**Supplemental Table 8** Body weight and body weight gain in female BALB/c mice exposed to BMIM dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(AOO) | BMIM | 0.15% DNFB | 25% HCA | Trend Analysis |
| 3.12% | 6.25% | 12.5% | 25% | p-value |
| *LLNA/IRR Study 1* |  |  |  |  |  |  |  |
| *Body weight (g)* |  |  |  |  |  |  |  |
|  Day 1 | 19.00 ± 0.29 | 18.89 ± 0.25 | 19.20 ± 0.16 | 18.58 ± 0.28 | 18.82 ± 0.24 | 19.21 ± 0.30 | 18.87 ± 0.35 | 0.440 |
|  Day 6 | 19.66 ± 0.21 | 19.49 ± 0.25 | 19.75 ± 0.21 | 19.66 ± 0.28 | 19.56 ± 0.28 | 19.70 ± 0.11 | 20.03 ± 0.37 | 0.853 |
| *Body weight gain (g)* | 0.66 ± 0.18 | 0.60 ± 0.10 | 0.55 ± 0.11 | 1.09 ± 0.33 | 0.74 ± 0.23 | 0.48 ± 0.23 | 1.16 ± 0.18 | 0.072 |
|  |  |  |  |  |  |  |  |  |
| *LLNA/IRR Study 2* |  |  |  |  |  |  |  |  |
| *Body weight (g)* |  |  |  |  |  |  |  |  |
|  Day 1 | 20.29 ± 0.21 | 20.50 ± 0.12 | 20.35 ± 0.16 | 20.09 ± 0.27 | 20.41 ± 0.28  | 20.35 ± 0.21 | 19.98 ± 0.22 | 0.253 |
|  Day 6 | 20.58 ± 0.18 | **21.23 ± 0.13\*** | 20.50 ± 0.19 | 21.10 ± 0.19 | 20.59 ± 0.47 | 20.98 ± 0.24 | 20.92 ± 0.19 | 0.410 |
| *Body weight gain (g)* | 0.29 ± 0.22 | 0.73 ± 0.16 | 0.15 ± 0.22 | **1.02 ± 0.13\*** | 0.17 ± 0.35 | 0.63 ± 0.19 | **0.94 ± 0.19\*** | 0.055 |
|  |  |  |  |  |  |  |  |  |
| *MEST Study* |  |  |  |  |  |  |  |  |
| *Sensitization* | AOO | AOO | 3.12% BMIM | 6.25% BMIM |  | AOO | 0.15% DNFB |  |
| *Challenge* | AOO | 6.25% BMIM | 6.25% BMIM | 6.25% BMIM |  | 0.15% DNFB | 0.15% DNFB |  |
| *Body weight (g)* |  |  |  |  |  |  |  |  |
|  Day 1 | 18.77 ± 0.10 | 18.75 ± 0.18 | 18.61 ± 0.19 | 18.67 ± 0.10 |  | 18.69 ± 0.21 | 18.76 ± 0.21 | 0.771 |
|  Day 9 | 19.58 ± 0.13 | 19.68 ± 0.22 | 19.51 ± 0.16 | 19.89 ± 0.20 |  | 20.18 ± 0.30 | 19.87 ± 0.20 | 0.492 |
| *Body weight gain (g)* | 0.82 ± 0.12 | 0.92 ± 0.11 | 0.90 ± 0.08 | 1.22 ± 0.17 |  | 1.49 ± 0.17 | 1.10 ± 0.23 | 0.169 |

Values represent the mean ± SE. N = 8 animals per dose group. N = 7 animals/group for LLNA/IRR Study 1 25% BMIM, N = 5 for LLNA/IRR Study 2 25% BMIM.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate BMIM-exposed groups that differ significantly from the vehicle controls or significant trends across BMIM-exposed groups and vehicle controls.

