**Reproductive Toxicity Screen for SF-005**

We have completed a Reproduction/Developmental Toxicity Screening Test of SF-005 in accordance with OECD Test Guideline (TG) 421 for purposes of dose range finding and screening for potential effects.[[1]](#footnote-1) We used dietary concentrations of SF-005 up to the European Food Saftet Authority (EFSA) maximum feasible dose (MFD) of 5% (w/w) in the diet, or 50,000 ppm, which is equivalent to 3,224 mg/kg-bw/day over 28 days for males, 3,934 mg/kg-bw/day for females during pregnancy, and 7,292 mg/kg-bw/day for females during lactation. The dietary concentrations used resulted in top doses of SF-005 that far exceeded the ECHA 1,000 mg/kg-bw/day limit dose for chemical exposures. Thus, this is a high dose, in particular for a flavour, and represents exposure that is >7,700 times higher than what is likely to occur in humans.[[2]](#footnote-2) Our findings suggest very low potential for reproductive or developmental toxicity of SF-005. Key findings include:

* No effect on adult male body weight or food intake over 28 days of exposure was observed at any dietary concentration up to and including 50,000 ppm, which was equivalent to an estimated mean intake of 3,224 mg SF-005/kg-bw/day).
* A decrease in maternal body weight and food intake during pregnancy was observed only at the highest concentration used (50,000 ppm), with estimated mean intake of 3,934 mg SF-005/kg bw/day.
* Effects on maternal body weight and food intake during lactation only at 50,000 ppm, which was equivalent to an estimated mean intake of 7,292 mg SF-005/kg bw/day.
* No effects on male or female offspring body weights were observed at any dietary concentration up to and including 50,000 ppm, which was equivlanent to 7,292 mg SF-005/kg-bw/day.
* No statistically significant effect on anogenital distance (AGD) in male offspring was observed at any dose.

Overall, few adverse effects were observed, and only at at extremely high dietary concentrations of SF-005. The effects on body weight and food intake observed in adult females in the 50,000 ppm diet group during pregnancy and laction suggest maternal toxicity of this extremely high dose.

An OECD TG 443 study requires many animals, at high cost and many months to complete. The results of our OECD TG 421 study show very low potential for reproductive or developmental toxicity of SF-005. This is further supported by a previously run 90-day study (OECD TG 408) that did not indicate reproductive toxicity.

**Background:**

Under the new Smoke Guidance (<https://www.efsa.europa.eu/en/efsajournal/pub/6435>), an OECD TG 443 Extended One-Generation Reproductive Toxicity Study (EOGRTS) is required to fulfil EFSA’s requirements for the reauthorization of smoke flavours. A dose-range finder (DRF) study was needed to select doses for an OECD TG 443 study of Kerry smoke flavour primary product (SFPP) SF-005. A previous 90-day study (OECD TG 408) of SF-005 did not indicate a potential for reproductive toxicity; however, the OECD TG 408 study does not address developmental toxicity. In addition, the OECD TG 408 study of SF-005 did not clearly define a No Observed Adverse Effect Level (NOAEL) for adult rats following 90 days of exposure (i.e., the Study Director concluded that no adverse effects were observed at the highest dose tested). Therefore, we carried out a DRF, based on the OECD TG 421 Reproduction/Developmental Toxicity Screening Test, for SF-005. Importantly, the OECD TG 421 study also can provide clear indications of potential reproductive and developmental toxicity.

**Objective:**

The objectives were to provide information for the selection of dietary concentrations of SF-005 for an EOGRTS in rats, and to assess potential reproductive or developmental toxicity of SF-005.

**Study Design:**

The dietary concentrations in this study were selected to be 0, 12,500, 25,000 and 50,000 ppm, based on a prior 90-day repeated dose toxicity study with dietary administration of SF-005 in rats. The highest concentration (5% in the diet, 50,000 ppm) is the EFSA MFD for dietary studies, which is as high as the dose can go before nutritional impacts are made to the diet. Dietary concentrations were not adjusted during the lactation period. Chemical analyses of the diets were performed once during the study and accuracy and homogeneity were confirmed.

The design of the study, based on the OECD TG 421, included 10 male and 10 female Wistar Han rats at each dietary concentration level. The following parameters and endpoints were evaluated in this study: mortality/moribundity, clinical signs, body weight and food consumption, test material intake, macroscopic examination, organ weights and microscopic examination. The following reproduction/developmental parameters were determined: estrous cycle, mating, pregnancy, and fertility indices, precoital time, number of implantation sites, gestation index and duration, viability and survival indices, parturition, maternal care, sex ratio and early postnatal pup development (i.e., mortality, clinical signs, body weights, anogenital distance, areola/nipple retention and macroscopic examination). Detailed materials and methods are provided in the full study report (Annex D-4).

**Results:**

**Parental body weights and food intake**

Males: Over 28 days of exposure, body weights of males were similar in all dietary groups. Male food intake was not significantly different from controls in any dietary group compared to controls at any interval during the 28 day dietary exposure.

Females: Pregnant females in the 50,000 ppm diet group weighed less than controls when weighed on days 11, 14, 17, and 20 of gestation. The other diet groups were similar to controls. Pregnant females in the 50,000 ppm diet group had lower food intake than controls during days 11-14, 14-17, 17-20, and 0-20 of gestation. During lactation, females in the 50,000 ppm diet group gained less weight than controls, with statistically significant lower body weight than controls on days 1, 4, 7, and 13 of lactation. Food intake during lactation was higher than in pregnancy, and the 50,000 ppm diet group had significantly lower daily food intake than controls during the periods of days 1-4, 4-7, 7-13, and days 1-13 of lactation. Food intake was also lower in the 12,500 ppm diet group than in controls on days 1-4, 10-13, and 1-13, but this finding is considered spurious given that food intake was not affected at 25,000 ppm.

