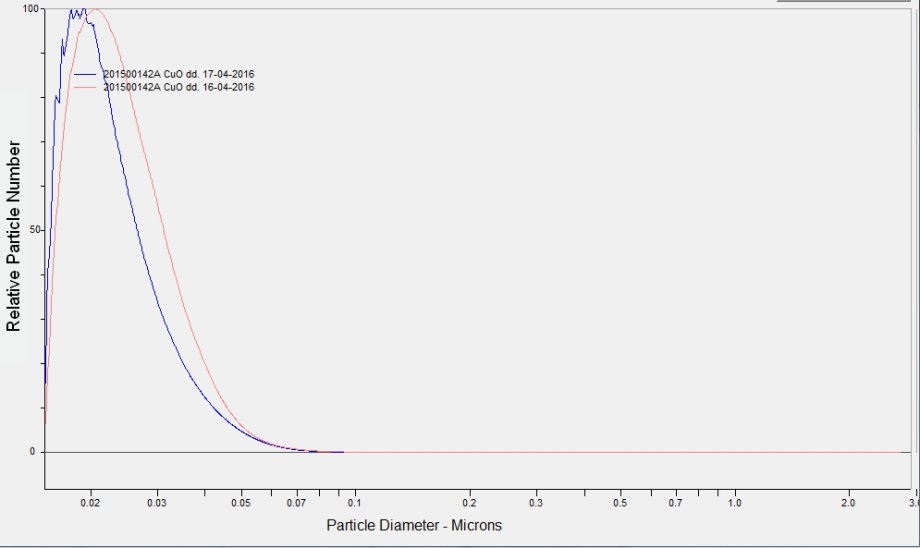
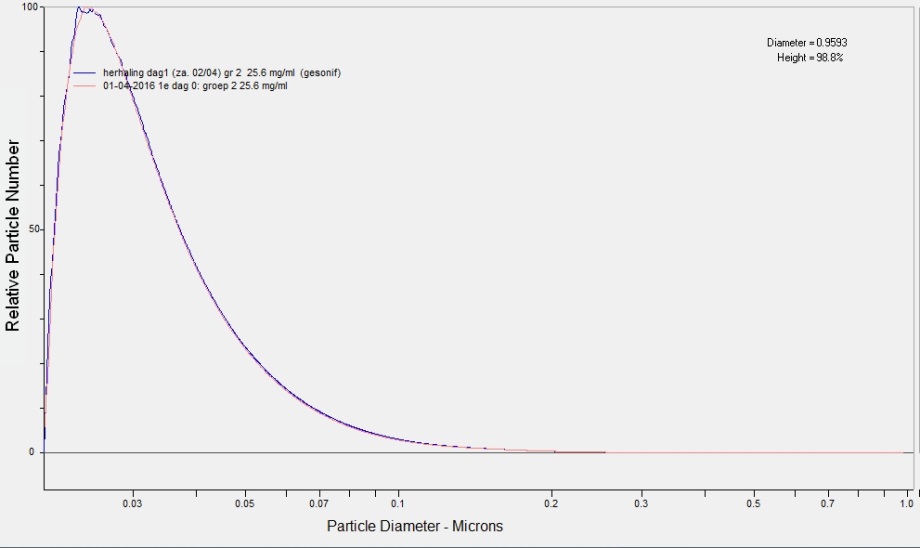
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TOXICITY OF COPPER OXIDE AND BASIC COPPER CARBONATE NANOPARTICLES AFTER SHORT TERM ORAL EXPOSURE IN RATS

Supplementary Figures and Tables.



Comparison CuO-NP dispersion freshly prepared versus 24 hours after preparation (102.4 mg/mL).



Comparison Cu2CO3(OH)2-NP dispersion freshly prepared versus 72 hours after preparation (25.6 mg/mL).

Supplementary Figure 1. Size distribution of CuO NP (top image ) and Cu2CO3(OH)2 NP (bottom image) dispersions freshly prepared and after storage for 24 hours and 72 hours, respectively, as measured by CPS Disc Centrifuge™. Data are presented in number metrics.

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Supplementary Figure 2. Morphology of pristine and transformed NPs by transmission electron microscopy (TEM). The top row depicts Cu2CO3(OH)2 NPs (a,b,c), while the bottom row is CuO NPs (d, e, f). The particles are illustrated before (a,b) and after dissolution testing at 37°C in either pH1.6 stomach simulant (b,e) or pH5.8 FeSSIF-V2 middle intestine simulant (c,f.). EDXS was unable to detect Cu content in the samples b and e.

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Supplementary Figure 3 Effects on bone marrow. The left image represents the bone marrow of a control animal. The right image indicates myeloid cell proliferation of the bone marrow at day 6 of an animal treated with Cu2CO3(OH)2 NPs 128 mg/kg b.w. for 5 consecutive days (days 1-5). Note abundance of myeloid cell lineage (\*) compared to erythroid cell lineage (#).

**Supplementary Table 1. Study design of five day oral toxicity study in rats.**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Study day** | **Days 1 to 5** | **Day 6** | **Day 7-25** | **Day 26** |
| **CuO NPs** | **Exposure by oral gavage (dose mg/kg)** | **Autopsy** | **Recovery** | **Autopsy** |
|  | 0 | N=4 |  | N=4 |
|  | 1 | N=4 |  | N=4 |
|  | 2 | N=4 |  | N=4 |
|  | 4 | N=4 |  | N=4 |
|  | 8 | N=4 |  | N=4 |
|  | 16 | N=4 |  | N=4 |
|  | 32 | N=4 |  | N=4 |
| **Pilot study** | 64 | N=2 |  |  |
| **Extra study** | 512 | N=4 |  |  |
| **Cu2CO3(OH)2 NPs** | **Exposure by oral gavage (dose mg/kg)** | **Autopsy** | **Recovery** | **Autopsy** |
| **Dose (mg/kg)** | 0 | N=4 |  | N=4 |
|  | 4 | N=4 |  | N=4 |
|  | 8 | N=4 |  | N=4 |
|  | 16 | N=4 |  | N=4 |
|  | 32 | N=4 |  | N=4 |
|  | 64 | N=4 |  | N=4 |
|  | 128 | N=4+4a |  |  |