The 25% BMIM dose group was excluded from the trend and pairwise statistical analyses for LLNA/IRR studies due to high mortality.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies. The AOO/AOO, AOO/0.15% DNFB, and 0.15% DNFB/0.15% DNFB were excluded from the trend analysis for the MEST study.

For the MEST study, the VHIC (vehicle irritancy control) was the AOO/6.25% BMIM group.

**Supplemental Table 9** Body weight and body weight gain in female BALB/c mice exposed to EMIM dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(AOO) | EMIM | 0.15% DNFB | 25% HCA | Trend Analysis |
| 6.25% | 12.5% | 25% | 50% | p-value |
| *LLNA/IRR Study 1* |  |  |  |  |  |  |  |
| *Body weight (g)* |  |  |  |  |  |  |  |
|  Day 1 | 19.66 ± 0.29 | **20.57 ± 0.22\*** | 19.90 ± 0.26 | 20.20 ± 0.18 | 19.92 ± 0.22 | 19.95 ± 0.28 | 19.82 ± 0.28 | 1.000 |
|  Day 6 | 19.99 ± 0.32 | 20.72 ± 0.14 | 20.29 ± 0.29 | 20.70 ± 0.23 | 20.81 ± 0.29 | 20.62 ± 0.28 | 20.77 ± 0.26 | 0.086 |
| *Body weight gain (g)* | 0.33 ± 0.13 | 0.16 ± 0.16 | 0.39 ± 0.19 | 0.50 ± 0.23 | 0.89 ± 0.17 | 0.67 ± 0.14 | **0.96 ± 0.17\*** | 0.016 |
|  |  |  |  |  |  |  |  |  |
| *MEST Study* |  |  |  |  |  |  |  |  |
| *Sensitization* | AOO | AOO | 25% EMIM | 50% EMIM |  | AOO | 0.15% DNFB |  |
| *Challenge* | AOO | 50% EMIM | 50% EMIM | 50% EMIM |  | 0.15% DNFB | 0.15% DNFB |  |
| *Body weight (g)* |  |  |  |  |  |  |  |  |
|  Day 1 | 18.00 ± 0.24 | 18.03 ± 0.23 | 17.81 ± 0.10 | 18.05 ± 0.25 |  | 18.18 ± 0.28 | 18.10 ± 0.28 | 0.812 |
|  Day 9 | 18.91 ± 0.23 | 18.79 ± 0.30 | 18.73 ± 0.19 | 19.09 ± 0.27 |  | 18.96 ± 0.22 | 18.57 ± 0.34 | 0.290 |
| *Body weight gain (g)* | 0.91 ± 0.05 | 0.76 ± 0.19 | 0.92 ± 0.18 | 1.04 ± 0.13 |  | 0.78 ± 0.12 | 0.47 ± 0.17 | 0.186 |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate EMIM-exposed groups that differ significantly from the vehicle controls or significant trends across EMIM-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies. The AOO/AOO, AOO/0.15% DNFB, and 0.15% DNFB/0.15% DNFB were excluded from the trend analysis for the MEST study.

For the MEST study, the VHIC (vehicle irritancy control) was the AOO/50% EMIM group.

**Supplemental Table 10** Local Lymph Node Assay (LLNA) in female BALB/c mice exposed to BMIM dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(AOO) | BMIM | 0.15% DNFB | 25% HCA | Trend Analysis |
| 3.12% | 6.25% | 12.5% | 25% | p-value |
| *LLNA Study 1* |  |  |  |  |  |  |  |
| DPM | 203.68 ± 21.20 | 242.87 ± 57.48 | 333.20 ± 84.07 | **386.07 ± 29.99\*\*** | 337.93 ± 70.01 | **2078.2 ± 338.4\*\*** | **570.33 ± 104.9\*** | **0.006** |
| SI | 1.0 | 1.2 | 1.6 | 1.9 | 1.7 | 10.2 | 2.8 |  |
|  |  |  |  |  |  |  |  |  |
| *LLNA Study 2* |  |  |  |  |  |  |  |
| DPM | 139.32 ± 61.25 | **231.80 ± 33.93\*** | 152.59 ± 17.50 | **207.75 ± 22.27\*** | 115.69 ± 33.48  | **1245.7 ± 313.4\*\*** | **433.13 ± 80.88\*\*** | 0.075 |
| SI | 1.0 | 1.7 | 1.1 | 1.5 | 0.8 | 8.9 | 3.1 |  |

