the samples (17).

***Necropsy and gross pathology***

All rats were euthanized by using carbon dioxide by a competent assistant veterinary on day 15 and necropsy was performed. Gross examination was carried out including the examination of the external surface of bodies, orifices, abdominal content, and physical abnormalities of vital organs. The vital organs such as liver, lung, kidney, spleen, brain (cerebrum and cerebellum) were removed through a midline incision of the rat's abdomen and weighted wet as soon as after dissection to avoid drying. The wet weights of the vital organs were recorded as absolute weight and relative organ weight.

## *Statistical analysis*

The skin irritation category will be determined based on the mean score of skin reaction according to Draize criteria and the number of animals showing the irritation effect (18). The result of the skin reaction grading also can be used to determine whether further skin irritation test is needed.

The rats' body weight, food & water consumption and organs weight was carried out using Microsoft Excel (version 16). All the results were written as mean ± standard deviation of the mean (SD). Relative organs weight was calculated by dividing absolute organ weight with terminal body weight x 100 (27).

Mean differences between KNCF 2.3 % w/v and KCNF+T 2.3 % w/v group for percentage body weight changes, food consumption, water intake and relative organ weight were analyzed using Independents-samples t-test with significant value at $p \leq 0.05$. Statistical analysis was carried out using IBM SPSS Statistics version 27 software.

The median lethal dose ($LD_{50}$) and acute dermal toxicity category of KNCF 2.3 % w/v and KCNF+T 2.3 % w/v were determined based on the number of rats showed mortality, moribund and severe pain/distress (17, 18).

## *Results*

***Nano physicochemical properties of KCNF***

The nano physicochemical properties of the KCNF were summarized in Table 1. The FESEM images of KCNF 2.3 % w/v and KCNF+T 2.3 % w/v were shown in Figure S1.

**Table 1**: Physicochemical properties of the KCNF

| Physicochemical properties | Value | Method |
|---|---|---|
| **Morphology** | Fibrous network structure | FESEM |
| **Particles size distribution** | Length: 179.71 ± 52.35 nm<br>Width: 6.26 ± 1.48 nm | TEM |
| **Functional group** | O-H groups<br>C-H symmetrical stretching<br>C-O-C stretching | FTIR |
| **Crystallinity Index (CrI)** | 65.78 % | XRD |
| **Specific surface area** | 2.18 $m^3 g^{-1}$ | BET |
| **Particle stability** | -21.8 ± 10.8 mV | Zeta potential |

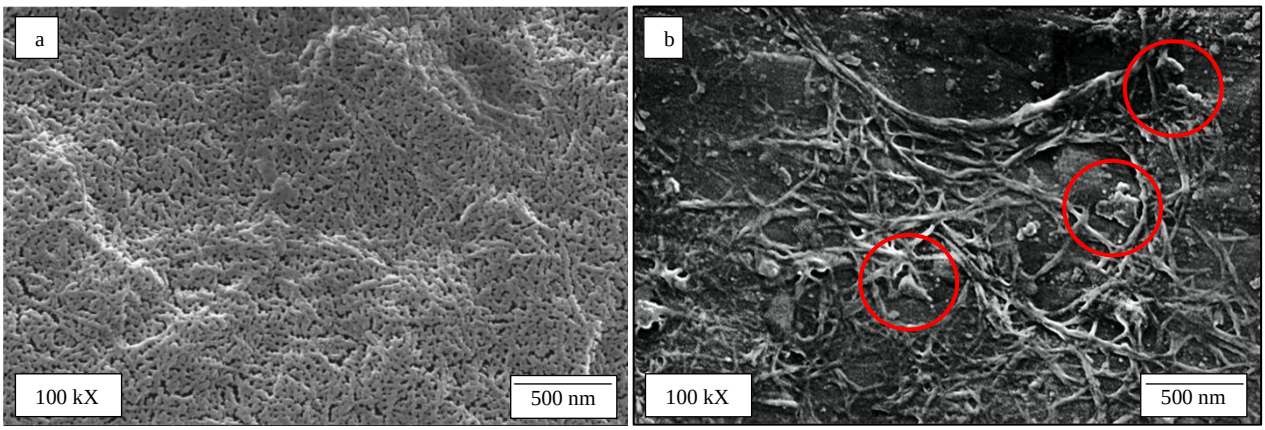

**Figure S1:** FESEM micrograph of KCNF 2.3 %w/w (a) and KCNF+T 2.3 %w/w (b) at 100.0 kX. Temephos impregnated on KCNF+T 2.3 %w/w was indicated by red circles