1. N=4 unscheduled autopsy in due to overt signs of toxicity

**Supplementary Table 2A Body and organ weight of male rats at day 6 after 5 consecutive days of oral administration of CuO NPs.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CuO NPs |  |  |  |  |  |  |  |  | Pilot Study |  | Extra Study |
| Dose | 0  mg/kg | 1  mg/kg | 2  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg |  | 64  mg/kg |  | 512  mg/kga |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Body weight (g) | 275 ± 8 | 275 ± 25 | 292 ±9 | 310 ± 46 | 288 ±8 | 290 ± 7 | 280 ± 23 |  | 288 ± 4 |  | 334 ± 43 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Lung | 1.30 ± 0.12 | 1.33 ± 0.10 | 1.37 ± 0.09 | 1.53 ± 0.09 (3) | 1.53 ± 0.08 | 1.39 ± 0.08 | 1.35 ± 0.10 |  | 1.29 (1) |  | 1.48 ± 0.09 |
| Heart | 0.881 ± 0.02 | 0.938 ± 0.06 | 0.957 ± 0.02 | 0.934 ± 0.06 | 0.945 ± 0.02 | 0.976 ± 0.03 | 0.931 ± 0.06 |  | 0.934 ± 0.08 |  | 1.097 ± 0.16 |
| Liver | 10.67 ± 0.52 | 11.00 ± 0.47 (3) | 11.19 ± 0.62 | 11.01 ± 0.63 | 11.22 ± 0.39 | 10.52 ±0.80 | 10.89 ± 1.14 |  | 11.01 ± 0.19 |  | 12.06 ± 2.21 |
| Kidney (L+R) | 2.18 ± 0.08 | 2.28 ± 0.14 (3) | 2.31 ± 0.10 | 2.22 ± 0.18 | 2.33 ± 0.06 | 2.33 ± 0.09 | 2.14 ± 0.26 |  | 2.18 ± 0.08 |  | 2.25 ± 0.25 |
| Spleen | 0.50 ± 0.04 | 0.50 ± 0.04 | 0.53 ± 0.03 | 0.52 ± 0.04 | 0.53 ±0.03 | 0.54 ± 0.03 | 0.54 ± 0.04 |  | 0.51 ± 0.06 |  | 0.855 ± 0.22 |
| Thymus | 0.503 ± 0.04 | 0.537 ± 0.09 | 0.552 ± 0.04 | 0.591 ± 0.02 | 0.544 ± 0.04 (3) | 0.530 ±0.07 | 0.527 ± 0.06 |  | 0.478 ± 0.03 |  | 0.526 ± 0.21 |
| Mesenteric Lymph Node | 0.126 ± 0.01 | 0.157 ± 0.05 | 0.161 ± 0.03 | 0.154 ± 0.02 | 0.144 ± 0.02 | 0.164 ± 0.03 | 0.155 ± 0.02 |  | 0.125 ± 0.02 |  | 0.263 ± 0.07 |
| Testes (L+R) | 2.66 ± 0.18 | 2.50 ±0.64 | 2.83 ± 0.11 | 2.93 ± 0.10 | 2.71 ± 0.22 | 2.80 ± 0.11 | 2.78 ± 0.14 |  | 2.79 ± 0.06 |  | 3.31 ± 0.30 |
| Brain | 2.00 ± 0.03 | 2.03 ± 0.01 | 20.6 ± 0.02 | 2.02 ± 0.03 | 2.04 ±0.04 | 2.07 ± 0.04(3) | 20.3 ± 0.06 |  | 2.06 (1) |  | 2.11 ± 0.03 |
| Adrenals | 0.032 ± 0.001 | 0.034 ± 0.005 | 0.037 ± 0.003 | 0.037 ± 0.002 | 0.039 ± 0.004 (2) | 0.037 ± 0.003 | 0.039 ± 0.003 |  | - |  | 0.058 ± 0.005 |

Values represent mean ± SD (weight in g). The number of animals per group is 4 (n=4) unless otherwise indicated in the table within brackets. For the dose of 64 mg/kg in the pilot study two (n=2) animals were evaluated. Autopsy was conducted at day 6 (24 hours after the last oral administration).

a) Animals with dose 512 mg/kg as extra group were treated without concurrent contemporary control.

**Supplementary Table 2B Body and organ weights of male rats at day 26 after 5 consecutive days of oral administration of CuO NPs.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| CuO NPs |  |  |  |  |  |  |  |
| Dose | 0  mg/kg | 1  mg/kg | 2  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg |
|  |  |  |  |  |  |  |  |
| Body weight (g) | 343 ± 7 | 332 ± 9 | 351 ±14 | 318 ± 56 | 345 ± 12 | 329 ± 41 | 341 ± 14 |
|  |  |  |  |  |  |  |  |
| Lung | 1.47 ± 0.08 | 1.54 ± 0.20 | 1.51 ± 0.07 | 1.50 ± 0.16 (3) | 1.53 ± 0.10 | 1.41 ± 0.18 | 1.52 ± 0.17 |
| Heart | 1.011 ± 0.04 | 0.987 ± 0.08 | 1.037 ± 0.08 | 0.992 ± 0.07 | 1.012 ± 0.08 | 1.052 ± 0.04 | 1.019 ± 0.06 |
| Liver | 12.36 ± 0.27 | 11.67 ±0.72 | 12.40 ± 0.70 | 11.03 ± 1.99 | 12.80 ± 1.12 | 11.18 ± 2.24 | 11.92 ± 0.42 |
| Kidney (L+R) | 2.61 ±0.06 | 2.46 ± 0.25 | 2.61 ± 0.12 | 2.38 ± 0.42 | 2.38 ± 0.32 | 2.45 ± 0.52 | 2.78 ± 0.39 |
| Spleen | 0.582 ± 0.03 | 0.521 ±0.04 | 0.582 ± 0.03 | 0.436 ± 0.17 | 0.595 ± 0.03 | 0.540 ± 0.05 | 0.599 ± 0.11 |
| Thymus | 0.483 ± 0.07 | 0.407 ± 0.04 | 0.486 ± 0.04 | 0.466 ± 0.06 | 0.485 ± 0.03 | 0.433 ± 0.13 | 0.493 ± 0.06 |
| Mesenteric Lymph Node | 0.189 ± 0.04 | 0.173 ± 0.01 | 0.200 ± 0.01 | 0.169 ± 0.01 | 0.161 ± 0.02 | 0.159 ± 0.02 | 0.204 ± 0.001 |
| Testes (L+R) | 2.97 ± 0.09 | 2.86 ± 0.06 | 3.10 ± 0.21 | 2.77 ± 0.41 | 2.89 ± 0.24 | 2.91 ± 0.37 | 2.98 ±0.07 |
| Brain | 2.14 ± 0.02 | 2.09 ± 0.04 | 2.15 ± 0.02 | 2.08 ± 0.09 | 2.10 ± 0.03 | 2.08 ± 0.11 | 2.18 ± 0.07 |
| Adrenals | 0.041 ± 0.004 | 0.037 ± 0.003 | 0.042 ± 0.002 | 0.042 ± 0.002 | 0.040 ± 0.007 | 0.040 ± 0.005 | 0.040 ± 0.003 |
|  |  |  |  |  |  |  |  |

Values represent mean ± SD (weight in g). The number of animals per group is 4 (n=4) unless otherwise indicated in the table within brackets. Autopsy was conducted at day 26 (21 days after the last oral administration).

**Supplementary Table 2C Body and organ weight in male rats at days 6/7 after 5 consecutive days of oral administration of Cu2CO3(OH)2 NPs.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cu2CO3(OH)2 NPs |  |  |  |  |  |  |  |  |  |  |  |
| Dose | 0  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg | 64  mg/kg | 128  mg/kg |  | 128  mg/kga |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Body weight (g) | 345 ± 11 | 385 ±24 | 394 ± 58 | 367 ± 15 | 362 ±11 | 344 ± 29 | 327 ± 73 |  | 290 ± 16\*\* |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |
| Lung | 1.48 ± 0.11 | 1.56 ± 0.15 | 1.50 ± 0.04 | 1.50 ± 0.08 | 1.36 ± 0.35 | 1.51 ± 0.53 | 1.46 ± 0.17 |  | 1.34 ± 0.16 |  |  |
| Heart | 1.210 ± 0.05 | 1.279 ± 0.07 | 1.300 ± 0.08 | 1.244 ±0.09 | 1.140 ± 0.06 | 1.185 ± 0.16 | 1.002 ± 0.14\* |  | 0.928 ± 0.02\*\* |  |  |
| Liver | 13.40 ± 0.57 | 15.68 ± 1.52 | 15.14 ± 1.24 | 14.79 ± 0.25 | 14.42 ± 0.90 | 14.15 ± 2.22 | 10.79 ± 1.22\*\* |  | 10.43 ± 1.33\*\* |  |  |
| Kidney (L+R) | 2.23 ± 0.15 | 2.35 ± 0.14 | 2.38 ± 0.05 | 2.26 ± 0.11 | 2.21 ± 0.25 | 2.29 ± 0.30 | 2.00 ± 0.75 |  | 2.14 ± 0.45 |  |  |
| Spleen | 0.992 ± 0.09 | 1.040 ± 0.16 | 1.032 ± 0.20 | 0.955 ± 0.09 | 1.020 ± 0.26 | 1.059 ± 0.15 | 0.508 ± 0.08\*\* |  | 0.540 ± 0.16\*\* |  |  |
| Thymus | 0.706 ± 0.13 | 0.789 ± 0.10 | 0.826 ± 0.04 | 0.745 ± 0.16 | 0.798 ± 0.21 | 0.717 ± 0.19 | 0.221 ± 0.06\*\* |  | 0.192 ± 0.06\*\* |  |  |
| Mesenteric Lymph Node | 0.254 ± 0.06 | 0.265 ± 0.05 | 0.227 ± 0.03 | 0.188 ± 0.03 | 0.263 ± 0.03 | 0.224 ± 0.08 | 0.210 ± 0.04 |  | 0.222 ± 0.06 |  |  |
| Testes (L+R) | 3.33 ± 0.23 | 3.42 ± 0.37 | 3.26 ± 0.38 | 2.66 ± 0.17 | 3.11 ± 0.28 | 3.29 ± 0.37 | 3.02 ± 0.29 |  | 3.06 ± 0.34 |  |  |
| Brain | 1.92 ± 0.03 | 1.94 ± 0.05 | 1.88 ± 0.02 | 1.93 ± 0.08 | 1.88 ± 0.11 | 1.91 ± 0.08 | 1.86 ± 0.07 |  | 1.84 ± 0.11 |  |  |
| Adrenals | 0.043 ± 0.004 | 0.051 ± 0.009 | 0.051 ± 0.006 | 0.051 ± 0.006 | 0.052 ± 0.005 | 0.051 ± 0.004 | 0.071 ± 0.015\* |  | 0.063 ± 0.008\*\* |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |

Values represent mean ± SD (weight in g). The number of animals per group is 4 (n=4) unless otherwise indicated in the table within brackets. Significant differences compared to vehicle treated animals. \*p<0.05, \*\*P<0.01 (ANOVA). Autopsy was conducted at day 6/ 7 (128 mg/kg b.w.) (24 or 48 hours after the last oral administration).

a)Unscheduled autopsy of dose group of 128 mg/kg at days 6/7 after treatment in view of poor condition of the animals.

**Supplementary Table 2D Body and organ weight in male rats at day 26 after 5 consecutive days of oral administration of Cu2CO3(OH)2 NPs -At day 26 (21 days after the last administration).**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cu2CO3(OH)2 NPs |  |  |  |  |  |  |
| Dose | 0  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg | 64  mg/kg |
|  |  |  |  |  |  |  |
| Body weight (g) | 457 ± 13 | 456 ± 24 | 426 ± 28 | 465 ± 50 | 448 ± 37 | 455 ± 26 |
|  |  |  |  |  |  |  |
| Lung | 1.81 ± 0.18 | 1.74 ± 0.03 | 1.44 ± 0.33 | 1.77 ± 0.20 | 1.60 ± 0.09 | 1.46 ± 0.24 |
| Heart | 1.356 ± 0.06 | 1.429 ± 0.08 | 1.342 ± 0.05 | 1.443 ± 0.16 | 1.402 ± 0.04 | 1.414 ± 0.11 |
| Liver | 16.27 ± 1.71 | 15.82 ± 0.85 | 14.73 ± 1.10 | 17.72 ± 3.15 | 15.82 ± 2.39 | 16.56 ± 1.94 |
| Kidney (L+R) | 2.70 ± 0.19 | 2.68 ± 0.26 | 2.58 ± 0.39 | 2.59 ± 0.15 | 2.62 ± 0.24 | 2.56 ± 0.30 |
| Spleen | 1.111 ± 0.17 | 1.132 ± 0.20 | 1.066 ± 0.10 | 1.145 ± 0.22 | 1.063 ± 0.11 | 1.034 ± 0.12 |
| Thymus | 0.777 ± 0.20 | 0.575 ± 0.07 | 0.629 ± 0.05 | 0.721 ± 0.15 | 0.720 ± 0.15 | 0.695 ± 0.12 |
| Mesenteric Lymph Node | 0.208 ± 0.02 | 0.210 ± 0.04 | 0.194 ± 0.02 | 0.227 ± 0.05 | 0.216 ± 0.05 (3) | 0.226 ± 0.03 |
| Testes (L+R) | 3.50 ± 0.17 | 3.12 ± 0.35 | 3.53 ± 0.06 | 3.64 ± 0.21 | 3.40 ± 0.13 | 3.42 ± 0.26 |
| Brain | 1.99 ± 0.08 | 2.00 ± 0.05 | 1.97 ± 0.04 | 2.03 ± 0.02 | 1.97 ± 0.07 | 1.99 ± 0.09 |
| Adrenals | 0.053 ± 0.005 (3) | 0.053 ± 0.005 | 0.051 ± 0.010 | 0.046 ± 0.010 | 0.050 ± 0.006 | 0.052 ± 0.004 |
|  |  |  |  |  |  |  |

Values represent mean ± SD (weight in g). The number of animals per group is 4 (n=4) unless otherwise indicated in the table within brackets. Autopsy was conducted at day 26 (21 days after the last oral administration).