Values represent the mean ± SE. N = 8 animals per dose group. N = 7 animals/group for LLNA/IRR Study 1 25% BMIM, N = 5 for LLNA/IRR Study 2 25% BMIM.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate BMIM-exposed groups that differ significantly from the vehicle controls or significant trends across BMIM-exposed groups and vehicle controls.

The 25% BMIM dose group was excluded from the trend and pairwise statistical analyses for LLNA/IRR studies due to high mortality.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies.

**Supplemental Table 11** Local Lymph Node Assay (LLNA) in female BALB/c mice exposed to EMIM dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(AOO) | EMIM | 0.15% DNFB | 25% HCA | Trend Analysis |
| 6.25% | 12.5% | 25% | 50% | p-value |
| *LLNA Study 1* |  |  |  |  |  |  |  |
| DPM | 69.32 ± 17.43 | 53.45 ± 14.28 | 45.26 ± 13.62 | 63.09 ± 6.97 | 60.07 ± 13.58 | **1209.1 ± 128.6\*\*** | **341.84 ± 42.08\*** | 0.748 |
| SI | 1.0 | 0.8 | 0.7 | 0.9 | 0.9 | 17.4 | 4.9 |  |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate EMIM-exposed groups that differ significantly from the vehicle controls or significant trends across EMIM-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies.

**Supplemental Table 12** Irritancy Assay (IRR) in female BALB/c mice exposed to BMIM dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(AOO) | BMIM | 0.15% DNFB | 25% HCA | Trend Analysis |
| 3.12% | 6.25% | 12.5% | 25% | p-value |
| *IRR Study 1* |  |  |  |  |  |  |  |
| % ear swelling, 24 hrs | 2.49 ± 0.50 | 3.67 ± 1.45 | 6.35 ± 1.78 | 6.59 ± 2.24 | 8.22 ± 2.43 | **72.33 ± 1.67\*\*** | 5.81 ± 1.28 | **0.045** |
|  |  |  |  |  |  |  |  |  |
| *IRR Study 2* |  |  |  |  |  |  |  |
| % ear swelling, 24 hrs | 3.06 ± 0.73 | 2.94 ± 0.92 | 4.55 ± 0.73 | 3.41 ± 0.88 | 5.63 ± 1.75  | **54.60 ± 4.19\*\*** | **9.67 ± 1.33\*\*** | 0.389 |

Values represent the mean ± SE. N = 8 animals per dose group. N = 7 animals/group for LLNA/IRR Study 1 25% BMIM, LLNA/IRR Study 2 25% BMIM

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate BMIM-exposed groups that differ significantly from the vehicle controls or significant trends across BMIM-exposed groups and vehicle controls.

The 25% BMIM dose group was excluded from the trend and pairwise statistical analyses for LLNA/IRR studies due to high mortality.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies.

**Supplemental Table 13** Irritancy Assay (IRR) in female BALB/c mice exposed to EMIM dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(AOO) | EMIM | 0.15% DNFB | 25% HCA | Trend Analysis |
| 6.25% | 12.5% | 25% | 50% | p-value |
| *IRR Study 1* |  |  |  |  |  |  |  |
| % ear swelling, 24 hrs | 6.56 ± 0.99 | 5.78 ± 1.47 | 6.50 ± 2.39 | 7.87 ± 1.97 | 4.65 ± 0.88 | **62.31 ± 2.66\*\*** | **13.17 ± 1.47\*** | 0.519 |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate EMIM-exposed groups that differ significantly from the vehicle controls or significant trends across EMIM-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies.