***Skin irritation***

Table 2 shows skin reaction upon dermal exposure with KCNF 2.3 % w/v and KCNF+T 2.3 % w/v at the dose of 2000 mg (kg body weight)$^{-1}$. The Draize mean score was 0 and there were no skin erythema, eschar or oedema observed in rats upon 24 hours post-exposure with KCNF 2.3 % w/v and KCNF+T 2.3 % w/v. Based on the mean score of the skin reaction according to Draize criteria, the KCNF 2.3 % w/v and KCNF+T 2.3 % w/v were not classified as skin irritation and thus, no further skin irritation test is required.

**Table 2**: Skin reaction exhibited by female Rattus norvegicus of Sprague Dawley (SD) rats treated KCNF 2.3 % w/v and KCNF+T 2.3 % w/v at the dose of 2000 mg/ kg body weight

| Skin reaction[a] | KCNF 2.3 % w/v | KCNF+T 2.3 % w/v |
|---|---|---|
| **Observation** | No abnormal lesion/irritation | No abnormal lesion/irritation |
| **24-hour** | 0 | 0 |
| **48-hour** | 0 | 0 |
| **72-hour** | 0 | 0 |
| **Mean score** | 0 | 0 |

[a]Skin reaction is based on the formation of erythema, eschar or oedema

Draize criteria for skin reaction grading:

No erythema/eschar/oedema - 0; Very slight erythema/oedema - 1; Well defined erythema/slight oedema - 2; Moderate to severe erythema/moderate oedema - 3; Severe erythema to eschar formation/ severe oedema - 4.

***Acute dermal toxicity***

***Body weight, food, and water consumption***

Table 3 shows body weight and body weight changes at weekly intervals of rats exposed with KCNF 2.3 % w/v and KCNF+T 2.3 % w/v. There was a gradual increase in mean body weight of the rats in KCNF 2.3 % w/v group with body weight changes percentage was 4.7 % (Week 2) as compared to initial body weight. Whereas, rats in KCNF+T 2.3 % w/v group showed a slightly decrease in body weight (-1.5 %) in Week 1 and increased to 0.9 % in Week 2. The Independent T-test showed no significant difference in the percentage of body weight change in both groups with $p \geq 0.05$.

The result of the food and water consumption as shown in Table 4 demonstrates that none of the rats suffered from reduced food and water consumption. Even though a slight decreased in water consumption for rats exposed with KCNF+T 2.3 % w/v, the Independent T-test analysis showed that there was no significant difference in food and water consumption among the rats in both groups with $p \geq 0.05$.

**Table 3:** Mean body weight of individual female Rattus norvegicus of Sprague Dawley rats dosed with 2000 mg/ kg body weight of KCNF 2.3 %w/v and KCNF+T 2.3 %w/v

| Body weight | KCNF 2.3 %w/v (Mean ± SD) | KCNF+T 2.3 %w/v (Mean ± SD) | P-value[b] |
|---|---|---|---|
| Initial (g) | 228.7 ± 5.5 | 249 ± 16.6 | |
| Week 1 (g) | 231.3 ± 4.0 | 245 ± 8.7 | |
| Week 1 (%)[a] | 1.2 ± 0.7 | -1.5 ± 3.2 | 0.241 |
| Week 2 (g) | 239.3 ± 6.5 | 251 ± 9.6 | |
| Week 2 (%)[a] | 4.7 ± 5.2 | 0.9 ±3.0 | 0.331 |

[a]Percentage of body weight change. Calculated as = Body weight at the end of each week– Initial body weight / Initial body weight x 100

[b]P-value for mean difference percentage of body weight change between KCNF 2.3 %w/v and KCNF+T 2.3 %w/v at Week 1 and Week 2. P-value less than 0.05 ($p \leq 0.05$), significant value