**Supplementary Table 3A Haematology in male rats at day 6 after 5 consecutive days of oral administration of CuO NPs.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CuO NPs |  |  |  |  |  |  |  |  | Pilot Study |  | Extra Study |
| Dose | 0  mg/kg | 1  mg/kg | 2  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg |  | 64  mg/kg |  | 512  mg/kga |
|  |  |  |  |  |  |  |  |  |  |  |  |
| WBC  x109/L | 5.07 ± 0.53 | 4.46 ± 0.52 | 5.27 ± 0.7 | 3.92 ± 0.40\*b | 4.40 ± 0.41 | 4.39 ± 0.51 | 3.98 ± 0.24\* |  | 2.89 ± 0.41\*\* |  | 9.53 ± 1.47 |
| RBC  x1012/L | 8.03 ± 0.29 | 7.96 ± 0.15 | 8.00 ± 0.12 | 8.12 ± 0.10 | 7.84 ± 0.27 | 7.81 ± 0.12 | 7.68 ± 0.21 |  | 7.50 ± 0.12\* |  | 8.33 ± 0.92 |
| HgB  Mmol/L | 8.60 ± 0.36 | 8.38 ± 0.40 | 8.60 ± 0.16 | 8.65 ± 0.13 | 8.43 ± 0.26 | 8.33 ± 0.15 | 8.05 ± 0.21 |  | 8.07 ± 0.19\* |  | 9.25 ± 0.85 |
| Hct  L/L | 0.415 ± 0.02 | 0.403 ± 0.02 | 0.410 ± 0.01 | 0.420 ± 0.02 | 0.402 ± 0.02 | 0.403 ± 0.01 | 0.400 ± 0.00 |  | 0.380 ±0.00\* |  | 0.218 ± 0.02 |
| MCV  fl | 52 ± 0.7 | 50 ± 1.7 | 51 ± 0.5 | 51 ± 0.6 | 52 ± 0.9 | 52 ± 0.4 | 52 ± 1.3 |  | 51 ± 0.1 |  | 52 ± 1.6 |
| MCH  fmol | 1.07 ± 0.01 | 1.05 ± 0.03 | 1.08 ± 0.02 | 1.07 ± 0.01 | 1.07 ± 0.01 | 1.07 ± 0.01 | 1.05 ± 0.00 |  | 1.07 ± 0.01 |  | 1.12 ± 0.02 |
| MCHC  Mmol/L | 20.62 ± 0.22 | 20.79 ± 0.20 | 20.97 ± 0.25 | 20.68 ± 0.24 | 20.76 ± 0.31 | 20.60 ± 0.17 | 20.46 ± 0.48 |  | 20.77 ± 0.17 |  | 21.24 ± 0.33 |
| RDW  % | 11.41 ± 0.19 | 11.69 ± 0.59 | 11.59 ± 0.19 | 11.44 ± 0.18 | 11.36 ± 0.19 | 11.44 ± 0.18 | 11.58 ± 0.11 |  | 11.60 ± 0.14 |  | 11.96 ± 0.21 |
| HDW  Mmol/L | 1.45 ± 0.06 | 1.50 ± 0.04 | 1.51 ± 0.01 | 1.47 ± 0.02 | 1.43 ± 0.04 | 1.48 ± 0.07 | 1.43 ± 0.10 |  | 1.43 ± 0.05 |  | 1.67 ± 0.08 |
| PLT  x109/L | 739 ± 36 | 577 ± 204 | 730 ± 32 | 728 ± 18 | 739 ± 9 | 692 ± 146 | 766 ± 41 |  | 739 ± 45 |  | 1196 ± 236 |
| MPV  fl | 6.70 ± 0.23 | 7.56 ± 0.97 | 6.90 ± 0.25 | 6.46 ± 0.29 | 6.90 ± 0.43 | 7.15 ± 0.95 | 6.45 ± 0.42 |  | 6.82 ± 0.26 |  | 7.56 ± 0.70 |
| Neutrophils  % | 9.9 ± 0.6 | 13.0 ± 3.0 | 11.1 ± 1.7 | 8.8 ± 1.1 | 11.7 ± 0.8 | 13.0 ± 2.1 | 11.2 ± 1.1 |  | 15.0 ± 2.1 |  | 27.5 ± 13.6 |
| Lymphocytes  % | 86.5 ± 0.7 | 80.8 ±4.6 | 84.7 ± 2.4 | 85.5 ± 2.0 | 83.0 ± 1.0 | 81.4 ± 2.8 | 83.0 ± 0.8\* |  | 81.4 ± 2.6 |  | 67.2 ± 15.4 |
| Monocytes  % | 1.54 ± 0.38 | 1.80 ± 0.54 | 2.01 ± 0.14 | 1.66 ± 0.21 | 1.37 ± 0.13 | 1.71 ± 0.20 | 1.78 ± 0.32 |  | 1.52 ± 0.26 |  | 2.56 ± 1.11 |
| Eosinophils  % | 1.61 ± 0.70 | 3.99 ± 1.51 | 1.66 0.69 | 3.65 ± 1.96 | 3.76 ± 0.32 | 3.56 ± 1.62 | 3.68 ± 0.18\*\* |  | 1.80 ± 0.80 |  | 0.64 ± 0.38 |
| LUC  % | 0.30 ± 0.12 | 0.33 ± 0.15 | 0.46 ± 0.08 | 0.41 ± 0.11 | 0.23 ± 0.05 | 0.25 ± 0.07 | 0.35 ± 0.28 |  | 0.18 ± 0.02 |  | 2.01 ± 1.38 |
| Basophils  % | 0.05 ± 0.04 | 006 ± 0.06 | 0.05 ± 0.10 | 0.03 ± 0.05 | 0.09 ± 0.04 | 0.06 ± 0.05 | 0.05 ± 0.00 |  | 0.10 ± 0.09 |  | 0.08 ± 0.03 |
| Reticulocytes  % | 2.70 ± 0.41 | 2.69 ± 0.44 | 2.85 ± 0.11 | 2.49 ± 0.34 | 2.47 ± 0.25 | 2.98 ± 0.38 | 2.57 ± 0.5 |  | 2.07 ± 0.30 |  | 1.66 ± 1.04 |
| Neutrophils  x109/L | 0.50 ± 0.08 | 0.58 ± 0.15 | 0.59 ± 0.16 | 0.35 ± 0.07 | 0.51 ± 0.07 | 0.58 ± 0.16 | 0.44 ± 0.01 |  | 0.44 ± 0.13 |  | 2.49 ± 1.09 |
| Lymphocytes  x109/L | 4.39 ± 0.44 | 3.62 ± 0.53 | 4.45 ± 0.48 | 3.36 ± 0.38 | 3.64 ± 0.33 | 3.56 ± 0.31 | 3.30 ± 0.23\* |  | 2.35 ± 0.26\*\* |  | 6.56 ± 2.54 |
| Monocytes  x109/L | 0.08 ± 0.02 | 0.08 ± 0.02 | 0.11 ± 0.02 | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.08 ± 0.02 | 0.07 ± 0.01 |  | 0.04 ± 0.00 |  | 0.23 ± 0.09 |
| Eosinophils  x109/L | 0.08 ± 0.04 | 0.17 ± 0.05 | 0.09 ± 0.05 | 0.14 ± 0.07 | 0.16 ± 0.00 | 0.16 ± 0.07 | 0.15 ± 0.02 |  | 0.05 ± 0.03 |  | 0.06 ± 0.03 |
| LUC  x109/L | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.00 | 0.02 ± 0.01 | 0.01 ± 0.00 | 0.01 ± 0.01 | 0.02 ± 0.01 |  | 0.00 ± 0.00 |  | 0.01 ± 0.01 |
| Basophils  x109/L | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |  | 0.00 ± 0.00 |  | 0.18 ± 0.10 |
| Reticulocytes  x1012/L | 0.22 ± 0.03 | 0.21 ± 0.03 | 0.23 ± 0.01 | 0.20 ± 0.03 | 0.19 ± 0.01 | 0.23 ± 0.03 | 0.20 ± 0.02 |  | 0.16 ± 0.02 |  | 0.13 ± 0.07 |

Values represent mean ± SD (n=4). The pilot study (dose 64 mg/kg b.w. was performed with n=2. For the dose of 32 mg/kg b.w. n=2 animals could be evaluated. Blood samples were obtained at day 6 (24 hours after the last administration).

1. Animals with dose 512 mg/kg as extra group were treated without concurrent contemporary control.
2. One sided students t-test, \* P<0.05, \*\* p<0.01, \*\*\* p<0.01.