**Supplemental Table 14** Mouse Ear Swelling Test (MEST) in female BALB/c mice exposed to BMIM dermally

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sensitization | AOO | AOO | 3.12% BMIM | 6.25% BMIM | AOO | 0.15% DNFB | Trend  |
| Challenge | AOO | 6.25% BMIM | 6.25% BMIM | 6.25% BMIM | 0.15% DNFB | 0.15% DNFB | Analysis |
| Parameter |  |  |  |  |  |  | p-value |
| % ear swelling, 24 hrs | 7.99 ± 1.36 | 6.40 ± 1.85 | **18.14 ± 2.43\*\*** | 11.70 ± 1.87 | 6.55 ± 1.76 | 12.89 ± 1.90 | 0.152 |
| % ear swelling, 48 hrs | 7.49 ± 1.23 | 7.37 ± 1.93 | **13.43 ± 1.75\*** | 9.28 ± 1.73 | 16.44 ± 1.19 | **34.20 ± 5.50\*** | 0.411 |

Values represent the mean ± SE. N = 8 animals per dose group. N = 7 animals/group for 3.12% BMIM + 6.25% BMIM 48 hrs

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate BMIM-exposed groups that differ significantly from the vehicle controls or significant trends across BMIM-exposed groups and vehicle controls.

The AOO/AOO, AOO/0.15% DNFB, and 0.15% DNFB/0.15% DNFB were excluded from the trend analysis for the MEST study. For the MEST study, the VHIC (vehicle irritancy control) was the AOO/6.25% BMIM group.

**Supplemental Table 15** Mouse Ear Swelling Test (MEST) in female BALB/c mice exposed to EMIM dermally

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sensitization | AOO | AOO | 25% EMIM | 50% EMIM | AOO | 0.15% DNFB | Trend |
| Challenge | AOO | 50% EMIM | 50% EMIM | 50% EMIM | 0.15% DNFB | 0.15% DNFB | Analysis |
| Parameter |  |  |  |  |  |  | p-value |
| % ear swelling, 24 hrs | -0.67 ± 1.55 | 1.85 ± 0.99 | 0.99 ± 1.35 | 2.45 ± 1.21 | 17.93 ± 2.99 | **57.19 ± 8.02\*\*** | 0.521 |
| % ear swelling, 48 hrs | 1.48 ± 1.70 | 5.32 ± 2.01 | 1.00 ± 1.57 | 4.43 ± 1.53 | 28.70 ± 5.54 | **62.22 ± 8.54\*\*** | 0.725 |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate EMIM-exposed groups that differ significantly from the vehicle controls or significant trends across EMIM-exposed groups and vehicle controls.

The AOO/AOO, AOO/0.15% DNFB, and 0.15% DNFB/0.15% DNFB were excluded from the trend analysis for the MEST study. For the MEST study, the VHIC (vehicle irritancy control) was the AOO/50% EMIM group.