**Table 4:** Mean food consumption and water intake of individual female Rattus norvegicus of Sprague Dawley rats dosed with 2000 mg/ kg body weight of KCNF 2.3 %w/v and KCNF+T 2.3 %w/v

| Food/water consumption [a] | KCNF 2.3 % w/v (Mean ± SD) | KCNF+T 2.3 % w/v (Mean ± SD) | P-value[b] |
|---|---|---|---|
| **Food (g)** | | | |
| Week 1 | 18.8 ± 6.6 | 18.2 ± 5.7 | 0.766 |
| Week 2 | 20.2 ± 4.2 | 21.7 ± 3.9 | 0.259 |
| **Water (ml)** | | | |
| Week 1 | 32.1 ± 10.5 | 34.0 ± 11.1 | 0.572 |
| Week 2 | 35.0 ± 9.5 | 32.3 ± 6.9 | 0.289 |

[a]Minimum to maximum food consumption and water intake of the rat within 14 days of the observation period

[b]P-value for mean difference food consumption and water intake between KCNF 2.3 % w/v and KCNF+T 2.3 % w/v at Week 1 and Week 2. P-value less than 0.05 ($p \leq 0.05$), significant value

***Clinical signs of toxicity***

A summary of the clinical signs of toxicity is presented in Table 5. The rats exposed with KCNF 2.3 % w/v did not show any significant clinical signs of toxicity while rats dosed with KCNF+T 2.3 % w/v showed various clinical signs of toxicity with very mild to mild effect. The toxicity signs were classical to cholinergic toxicity and were reversible within six hours to one day.

Therefore, these signs were insufficient to conclude the rats suffered from moribund/impending death or

physical, physiological, nervous system and behavioural abnormalities upon dermal exposure.

**Table 5**: Clinical signs of toxicity and severity in female Rattus norvegicus of Sprague Dawley rats upon dermal dosing of KCNF 2.3 % w/v and KCNF+T 2.3 % w/v at dose 2000 mg/ kg body weight

| Sample | Clinical signs of toxicity | Severity |
|---|---|---|
| KCNF 2.3 % w/v | Imbalance movement | Very mild |
| | Porphyrin secretion | Very mild |
| KCNF+T 2.3 % w/v | Imbalance movement | Very mild |
| | Light tremor | Very mild |
| | Piloerection | Mild |
| | Porphyrin secretion | Mild |
| | Nasal mucus discharge | Mild |
| | Soft stools | Mild |
| | Inactive | Mild |

***Gross examination and organ weight***

The gross examination carried out during necropsy found that no major changes were observed in rats of both groups. Nonetheless, enlarged blood vessel and redness at the duodenum, ileum, jejunum, caecum, and colon of gastrointestinal (GI) tract was found in two of the rats exposed with KCNF 2.3 % w/v and KCNF+T 2.3 % w/v (Figure S2). Whereas, no significant difference was found for all relative organ weights in both groups with $p \geq 0.05$ as shown in Table 6. However, the relative organ weight of the brain in rats exposed to KCNF+T 2.3 % w/v was slightly lower (0.66 % g) compared with rats exposed to KCNF 2.3 % w/v group (0.85 % g).

**Table 6:** Mean absolute and relative organ weights of female Rattus norvegicus of Sprague Dawley rats treated with the KCNF 2.3 %w/v and KCNF+T 2.3 %w/v at the dose of 2000 mg/ kg body weight.

| Organ weight | KCNF 2.3 %w/v (Mean ± SD) | KCNF+T 2.3 %w/v (Mean ± SD) | P-value[c] |
|---|---|---|---|
| Terminal body weight[a](g) | 229 ± 4.16 | 242 ± 6.66 | |
| **Kidney** | | | |
| Absolute (g) | 2.49 ± 0.42 | 2.37 ± 0.56 | |
| Relative[b] (%g) | 1.09 ± 0.20 | 0.98 ± 0.24 | 0.585 |
| **Spleen** | | | |
| Absolute (g) | 0.48 ± 0.02 | 0.55 ± 0.02 | |
| Relative (%g) | 0.21 ± 0.01 | 0.23 ± 0.01 | 0.132 |
| **Liver** | | | |
| Absolute (g) | 11.25 ± 1.18 | 11.88 ± 1.19 | |
| Relative (%g) | 4.90 ± 0.42 | 4.90 ± 0.41 | 1.000 |
| **Brain** | | | |
| Absolute (g) | 1.96 ± 0.21 | 1.60 ± 0.30 | |
| Relative (%g) | 0.85 ± 0.10 | 0.66 ± 0.14 | 0.124 |

[a]Weight after euthanized

[b]Relative organ weight calculated as: Absolute organ weight (g) / terminal body weight (g) x 100

[c]P-value for mean difference relative organ weight between KCNF 2.3 % w/v and KCNF+T 2.3 % w/v. P-value less than 0.05 ($p \leq 0.05$), significant value.