**Supplementary Table 3B Haematology in male rats at day 26 after 5 consecutive days of oral administration of CuO NPs.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CuO NPs |  |  |  |  |  |  |  |  |
| Dose | 0  mg/kg |  | 1  mg/kg | 2  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg |
|  |  |  |  |  |  |  |  |  |
| WBC  x109/L | 5.40 ± 0.99 |  | 5.95 ± 1.18 | 5.15 ± 0.31 | 4.87 ± 0.80 | 6.44 ± 0.96 | 5.60 ± 1.13 | 5.33 ± 0.99 |
| RBC  x1012/L | 8.54 ± 0.25 |  | 8.55 ± 0.06 | 8.59 ± 0.16 | 8.66 ± 0.27 | 8.69 ± 0.15 | 8.77 ± 0.31 | 8.28 ± 0.96 |
| HgB  Mmol/L | 8.73 ± 0.22 |  | 8.57 ± 0.06 | 8.55 ± 0.29 | 8.73 ± 0.31 | 8.85 ± 0.25 | 8.87 ± 0.31 | 8.44 ± 0.83 |
| Hct  L/L | 0.410 ± 0.01 |  | 0.413 ± 0.01 | 0.405 ± 0.01 | 0.413 ± 0.01 | 0.425 ± 0.01 | 0.427 ± 0.01 | 0.398 ± 0.04 |
| MCV  fl | 49 ± 0.5 |  | 48 ± 0.4 | 47 ± 0.3 | 47 ± 0.6 | 49 ± 0.3 | 48 ± 0.1 | 48 ± 0.8 |
| MCH  fmol | 1.02 ± 0.02 |  | 1.01 ± 0.02 | 1.00 ± 0.01 | 1.01 ± 0.01 | 1.02 ± 0.01 | 1.01 ± 0.01 | 1.02 ± 0.02 |
| MCHC  Mmol/L | 20.95 ± 0.14 |  | 20.92 ± 0.14 | 21.00 ± 0.28 | 21.26 ± 0.27 | 20.94 ± 0.14 | 21.07 ± 0.07 | 21.21 ± 0.19 |
| RDW  % | 11.49 ± 0.15 |  | 11.55 ± 0.09 | 11.59 ± 0.14 | 11.55 ± 0.22 | 11.48 ± 0.12 | 11.57 ± 0.15 | 11.59 ± 0.19 |
| HDW  Mmol/L | 1.71 ± 0.03 |  | 1.74 ± 0.01 | 1.73 ± 0.03 | 1.80 ± 0.05 | 1.69 ± 0.03 | 1.75 ± 0.03 | 1.69 ± 0.04 |
| PLT  x109/L | 707 ± 20 |  | 714 ± 28 | 693 ± 26 | 706 ± 23 | 678 ± 60 | 681 ± 19 | 749 ± 69 |
| MPV  fl | 7.14 ± 0.29 |  | 6.98 ± 0.24 | 6.93 ± 0.19 | 7.15 ± 0.44 | 7.11 ± 0.28 | 7.23 ± 0.51 | 6.83 ± 0.17 |
| Neutrophils  % | 11.4 ± 1.3 |  | 12.0 ± 0.2 | 10.8 ± 2.0 | 10.1 ± 1.3 | 12.0 ± 2.1 | 11.6 ± 2.9 | 10.8 ± 2.4 |
| Lymphocytes  % | 84.1 ± 1.8 |  | 84.0 ± 1.1 | 85.0 ± 2.6 | 86.6 ± 2.5 | 84.0 ± 2.4 | 85.0 ± 3.5 | 83.5 ± 4.2 |
| Monocytes  % | 1.41 ± 0.06 |  | 2.00 ± 0.43 | 1.88 ± 0.48 | 1.60 ± 0.40 | 1.78 ± 0.46 | 1.47 ± 0.38 | 1.85 ± 0.48 |
| Eosinophils  % | 2.68 ± 2.08 |  | 1.43 ± 0.68 | 1.66 ± 0.21 | 1.35 ± 0.74 | 1.75 ± 0.26 | 1.33 ± 0.25 | 3.25 ± 2.51 |
| LUC  % | 0.39 ± 0.22 |  | 0.45 ± 0.13 | 0.58 ± 0.21 | 0.38 ± 0.16 | 0.45 ± 0.19 | 0.55 ± 0.26 | 0.42 ± 0.31 |
| Basophils  % | 0.06 ± 0.03 |  | 0.07 ± 0.03 | 0.03 ± 0.03 | 0.08 ± 0.03 | 0.04 ± 0.05 | 0.07 ± 0.06 | 0.12 ± 0.04 |
| Reticulocytes  % | 2.15 ± 0.16 |  | 2.22 ± 0.18 | 2.12 ± 0.06 | 2.17 ± 0.27 | 2.00 ± 0.12 | 2.05 ± 0.20 | 2.24 ± 0.43 |
| Neutrophils  x109/L | 0.62 ± 0.17 |  | 0.72 ± 0.14 | 0.56 ± 0.12 | 0.48 ± 0.07 | 0.76 ± 0.04 | 0.67 ± 0.30 | 0.59 ± 0.24 |
| Lymphocytes  x109/L | 4.54 ± 0.86 |  | 5.01 ± 1.06 | 4.38 ± 0.21 | 4.22 ± 0.75 | 5.43 ± 0.97 | 4.73 ± 0.76 | 4.43 ± 0.62 |
| Monocytes  x109/L | 0.08 ± 0.01 |  | 0.12 ± 0.01 | 0.10 ± 0.03 | 0.08 ± 0.02 | 0.11 ± 0.01 | 0.08 ± 0.03 | 0.09 ± 0.00 |
| Eosinophils  x109/L | 0.14 ± 0.08 |  | 0.08 ± 0.02 | 0.08 ± 0.02 | 0.07 ± 0.03 | 0.12 ± 0.03 | 0.08 ± 0.03 | 0.19 ± 0.17 |
| LUC  x109/L | 0.02 ± 0.02 |  | 0.03 ± 0.01 | 0.03 ± 0.02 | 0.02 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.02 | 0.02 ± 0.01 |
| Basophils  x109/L | 0.00 ± 0.00 |  | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| Reticulocytes  x1012/L | 0.18 ± 0.01 |  | 0.19 ± 0.01 | 0.18 ± 0.00 | 0.19 ± 0.02 | 0.17 ± 0.01 | 0.18 ± 0.02 | 0.18 ± 0.01 |

Values represent mean ± SD (n=4). Blood samples were obtained at day 26 (21 days after the last administration).For some animals blood was not available for evaluation. The number of animals is n=3 for the dose groups of 1, 4 and 16 mg/kg b.w.