**Supplemental Table 16** Body weight and body weight gain in female BALB/c mice exposed to BMPY dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(DMF) | BMPY | 0.15% DNFB | 25% HCA | Trend Analysis |
| 0.825% | 1.65% | 3.12% | 6.25% | p-value |
| *LLNA/IRR Study 1* |  |  |  |  |  |  |  |
| *Body weight (g)* |  |  |  |  |  |  |  |
|  Day 1 | 20.11 ± 0.27 | 20.19 ± 0.16 | 19.97 ± 0.24 | 19.81 ± 0.16 | 20.14 ± 0.34 | 20.19 ± 0.26 | 20.12 ± 0.24 | 0.489 |
|  Day 6 | 20.71 ± 0.32 | 20.22 ± 0.19 | 20.27 ± 0.18 | 20.43 ± 0.23 | 20.86 ± 0.29 | 21.17 ± 0.37 | **19.56 ± 0.32\*** | 0.617 |
| *Body weight gain (g)* | 0.60 ± 0.12 | 0.03 ± 0.19 | 0.30 ± 0.12 | 0.62 ± 0.18 | 0.72 ± 0.18 | 0.98 ± 0.20 | **-0.56 ± 0.22\*\*** | 0.190 |
|  |  |  |  |  |  |  |  |  |
| *LLNA/IRR Study 2* |  |  |  |  |  |  |  |  |
| *Body weight (g)* |  |  |  |  |  |  |  |  |
|  Day 1 | 19.22 ± 0.25 | 19.40 ± 0.17 | 19.79 ± 0.15 | 19.22 ± 0.32 | 19.47 ± 0.15  | 19.51 ± 0.28 | 19.40 ± 0.25 | 0.603 |
|  Day 6 | 19.96 ± 0.33 | 20.19 ± 0.33 | 19.96 ± 0.40 | 20.05 ± 0.34 | 19.88 ± 0.25 | 20.71 ± 0.23 | 19.38 ± 0.40 | 0.776 |
| *Body weight gain (g)* | 0.74 ± 0.18 | 0.79 ± 0.32 | 0.17 ± 0.29 | 0.83 ± 0.22 | 0.42 ± 0.24 | 1.20 ± 0.21 | **-0.02 ± 0.19\*** | 0.400 |
|  |  |  |  |  |  |  |  |  |
| *MEST Study* |  |  |  |  |  |  |  |  |
| *Sensitization* | DMF | DMF | 1.56% BMPY | 3.12% BMPY |  | DMF | 0.15% DNFB |  |
| *Challenge* | DMF | 3.12% BMPY | 3.12% BMPY | 3.12% BMPY |  | 0.15% DNFB | 0.15% DNFB |  |
| *Body weight (g)* |  |  |  |  |  |  |  |  |
|  Day 1 | 18.61 ± 0.27 | 18.72 ± 0.21 | 18.23 ± 0.18 | 18.29 ± 0.17 |  | 18.74 ± 0.17 | 18.38 ± 0.18 | 0.161 |
|  Day 9 | 19.34 ± 0.34 | 19.58 ± 0.31 | 19.11 ± 0.17 | 19.61 ± 0.14 |  | 19.57 ± 0.29 | 19.44 ± 0.23 | 0.832 |
| *Body weight gain (g)* | 0.73 ± 0.18 | 0.86 ± 0.16 | 0.87 ± 0.26 | 1.32 ± 0.11 |  | 0.84 ± 0.17 | 1.07 ± 0.20 | 0.146 |

Values represent the mean ± SE. N = 8 animals per dose group. N = 7 animals/group for LLNA/IRR Study 1 0.15% DNFB weight, LLNA/IRR Study 2 1.65% BMPY

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate NBuPY-exposed groups that differ significantly from the vehicle controls or significant trends across NBuPY-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies. The DMF/DMF, DMF/0.15% DNFB, and 0.15% DNFB/0.15% DNFB were excluded from the trend analysis for the MEST study.

For the MEST study, the VHIC (vehicle irritancy control) was the DMF/3.12% BMPY group.