**Offspring body weights**

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Pups were weighed on postnatal days 1, 4, 7, and 13. Male and female offspring gained weight in all groups from birth to termination on postnatal day 13.

Males: Male offspring body weights were not statistically significantly different from controls in any dietary group at any age through termination at postnatal day 13.

Females: Female offspring body weights were not statistically significantly different from controls in any dietary group at any age through termination at postnatal day 13.

**Offspring anogenital distance and male offspring nipple retention**

Anogenital distance was measured on postnatal day 4, and values were normalized with the cube root of body weight. The normalized AGDs were similar in all dietary groups, with no statistically significant differences observed. As normal, no areolae or nipples were observed in male offspring on postnatal day 13 in any dietary group.

**Other endpoints assessed (data not shown, unaffected by SF-005)**

Additional adult endpoints evaluated in this study included mortality/moribundity, clinical signs, test material intake, macroscopic examination, organ weights and microscopic examination. No mortality nor test material-related changes were noted in any of these adult endpoints.

Additional reproductive/developmental endpoints included estrous cycle, mating, pregnancy, and fertility indices, precoital time, number of implantation sites, gestation index and duration, viability and survival indices, parturition, maternal care, sex ratio, and early postnatal developmental endpoints (mortality, clinical signs, areola/nipple retention and macroscopic examination). No test material-related effects were observed for any of these additional endpoints.

**Test material intake (mean mg/kg-bw/day)**

The dietary concentrations used in this study were combined with the measured food intake by the parental animals to calculate estimated daily intake of SF-005. The highest dietary concentration of SF-005 used in this study was selected to meet the EFSA MFD of 5% (w/w), 50,000, in the diet. The estimated mean intakes of SF-005 at the highest dose tested were 3,244 mg/kg-bw/day over 28 days for adult males, 3.934 mg/kg-bw/day for females during pregnancy, and 7,292 mg/kg-bw/day for females during lactation, which far exceeded the ECHA limit dose of 1000 mg/kg-bw/day for chemical exposures in an OECD TG 421 or 443 study. As noted in our June 15, 2022, letter, we did not intend to use the results of the DRF to determine NOAELs or Lowest Observed Adverse Effect Level (LOAELs). However, given the relative absence of effects observed in this study at such high doses, NOAELs and/or LOAELs for adult males, adult females, and offspring are provided in Table 1. As shown in the table, all NOAELs and LOEALs are higher then the ECHA limit dose of 1,000 mg/kg-bw/day. The NOAELs in this study are also higher than the NOAEL of 1,351mg SF-005/kg-bw/day determined previously in an OECD TG 408 90-day exposure study in rats submitted in the original dossier submission.

**Table 1.** NOAELs and LOAELs for SF-005 based on dietary concentrations (ppm) and estimated mean daily intake of SF-005 (mg SF-005 /kg-bw/day)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | NOAEL(ppm in diet) | NOAEL(mg/kg-bw/day) | LOAEL (ppm in diet) | LOAEL (mg/kg-bw/day) | Critical Effect |
| Adult male | 50,000 | 3,244 | N/A | N/A | N/A |
| Maternal (pregnancy) | 25,000 | 2,021 | 50,000 | 3,934 | Body weight |
| Maternal (lactation) | 25,000 | 3,903 | 50,000 | 7,292 | Body weight |
| Developmental (Maternal intake during pregnancy)  | 50,000 | 3,934 | N/A | N/A | N/A |
| Developmental (Maternal intake during lactation)  | 50,000 | 7,292 | N/A | N/A | N/A (no effects on postnatal survival or growth of offspring) |

**Discussion:**

The adverse effects observed in this study were limited to lower body weights in pregnant females in the 50,000 ppm diet group (i.e., 3,934 mg/kg-bw/day), accompanied by significantly lower food intake than controls. Maternal body weights were also significantly lower during lactation in the at 50,000 ppm (i.e., 7,292 mg/kg-bw/day) diet group, indicating that the highest dose reached in the study is the maximum tolerated dose (MTD).

Despite the effects on maternal body weight and food intake during pregnancy and lactation, offspring body weights were not affected at the highest concentration tested.There was a trend toward lower AGD in male offspring in the 50,000 ppm group; however, the difference was not statistically significant. The lack of any retained nipples/areolae in male pups at PND 13 suggests that this statistically nonsignificant finding was not related to SF-005 exposure.

Overall, this TG 421 Reproduction/Developmental Toxicity Screening Test indicates a low potential for reproductive and developmental toxicity of SF-005. The effects observed were limited to decreases in adult female body weights and food consumption during gestation and/or lactation at extremely high doses of SF-005.

1. We acknowledge that our letter dated June 15, 2022, mistakenly stated that we planned to conduct a modified OECD TG 422 study. As noted in the letter, the purpose of the screening study was to inform dose levels for an OECD TG 443 study. The OECD TG 421 study is sufficient for this purpose and was the intended study and consistent with the notified studies to EFSA. [↑](#footnote-ref-1)
2. Calculated using EFSA’s Dietary Exposure model (DietEx) assuming typical use levels and 95th percentile exposure and adjusted for market share data. [↑](#footnote-ref-2)