**Supplementary Table 3C. Haematology in male rats at day 6 after 5 consecutive days of oral administration of Cu2CO3(OH)2 NPs.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cu2CO3(OH)2 NPs |  |  |  |  |  |  |  |  |  |
| Dose | 0  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg | 64  mg/kg | 128  mg/kg |  | 128  mg/kga |
|  |  |  |  |  |  |  |  |  |  |
| WBC  x109/L | 8.29 ± 1.56 (3) | 13.10 ± 1.75 | 11.46 ± 0.55 | 10.78 ± 1.72 | 13.48 ± 3.13 | 16.37 ± 4.85\* | 17.49 ± 7.8\* |  | 18.35 ± 6.8\* |
| RBC  x1012/L | 7.32 ± 0.26 | 7.57 ± 0.3 | 7.27 ± 0.4 | 7.20 ± 0.35 | 7.32 ± 0.36 | 7.51 ± 0.25 | 9.36 ± 1.5\*\* |  | 8.38 ± 0.17 |
| HgB  Mmol/L | 8.23 ± 0.4 | 8.48 ± 0.56 | 8.45 ± 0.31 | 8.30 ± 0.35 | 8.20 ± 0.39 | 8.68 ± 0.22 | 10.0 ± 0.92\*\* |  | 9.43 ± 0.21\* |
| Hct  L/L | 0.40 ± 0.02 | 0.41 ± 0.03 | 0.41 ± 0.01 | 0.40 ± 0.02 | 0.40 ± 0.02 | 0.42 ± 0.01 | 0.48 ± 0.05\*\* |  | 0.45 ± 0.01\* |
| MCV  fl | 54 ± 1 | 54 ± 1 | 57 ± 3 | 55 ± 1 | 54 ± 1 | 55 ± 1 | 52 ± 4 |  | 54 ± 2 |
| MCH  fmol | 1.12 ± 0.02 | 1.12 ± 0.04 | 1.17 ± 0.06 | 1.15 ± 0.03 | 1.12 ± 0.01 | 1.16 ± 0.04 | 1.08 ± 0.11 |  | 1.12 ± 0.05 |
| MCHC  Mmol/L | 20.6 ± 0.11 | 20.7 ± 0.23 | 20.3 ± 0.36 | 20.8 ± 0.10 | 20.6 ± 0.11 | 20.9 ± 0.46 | 20.8 ± 0.86 |  | 20.8 ± 0.29 |
| RDW  % | 12.23 ± 0.37 | 12.75 ± 0.44 | 12.44 ± 0.63 | 12.30 ± 0.55 | 12.44 ± 0.43 | 12.10 ± 0.88 | 13.08 ± 1.44 |  | 13.37 ± 0.8 |
| HDW  Mmol/L | 1.62 ± 0.04 | 1.64 ± 0.06 | 1.65 ± 0.04 | 1.68 ± 0.06 | 1.66 ± 0.04 | 1.67 ± 0.12 | 2.01 ± 0.28\*\* |  | 1.91 ± 0.10\* |
| PLT  x109/L | 969 ± 79 | 956 ± 114 | 1160 ± 172 | 915 ± 200 | 1033 ±94 | 971 ± 241 | 1171 ± 102 |  | 1135 ± 35 |
| MPV  fl | 6.27 ± 0.50 | 6.58 ± 0.68 | 6.28 ± 0.58 | 6.46 ± 0.25 | 6.56 ± 0.73 | 7.16 ± 1.73 | 6.64 ± 1.35 |  | 6.33 ± 0.50 |
| Neutrophils  % | 16.4 ± 2.6 | 15.8 ± 1.5 | 13.2 ± 4.4 | 16.0 ± 3.2 | 17.0 ± 2.4 | 16.8 ± 3.7 | 55.5 ± 14.1\*\*\* |  | 49.5 ± 7.4\*\* |
| Lymphocytes  % | 78.9 ± 3.2 | 79.1 ± 1.8 | 82.3 ± 5.1 | 79.5 ± 3.3 | 78.0 ± 2.8 | 77.3 ± 4.2 | 35.1 ± 16\*\*\* |  | 39.4 ± 9.4\*\* |
| Monocytes  % | 2.2 ± 0.5 | 2.7 ± 0.5 | 2.5 ± 0.4 | 2.2 ± 0.3 | 2.7 ± 0.5 | 3.8 ± 0.3\*\* | 6.0 ± 0.7\*\*\* |  | 7.2 ± 0.5\*\*\* |
| Eosinophils  % | 2.0 ± 0.8 | 1.8 ± 0.4 | 1.5 ± 0.6 | 1.7 ± 0.1 | 1.6 ± 0.6 | 1.4 ± 0.4 | 0.7 ± 0.1 |  | 0.8 ± 0.4 |
| LUC  % | 0.5 ± 0.3 | 0.5 ± 0.1 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.4 | 0.6 ± 0.1 | 2.6 ± 2.1 |  | 3.2 ± 1.8 |
| Basophils  % | 0.03 ± 0.06 | 0.13 ± 0.05 | 0.09 ± 0.03 | 0.11 ± 0.03 | 0.08 ± 0.05 | 0.10 ± 0.09 | 0.08 ± 0.05 |  | 0.05 ± 0.00 |
| Reticulocytes  % | 3.29 ± 0.16 | 3.27 ± 0.22 | 4.05 ± 0.25 | 3.65 ± 0.62 | 3.30 ± 0.61 | 1.74 ± 0.25\*\* | 0.85 ± 0.11\*\*\* |  | 1.07 ± 0.12\*\*\* |
| Neutrophils  x109/L | 1.36 ± 0.4 | 2.06 ± 0.3 | 1.53 ± 0.6 | 1.70 ± 0.2 | 2.25 ± 0.4 | 2.69 ± 0.7 | 10.1 ± 6.1\*\* |  | 9.3 ± 4.4\* |
| Lymphocytes  x109/L | 6.52 ± 1.1 | 10.37 ± 1.5 | 9.42 ± 0.4 | 8.60 ± 1.7 | 10.54 ± 2.6 | 12.75 ± 4.1\* | 5.59 ± 1.9 |  | 6.95 ± 2.3 |
| Monocytes  x109/L | 0.19 ± 0.08 | 0.36 ± 0.09 | 0.28 ± 0.05 | 0.24 ± 0.06 | 0.38 ± 0.14 | 0.61 ± 0.15 | 1.08 ± 0.56\*\* |  | 1.33 ± 0.55\*\* |
| Eosinophils  x109/L | 0.17 ± 0.05 | 0.23 ± 0.03 | 0.18 ± 0.08 | 0.18 ± 0.03 | 0.22 ± 0.09 | 0.21 ± 0.06 | 0.12 ± 0.04 |  | 0.15 ± 0.10 |
| LUC  x109/L | 0.05 ± 0.04 | 0.07 ± 0.02 | 0.06 ± 0.01 | 0.05 ± 0.02 | 0.09 ± 0.07 | 0.11 ± 0.05 | 0.57 ± 0.56\* |  | 0.60 ± 0.51\* |
| Basophils  x109/L | 0.00 ± 0.00 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.02 ± 0.02 | 0.01 ± 0.01 |  | 0.01 ± 0.01 |
| Reticulocytes  x1012/L | 0.24 ± 0.02 | 0.25 ± 0.02 | 0.29 ± 0.02\* | 0.26 ± 0.04 | 0.24 ± 0.04 | 0.13 ± 0.02\*\* | 0.08 ± 0.01\*\*\* |  | 0.09 ± 0.01\*\*\* |

Values represent mean ± SD (n=4). Blood samples were obtained at day 6/7 (24 or 48 hours after the last administration). For some animals blood was not available for evaluation. For the control group and the dose of 128 mg/kg (unscheduled autopsy the number of animals was n=3.

a)Unscheduled autopsy of dose group of 128 mg/kg at days 6/7 after treatment in view of poor condition of the animals.

**Supplementary Table 3D Haematology in male rats at day 26 after 5 consecutive days of oral administration of Cu2CO3(OH)2 NPs.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cu2CO3(OH)2 NPs |  |  |  |  |  |  |
| Dose | 0  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg | 64  mg/kg |
|  |  |  |  |  |  |  |
| WBC  x109/L | 10.50 ± 1.75 | 13.33 ± 2.62 | 10.76 ± 1.90 | 12.31 ± 1.47 | 11.93 ± 2.53 | 9.67 ± 1.38 |
| RBC  x1012/L | 7.90 ± 1.14 | 7.94 ± 0.21 | 7.95 ± 0.34 | 7.85 ±0.26 | 7.83 ± 0.21 | 7.84 ± 0.15 |
| HgB  Mmol/L | 8.40 ± 0.33 | 8.53 ± 0.36 | 8.55 ± 0.41 | 8.38 ± 0.26 | 8.28 ± 0.15 | 8.13 ± 0.33 |
| Hct  L/L | 0.42 ± 0.02 | 0.41 ± 0.02 | 0.41 ± 0.02 | 0.41 ± 0.01 | 0.41 ± 0.01 | 0.39 ± 0.01 |
| MCV  fl | 52 ±2 | 52 ±1 | 52 ±1 | 52 ±0 | 52 ±1 | 50 ± 2 |
| MCH  fmol | 1.07 ± 0.05 | 1.07 ± 0.03 | 1.08 ± 0.03 | 1.06 ± 0.01 | 1.06 ± 0.02 | 1.04 ± 0.04 |
| MCHC  Mmol/L | 20.4 ± 0.1 | 20.7 ± 0.3 | 20.8 ± 0.3 | 20.6 ± 0.2 | 20.4 ± 0.2 | 20.7 ± 0.2 |
| RDW  % | 12.71 ± 0.40 | 13.24 ± 0.41 | 12.64 ± 0.39 | 13.23 ±0.81 | 13.76 ± 0.64 | 13.61 ± 0.68 |
| HDW  Mmol/L | 1.79 ± 0.09 | 1.95 ± 0.13 | 1.91 ±0.04 | 1.90 ±0.09 | 1.98 ± 0.11 | 1.94 ±0.06 |
| PLT  x109/L | 915 ± 69 | 717 ± 128 | 932 ± 143 | 981 ± 48 | 919 ± 74 | 939 ± 78 |
| MPV  fl | 6.58 ± 0.53 | 6.93 ± 0.73 | 7.15 ± 1.01 | 6.71 ± 0.24 | 6.20 ± 0.35 | 6.67 ± 0.25 |
| Neutrophils  % | 17.5 ± 5.2 | 19.7 ± 4.5 | 13.4 ± 2.2 | 18.7 ± 1.3 | 20.2 ± 7.0 | 17.7 ± 3.7 |
| Lymphocytes  % | 79.0 ± 5.1 | 75.4 ± 3.3 | 82.6 ± 3.5 | 77.6 ± 1.7 | 75.2 ± 6.3 | 78.5 ± 3.4 |
| Monocytes  % | 2.03 ± 0.41 | 3.06 ± 1.29 | 2.40 ± 1.31 | 2.11 ± 0.44 | 2.69 ± 0.86 | 1.76 ± 0.29 |
| Eosinophils  % | 1.15 ± 0.25 | 1.40 ± 0.18 | 1.26 ± 0.27 | 1.38 ± 0.29 | 1.40 ± 0.44 | 1.60 ± 0.56 |
| LUC  % | 0.2 ± 0.1 | 0.3 ± 0.2 | 0.3 ± 0.2 | 0.2 ± 0.0 | 0.4 ± 0.2 | 0.3 ± 0.2 |
| Basophils  % | 0.11 ± 0.03 | 0.10 ± 0.04 | 0.06 ± 0.05 | 0.08 ± 0.03 | 0.10 ± 0.04 | 0.09 ± 0.02 |
| Reticulocytes  % | 3.07 ± 0.28 | 3.05 ± 0.42 | 2.68 ± 0.35 | 3.38 ± 0.37 | 3.28 ± 0.40 | 3.31 ± 0.39 |
| Neutrophils  x109/L | 1.86 ± 0.75 | 2.56 ± 0.45 | 1.47 ± 0.48 | 2.29 ± 0.24 | 2.29 ± 0.42 | 1.73 ± 0.51 |
| Lymphocytes  x109/L | 8.27 ± 1.34 | 10.10 ± 2.29 | 8.84 ± 1.20 | 9.56 ± 1.18 | 9.08 ± 2.62 | 7.56 ± 0.91 |
| Monocytes  x109/L | 0.22 ± 0.06 | 0.43 ± 0.22 | 0.27 ± 0.18 | 0.26 ± 0.09 | 0.34 ± 0.17 | 0.17 ± 0.05 |
| Eosinophils  x109/L | 0.12 ± 0.01 | 0.19 ± 0.05 | 0.14 ± 0.06 | 0.17 ± 0.04 | 0.16 ± 0.04 | 0.15 ± 0.05 |
| LUC  x109/L | 0.03 ± 0.01 | 0.05 ± 0.03 | 0.04 ± 0.02 | 0.02 ± 0.00 | 0.06 ± 0.04 | 0.03 ± 0.03 |
| Basophils  x109/L | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.01 ± 0.01 | 0.00 ± 0.00 |
| Reticulocytes  x1012/L | 0.24 ± 0.02 | 0.24 ± 0.03 | 0.21 ± 0.03 | 0.27 ± 0.02 | 0.26 ± 0.03 | 0.26 ± 0.03 |