**Supplemental Table 17** Body weight and body weight gain in female BALB/c mice exposed to NBuPY dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(DMF) | NBuPY | 0.15% DNFB | 25% HCA | Trend Analysis |
| 0.825% | 1.65% | 3.12% | 6.25% | 12.5% | p-value |
| *LLNA/IRR Study 1* |  |  |  |  |  |  |  |  |
| *Body weight (g)* |  |  |  |  |  |  |  |  |
|  Day 1 | 19.16 ± 0.24 | n/a | 18.96 ± 0.24 | 19.02 ± 0.30 | 19.06 ± 0.25 | 18.89 ± 0.29 | 18.92 ± 0.24 | 18.96 ± 0.27 | 0.528 |
|  Day 6 | 19.73 ± 0.19 | n/a | 19.53 ± 0.37 | 19.89 ± 0.33 | 19.93 ± 0.33 | 19.06 ± 0.25 | 20.08 ± 0.31 | 19.66 ± 0.30 | 0.182 |
| *Body weight gain (g)* | 0.58 ± 0.11 | n/a | 0.56 ± 0.15 | 0.87 ± 0.19 | 0.88 ± 0.18 | 0.16 ± 0.19 | **1.15 ± 0.22\*** | 0.69 ± 0.23 | 0.575 |
|  |  |  |  |  |  |  |  |  |  |
| *LLNA/IRR Study 2* |  |  |  |  |  |  |  |  |  |
| *Body weight (g)* |  |  |  |  |  |  |  |  |  |
|  Day 1 | 19.35 ± 0.32 | 19.19 ± 0.28 | 19.38 ± 0.39 | 19.30 ± 0.40 | 19.23 ± 0.32 | n/a | 19.33 ± 0.36 | 19.46 ± 0.33 | 0.634 |
|  Day 6 | 20.14 ± 0.28 | 19.87 ± 0.39 | 20.21 ± 0.48 | 19.80 ± 0.53 | 20.11 ± 0.28 | n/a | 20.35 ± 0.52 | 19.39 ± 0.40 | 0.962 |
| *Body weight gain (g)* | 0.79 ± 0.12 | 0.67 ± 0.19 | 0.83 ± 0.13 | 0.50 ± 0.23 | 0.88 ± 0.21 | n/a | 1.02 ± 0.21 | **-0.07 ± 0.15\*** | 0.981 |
|  |  |  |  |  |  |  |  |  |  |
| *MEST Study* |  |  |  |  |  |  |  |  |  |
| *Sensitization* | DMF | DMF | 3.12% NBuPY | 6.25% NBuPY | 12.5% NBuPY |  | DMF | 0.15% DNFB |  |
| *Challenge* | DMF | 12.5% NBuPY | 12.5% NBuPY | 12.5% NBuPY | 12.5% NBuPY |  | 0.15% DNFB | 0.15% DNFB |  |
| *Body weight (g)* |  |  |  |  |  |  |  |  |  |
|  Day 1 | 18.32 ± 0.35 | 18.60 ± 0.24 | 18.48 ± 0.31 | 19.22 ± 0.21 | 18.25 ± 0.29 |  | 18.62 ± 0.15 | 18.36 ± 0.30 | 0.893 |
|  Day 9 | 19.14 ± 0.45 | 19.85 ± 0.31 | 19.77 ± 0.33 | 20.47 ± 0.33 | 19.74 ± 0.33 |  | 19.84 ± 0.20 | 20.08 ± 0.42 | 0.893 |
| *Body weight gain (g)* | 0.82 ± 0.16 | 1.26 ± 0.14 | 1.29 ± 0.13 | 1.25 ± 0.25 | 1.49 ± 0.20 |  | 1.22 ± 0.11 | 1.72 ± 0.41 | 0.545 |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate NBUPY-exposed groups that differ significantly from the vehicle controls or significant trends across NBUPY-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies. The DMF/DMF, DMF/0.15% DNFB, and 0.15% DNFB/0.15% DNFB were excluded from the trend analysis for the MEST study.

For the MEST study, the VHIC (vehicle irritancy control) was the DMF/12.5% NBuPY group.