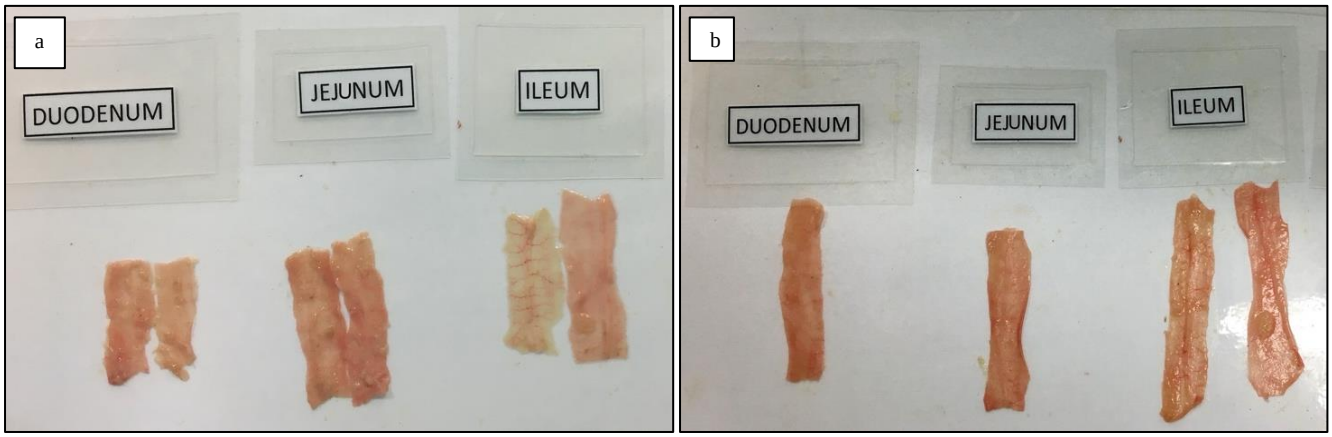


**Figure S2:** Example of engorged blood vessels and redness at duodenum, jejunum, and ileum of the rats exposed with KCNF 2.3 %w/w (a) and KCNF+T 2.3 %w/w (b) at the dose level of 2000 mg/kg body weight

***Mortality, moribund and severe pain/distress***

All the six female rats dosed with KCNF 2.3 % w/v and KCNF+T 2.3 % w/v showed no mortality, moribund or severe pain/distress throughout the 14 days of observation. Based on OECD test guideline for acute dermal toxicity, $LD_{50}$ for acute dermal exposure of KCNF 2.3 % w/v and KCNF+T 2.3 % w/v is > 2000 mg (kg body weight)$^{-1}$ (17).

## *Discussion*

The result of KCNF 2.3 % w/v and KCNF+T 2.3 % w/v skin irritation showed Draize mean scores of 0 with the absence of oedema, erythema, and eschar formation

(Table 2). The result of this study was consistent with other studies, whereby the null skin irritation index for temephos and CNF has been described in vivo and in vitro (28-30). Based on the result, the test items did not produce skin irritation upon contact with skin. Therefore, KCNF 2.3 % w/v and KCNF+T 2.3 % w/v were not classified as skin irritants (18).

The result of the percentage body weight changes in Table 3 shows that all rats had increased in percentage of body weight changes with no significant difference between these two groups. However, two rats in the KCNF+T 2.3 % w/v group showed a slight decrease in body weight changes proposed an inefficient food utilisation, despite the mean food consumption was similar to other rats. The reduction in body weight was reported in rats following repeated dermal exposure of temephos with dose 60 mg (kg body weight$^{-1}$) for three weeks (31). Nonetheless, the decrease in body weight change of the two rats was less than 20 % (25) and insignificant ($p > 0.05$). Therefore, it cannot be regarded as a sign of toxicity.