Values represent mean ± SD (n=4). Blood samples were obtained at day 26 (21 days after the last administration).For some animals blood was not available for evaluation. The number of animals is n=3 for the dose groups of 1, 4 and 16 mg/kg b.w.

**Supplementary Table 4 Bone marrow cellularity in male rats after 5 consecutive days of oral administration of CuO NPs and Cu2CO3(OH)2 NPs.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CuO NPs |  |  |  |  |  |  |  |  | Pilot Study |  | Extra Study |
| Dose | 0  mg/kg | 1  mg/kg | 2  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg |  | 64  mg/kg |  | 512  mg/kga |
| Day 6 |  |  |  |  |  |  |  |  |  |  |  |
| Bone marrow cells  Numberx109/L | 8.56 ± 2.67 | 9.45 ± 4.80 | 4.79 ± 2.99 | 12.78 ± 11.86 | 11.35 ± 8.16 | 8.19 ±2.45 | 7.94 ± 5.50 |  | 13.72 ± 13.26 (2) |  | 10.45 ± 4.97 |
| Day 26 |  |  |  |  |  |  |  |  |  |  |  |
| Bone marrow cells  Numberx109/L | 12.91 ± 5.51 | 13.18 ± 6.91 (3) | 12.20 ± 6.53 | 11.19 ± 7.56 (3) | 6.11 ± 3.01 | 12.10 ± 2.44 (3) | 12.36 ± 2.01 |  | NP |  | NP |
|  |  |  |  |  |  |  |  |  |  |  |  |

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cu2CO3(OH)2 NPs |  |  |  |  |  |  |  |  |  |
| Dose | 0  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg | 64  mg/kg | 128  mg/kg |  | 128  mg/kgb |
| Day 6 |  |  |  |  |  |  |  |  |  |
| Bone Marrow cells  Number x109/L | 21.25 ± 6.72 | 22.90 ± 8.84 | 22.88 ± 2.27 | 18.80 ± 11.21 | 15.90 ± 3.80 | 14.68 ± 3.37 | 13.60 ± 2.72 |  | 18.09 ±2.09 |
| Day 26 |  |  |  |  |  |  |  |  |  |
| Bone Marrow cells  Number x109/L | 16.71 ± 4.09 | 23.29 ± 6.18 | 18.86 ± 4.63 | 17.94 ± 8.94 | 18.82 ± 6.32 | 17.47 ± 4.09 | NP |  | NP |
|  |  |  |  |  |  |  |  |  |  |
| Erythroid BM cells (%) | 45.8 ± 4.3 |  |  |  |  |  | 16 ± 4.4 (3)\*\*\* |  | 22.8 ± 8.7\*\* |
| Myeloid/  Lymphoid BM cells (%) | 54.2 ± 4.3 |  |  |  |  |  | 84 ± 4.4 (3)\*\*\* |  | 77.2 ± 8.7\*\* |

Values represent mean ± SD (n=4).

a) Animals with dose 512 mg/kg b.w. CuO NPs as extra group were treated without concurrent control. For the dose of 64 mg/kg b.w. CuO NPs in the pilot study two (n=2) animals were evaluated. b) Unscheduled autopsy of dose group of 128 mg/kg at days 6/7 after treatment in view of poor condition of the animals.

The number of animals per group is 4 (n=4) unless otherwise indicated in the table as for some animals Bone Marrow cells were not available for evaluation. NP, not present. Significantly different compared to vehicle control treated animals : \*\* p<0.01, \*\*\* P<0.001.