 **Supplemental Table 18** Local Lymph Node Assay (LLNA) in female BALB/c mice exposed to BMPY dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(DMF) | BMPY | 0.15% DNFB | 25% HCA | Trend Analysis |
| 0.825% | 1.65% | 3.12% | 6.25% | p-value |
| *LLNA Study 1* |  |  |  |  |  |  |  |
| DPM | 41.62 ± 7.41 | 43.56 ± 4.90 | 53.41 ± 4.27 | 50.80 ± 5.34 | **83.90 ± 12.65\*\*** | **966.38 ± 151.8\*\*** | **238.31 ± 29.37\*** | **0.003** |
| SI | 1.0 | 1.0 | 1.3 | 1.2 | 2.0 | 23.2 | 5.7 |  |
|  |  |  |  |  |  |  |  |  |
| *LLNA Study 2* |  |  |  |  |  |  |  |
| DPM | 122.29 ± 20.50 | 138.84 ± 19.29 | 95.85 ± 19.99 | 182.86 ± 23.76 | **232.43 ± 18.67\*\*** | **3149.4 ± 742.5\*\*** | **634.62 ± 82.88\*\*** | **0.002** |
| SI | 1.0 | 1.1 | 0.8 | 1.5 | 1.9 | 25.8 | 5.2 |  |

Values represent the mean ± SE. N = 8 animals per dose group. N = 7 animals/group for LLNA/IRR Study 2 1.65% BMPY

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate BMPY-exposed groups that differ significantly from the vehicle controls or significant trends across BMPY-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies.

**Supplemental Table 19** Local Lymph Node Assay (LLNA) in female BALB/c mice exposed to NBuPY dermally

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(DMF) | NBuPY |  | 0.15% DNFB | 25% HCA | Trend Analysis |
| 0.825% | 1.65% | 3.12% | 6.25% | 12.5% | p-value |
| *LLNA Study 1* |  |  |  |  |  |  |  |  |
| DPM | 67.64 ± 14.82 | n/a | 65.82 ± 15.34 | 56.51 ± 5.39 | 100.81 ± 11.08 | 87.58 ± 13.77 | **1277.0 ± 174.7\*\*** | **139.21 ± 17.06\*\*** | **0.025** |
| SI | 1.0 | n/a | 1.0 | 0.8 | 1.5 | 1.3 | 18.9 | 2.1 |  |
|  |  |  |  |  |  |  |  |  |  |
| *LLNA Study 2* |  |  |  |  |  |  |  |  |
| DPM | 143.18 ± 27.13 | 118.65 ± 0.81 | 129.2 ± 23.27 | 79.18 ± 8.12 | 140.04 ± 50.94 | n/a | **2559.3 ± 171.1\*\*** | **491.74 ± 102.2\*\*** | 0.093 |
| SI | 1.0 | 0.8 | 0.9 | 0.6 | 1.0 | n/a | 17.9 | 3.4 |  |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate NBuPY-exposed groups that differ significantly from the vehicle controls or significant trends across NBuPY-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies.

 **Supplemental Table 20** Irritancy Assay (IRR) in female BALB/c mice exposed to BMPY dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(DMF) | BMPY | 0.15% DNFB | 25% HCA | Trend Analysis |
| 0.825% | 1.65% | 3.12% | 6.25% | p-value |
| *IRR Study 1* |  |  |  |  |  |  |  |
| % ear swelling, 24 hrs | 2.55 ± 1.41 | 4.17 ± 1.38 | 6.08 ± 1.98 | 7.29 ± 2.08 | 5.46 ± 1.50 | **20.41 ± 1.79\*\*** | **38.21 ± 3.13\*\*** | 0.081 |
|  |  |  |  |  |  |  |  |  |
| *IRR Study 2* |  |  |  |  |  |  |  |
| % ear swelling, 24 hrs | 2.83 ± 1.52 | 3.16 ± 1.74 | 7.32 ± 0.97 | 2.67 ± 1.09 | 6.12 ± 1.91 | **16.80 ± 1.43\*\*** | **23.83 ± 1.50\*\*** | 0.416 |

Values represent the mean ± SE. N = 8 animals per dose group

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate BMPY-exposed groups that differ significantly from the vehicle controls or significant trends across BMPY-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies.