There were no treatment-related mortality or unscheduled death attributed to the dermal exposure of KCNF 2.3 % w/v and KCNF+T 2.3 % w/v throughout the 14 days observation period amongst the rats. The clinical observations showed very mild to mild clinical signs of toxicity in both treatment groups, and thus not considered as severe pain/distress which required early euthanisation (32, 33). Comparing with clinical signs of toxicity presented by the rats exposed with KCNF 2.3 % w/v, the rats in KCNF+T 2.3 % w/v group presented various mild clinical signs, such as light tremors, inactive, piloerection, and soft stool. These mild clinical signs of toxicity were suggested as classical to acute organophosphate intoxication (34). Therefore, it was postulated that single administration of KCNF+T 2.3 % w/v via dermal did not induce significant temephos intoxication in the experimental animals in terms of neuromuscular and behavioural changes. Though no significant temephos intoxication was reported in the current study, the occurrence of mild clinical signs of toxicity in the KCNF+T 2.3 % w/v group requires further research to be done to elucidate potential repeated dose toxicity following exposure with KCNF+T 2.3 % w/v.

The gross necropsy and pathology findings showed no abnormal lesion of the organs, except for blood vessel engorgement and redness at the duodenum, jejunum, ileum, caecum, and colon in rats of both treatment groups. The engorgement of blood vessels and redness indicated possible inflammation at the small and large intestines (35, 36). It was postulated that percutaneous absorption of KCNF occurred through hair follicle pathway mainly due to the skin hydration capability of CNF and distributed to the GI tract which resulted in the blood vessels engorgement and redness of the intestines (37, 38). The biodistribution of nanomaterial such as zinc oxide nanoparticle to the GI tract upon subcutaneous exposure has been previously reported whereby 28 % of the zinc oxide translocated at stomach and intestines after 25 hours of exposure (39). Therefore, further study is required to elucidate biodistribution and translocation of KCNF to other organs especially to the GI tract following absorption from dermal exposure.

Relative organ weight and absolute organ weight are used to evaluate the chemical-related adverse effect in rats and are one of the important endpoints in toxicity studies (27). Changes in organ size and weight can be one of the early indicators for chemically induced effect in the absence of organ morphological changes and are valuable for recognising target organ toxicity (40). Based on the result of this study, there was no significant difference in relative organ weight between rats in KCNF 2.3 % w/v and KCNF+T 2.3 % w/v group (Table 6). However, it was noted that the relative organ weight for the brain in rats exposed with KCNF+T 2.3 %w/v was slightly lower (0.66 % g) than relative brain weight in KCNF 2.3 % w/v group (0.85 % g). The slightly lower relative brain weight in KCNF+T 2.3 % w/v indicated possible adverse effects to the organ since the brain is the target organ toxicity for temephos even though gross necropsy found no morphological changes (41). There is a possibility the KCNF enhances the absorption of temephos due to skin hydration capability, which leads to skin swelling, improves skin permeability, and eventually allows temephos absorption through enlarged skin pores. Repeated dose toxicity study of the KCNF 2.3 % w/v and KCNF+T 2.3 % w/v is recommended to ascertain long term toxicity to induce pathological effects.

Based on the mortality/moribund and severe pain/distress result, the median lethal dose ($LD_{50}$) of the KCNF 2.3 % w/v and KCNF+T 2.3 % w/v in the rats was found to be > 2000 mg/kg body weight for dermal exposure. Therefore, the KCNF 2.3 % w/v and KCNF+T 2.3 % w/v are categorised into Category 5 according to Globally Harmonised System (GHS), which was considered as non-classified as hazardous material (18).

## *Conclusion*

The KCNF 2.3 % w/v and KCNF+T 2.3 % w/v are categorised as Category 5 for acute toxicity under GHS i.e., non-classified as a hazardous material. The KCNF is unlikely to cause skin irritation or acute toxicity via dermal exposure under typical use. Nevertheless, repeated dose study will be beneficial to determine whether the presence of temephos impregnated onto the nanofiber (KCNF+T 2.3 % w/v) could potentially induce mild clinical signs of cholinergic toxicity and effect specific e.g., target organ toxicity compared to KCNF without temephos (KCNF 2.3 % w/v).

## *Ethical Clearance*

Animal ethic approval has been obtained from USM

Animal Ethics Committee with approval number USM/IACUC/2019/(119)(1016). The study was carried out following OECD test guideline 402 Acute Dermal Toxicity-Fixed Dose Procedure which complied with Directive 2010/63/EU.

## *Competing interests*

The authors declare no conflicts of interest in the conduct of the study.

## *Acknowledgements*

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