**Supplemental Table 21** Irritancy Assay (IRR) in female BALB/c mice exposed to NBuPY dermally

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameter | Vehicle(DMF) | NBuPY | 0.15% DNFB | 25% HCA | Trend Analysis |
| 1.65% | 3.12% | 6.25% | 12.5% |  |  | p-value |
| *IRR Study 1* |  |  |  |  |  |  |  |
| % ear swelling, 24 hrs | 0.03 ± 0.72 | 2.36 ± 1.24 | **5.19 ± 1.18\*\*** | **4.75 ± 1.38\*\*** | **10.31 ± 2.10\*\*** | **16.48 ± 2.16\*\*** | **31.99 ± 2.48\*\*** | **0.001** |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate NBuPY-exposed groups that differ significantly from the vehicle controls or significant trends across NBuPY-exposed groups and vehicle controls.

The 0.15% DNFB and 25% HCA positive controls were excluded from the trend analysis for the LLNA/IRR studies.

**Supplemental Table 22** Mouse Ear Swelling Test (MEST) in female BALB/c mice exposed to BMPY dermally

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Sensitization | DMF | DMF | 1.56% BMPY | 3.12% BMPY | DMF | 0.15% DNFB | Trend  |
| Challenge | DMF | 3.12% BMPY | 3.12% BMPY | 3.12% BMPY | 0.15% DNFB | 0.15% DNFB | Analysis |
| Parameter |  |  |  |  |  |  | p-value |
| % ear swelling, 24 hrs | -0.41 ± 1.66 | 0.07 ± 1.38 | 4.90 ± 1.81 | 1.94 ± 1.46 | 11.73 ± 3.45 | 28.53 ± 6.93 | 0.520 |
| % ear swelling, 48 hrs | 0.52 ± 1.12 | 0.10 ± 1.57 | 2.98 ± 1.96 | 3.83 ± 1.44 | 3.98 ± 2.47 | **30.83 ± 7.97\*\*** | 0.074 |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate BMPY-exposed groups that differ significantly from the vehicle controls or significant trends across BMPY-exposed groups and vehicle controls.

The DMF/DMF, DMF/0.15% DNFB, and 0.15% DNFB/0.15% DNFB were excluded from the trend analysis for the MEST study.

For the MEST study, the VHIC (vehicle irritancy control) was the DMF/3.12% BMPY group.

**Supplemental Table 23** Mouse Ear Swelling Test (MEST) in female BALB/c mice exposed to NBuPY dermally

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Sensitization | DMF | DMF | 3.12% NBuPY | 6.25% NBuPY | 12.5% NBuPY | DMF | 0.15% DNFB | Trend |
| Challenge | DMF | 12.5% NBuPY | 12.5% NBuPY | 12.5% NBuPY | 12.5% NBuPY | 0.15% DNFB | 0.15% DNFB | Analysis |
| Parameter |  |  |  |  |  |  |  | p-value |
| % ear swelling, 24 hrs | **1.13 ± 1.98\*** | 7.65 ± 1.84 | 5.57 ± 1.80 | **1.55 ± 1.94\*** | **1.09 ± 2.12\*** | 12.79 ± 2.12 | 22.98 ± 3.95 | **0.006** |
| % ear swelling, 48 hrs | 3.24 ± 2.12 | 7.23 ± 2.81 | 6.85 ± 2.33 | 4.96 ± 2.04 | 1.09 ± 2.04 | 11.87 ± 3.03 |  25.94 ± 5.55 | 0.053 |

Values represent the mean ± SE. N = 8 animals per dose group.

\*p < 0.05; \*\*p < 0.01. Bolded entries indicate NBuPY-exposed groups that differ significantly from the vehicle controls or significant trends across NBuPY -exposed groups and vehicle controls.

The DMF/DMF, DMF/0.15% DNFB, and 0.15% DNFB/0.15% DNFB were excluded from the trend analysis for the MEST study.

For the MEST study, the VHIC (vehicle irritancy control) was the DMF/12.5% NBuPY group.