**Supplementary Table 5A Clinical chemistry in male rats at day 6 after 5 consecutive days of oral administration of CuO NPs.**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| CuO NPs |  |  |  |  |  |  |  |  | Pilot Study |  | Extra Study |
| Dose | 0  mg/kg | 1  mg/kg | 2  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg |  | 64  mg/kg |  | 512  mg/kga |
|  |  |  |  |  |  |  |  |  |  |  |  |
| TP  g/L | 48 ± 2 | 45 ± 2 | 47 ± 1 | 47 ± 1 | 47 ± 0.5 | 46 ± 1 | 44 ± 2 |  | 46 ± 0.4 |  | 52 ± 3 |
| ALB g/L | 13.3 ±  0.6 | 12.7 ±  0.5 | 12.9 ±  0.4 | 13.1 ±  0.3 | 13.2 ±  0.2 | 12.7 ±  0.2 | 12.5 ±  0.4 |  | 12.9 ±  0.2 |  | 13.7 ± 0.9 |
| ALP  IU/L | 327 ±  27 | 301 ±  33 | 268 ±  10\* | 277 ±  9\* | 338 ±  14 | 283 ±  10\* | 262 ±  29\* |  | 244 ±  13\* |  | 176 ± 76 |
| ALT  IU/L | 51 ± 6 | 51 ± 5 | 45 ± 2 | 47 ± 3 | 49 ± 4 | 47 ± 1 | 52 ± 4 |  | 53 ± 1 |  | 704 ± 451 |
| AST  IU/L | 59 ± 2 | 62 ± 9 | 55 ± 2 | 53 ± 4 | 58 ± 4 | 55 ± 4 | 78 ± 7\*\* |  | 84 ± 0.4\*\* |  | 899 ± 653 |
| GGT  U/L | 0.48 ±  0.3 | 0.53 ±  0.4 | 0.75 ±  0.6 (3) <DL(1) | 0.51 ±  0.3 (3) <DL(1) | 0.30 ±  0.2 (2) <DL(2) | 0.35 ±  0.2 (3) <DL(1) | 0.59 (1) <DL(2) |  | 0.16 +  0.1 |  | 0.26 ± 0.2 (3) |
| LDH  IU/L | 573 ± 175 | 399 ± 78\* | 340 ± 24 | 435 ± 222 | 622 ± 3.26 | 390 ± 110 | 797 ± 812 |  | 1788 ± 64\*\* |  | 4452 ± 1622 |
| CHOL  mmol/L | 1.25 ±  0.05 | 1.25 ±  0.1 | 1.24 ±  0.1 | 1.19 ±  0.1 | 1.22 ±  0.1 | 1.28 ±  0.03 | 1.21±  0.1 |  | 1.20 ±  0.02 |  | 1.64 ± 0.3 |
| TG  mmol/L | 1.13 ± 0.21 | 0.97 ± 0.37 | 1.44 ± 0.24 | 1.28 ± 0.37 | 1.21 ± 0.21 | 1.10 ± 0.31 | 0.91 ± 0.29 |  | 1.26 ± 0.45 |  | 1.11 ± 0.4 |
| CRE  µmol/L | 14.0 ±  1.52 | 14.2 ±  2.2 | 15.3 ±  1.1 | 14.1 ±  2.0 | 15.2 ±  2.2 | 15.1 ±  1.4 | 15.2 ±  2.4 |  | 12.4 ±  0.5 |  | 28.7 ± 6.4 |
| GLU  mmol/L | 10.8 ±  0.8 | 10.6 ±  1.5 | 11.1 ±  0.2 | 11.8 ±  0.8 | 11.2 ±  0.6 | 11.4 ±  0.4 | 11.2±  1.7 |  | 10.7 ±  0.5 |  | 8.21 ± 1.2 |
| K  mmol/L | 4.5 ±  0.3 | 4.5 ±  0.2 | 5.1 ±  0.2 | 4.6 ±  0.2 | 4.9 ±  0.3 | 4.7 ±  0.2 | 4.6 ±  0.4 |  | 4.3 ±  0.01 |  | 5.0 ± 0.2 |
| Ca  mmol/L | 2.57 ± 0.07 | 2.49 ± 0.08 | 2.50 ± 0.04 | 2.51 ± 0.04 | 2.57 ± 0.02 | 2.49 ± 0.03 | 2.42 ± 0.04 |  | 2.46 ± 0.00 |  | 2.4 ± 0.07 |
| Cl  mmol/L | 99.3 ± 2.8 | 99.5 ± 1.2 | 99.4 ± 1.0 | 98.4 ± 0.4 | 97.5 ± 1.2 | 97.4 ± 0.8 | 98.98 ± 1.7 |  | 99 ± 1.2 |  | 103 ± 1.9 |
| Fe  µmol/L | 32.3 ± 1.9 | 33.2 ± 1.2 | 31.4 ± 2.6 | 31.9 ± 3.7 | 37.2 ± 6.1 | 37.4 ± 5.9 | 56.3 ± 18.2\* |  | 68.7 ± 7.7\*\* |  | 18.7 ± 8.0 |
| Na  mmol/L | 142 ± 3.0 | 141 ± 0.8 | 139 ± 0.4 | 140 ± 0.7 | 141 ± 0.4 | 139 ± 0.5 | 140 ± 2.1 |  | 142 ± 0.7 |  | 143 ± 2.8 |
| Urea  mmol/L | 5.7 ± 0.6 | 5.4 ± 0.7 | 5.1 ± 0.4 | 5.8 ± 0.2 | 6.3 ± 0.5 | 5.3 ± 0.1 | 5.4 ± 0.7 |  | 5.5 ± 0.7 |  | 6.6 ± 0.7 |
| Uric Acid | 41 ± 3.1 | 43 ± 14 | 44 ± 1.4 | 34 ± 4.3 | 44 ± 14 | 39 ± 3.9 | 60 ± 3.1\*\* |  | 43 ± 6.1 |  | 74 ± 14 |
| TBAX  µmol/L | 41 ± 12 | 62 ± 71 | 25 ± 12 | 31 ± 9 | 24 ± 13 | 21 ± 18 | 35 ± 8 |  | 8 ± 1\* |  | nd |
| TBIL  µmol/L | 8.3 ± 1.2 | 7.8 ± 0.9 | 8.0 ± 0.6 | 7.1 ± 0.2 | 8.5 ± 0.2 | 7.4 ± 0.8 | 9.5 ± 0.2 |  | 7.1 ± 1.5 |  | 6.6 ± 2.5 |
|  |  |  |  |  |  |  |  |  |  |  |  |
| ROM  IU/L | 233 ± 26 | 216 ± 38 | 232 ± 14 | 246 ± 8 | 231 ± 21 | 230 ± 13 | 224 ± 50 |  | 265 ± 28 |  | 342 ± 75 |
| SHP  µmol/L | 310 ± 19 | 297 ± 22 | 290 ± 28 | 295 ± 21 | 291 ± 30 | 269 ± 14\* | 283 ± 9 |  | 224 ± 19\* |  | 111 ± 45 |

Values represent mean ± SD. Blood was obtained at day 6 (24 hours after the last administration).

Abbreviations: ALB, albumin; ALP, alkaline phosphatase, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAP, biological anto-oxidant potential; Ca, calcium; Cl, chloride; ; CHOL, cholesterol; CRE, creatinine; Fe, iron; GGT, gamma glutamyltransferase; GLU, glucose; K, potassium; LDH, lactate dehydrogenase; Na, sodium; ROM, reactive oxygen metabolites; SHP, plasmatic thiol groups; TBAX, total bile acids; TBIL, total bilirubin; TG, triglycerides; TP, total protein; TTT, total thiols.

a) Animals with dose 512 mg/kg b.w. CuO NPs as extra group were treated without concurrent control. For the dose of 64 mg/kg b.w. CuO NPs in the pilot study two (n=2) animals were evaluated. In other dose groups the number of animals is n=4. Significant differences with non treated control animals \*p<0.05, \*\*p<0.01 (ANOVA).

**Supplementary Table 5B Clinical chemistry in male rats at day 26 after 5 consecutive days of oral administration of CuO-NP.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| CuO NPs |  |  |  |  |  |  |  |
| Dose | 0  mg/kg | 1  mg/kg | 2  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg |
|  |  |  |  |  |  |  |  |
| TP  g/L | 49 ± 1 | 49 ± 1 | 49 ± 2 | 50 ± 1 (3) <DL(1) | 51 ± 1 | 50 ± 1 | 52 ± 1 |
| ALB g/L | 13.6 ± 0.2 | 13.5 ± 0.6 | 13.2 ± 0.3 | 13.6 ± 0.3 (3) <DL(1) | 14.1 ± 0.3 | 13.8 ± 0.3 | 13.8 ± 0.3 |
| ALP  IU/L | 276 ± 19 | 290 ± 21 | 232 ± 19\* | 209 ± 62 | 274 ± 30 | 241 ± 29 | 236 ± 44 |
| ALT  IU/L | 49 ± 4.9 | 52 ± 4.0 | 46 ± 4.1 | 43 ± 5.4 | 50 ± 5.9 | 51 ± 2.1 | 46 ± 2.0 |
| AST  IU/L | 64 ± 3.7 | 70 ± 6.7 | 62 ± 1.4 | 63 ± 4.1 | 66 ± 3.7 | 65 ± 4.4 | 68 ± 6.0 |
| GGT  U/L | 0.44 ± 0.2 (2) <DL(2) | 0.51 ± 0.3 (3) <DL(1) | 0.15 ± 0.1 | 0.27 ± 0.1 | 0.73 ± 0.3 (2) <DL(2) | 0.26 ± 0.1 (3) <DL(1) | 0.54 ± 0.1 (2) <DL(2) |
| LDH  IU/L | 635 ± 129 | 634 ± 316 | 585 ± 223 | 694 ± 156 | 547 ± 242 | 573 ± 205 | 822 ± 131 |
| CHOL  mmol/L | 1.26 ± 0.06 | 1.26 ± 0..02 | 1.26 ± 0.09 | 1.26 ± 0.10 | 1.24 ± 0.05 | 1.30 ± 0.10 | 1.40 ± 0.20 |
| TG  mmol/L | 1.21 ± 0.15 | 1.17 ± 0.20 | 1.27 ± 0.30 | 1.22 ± 0.18 (3) <DL()1) | 1.49 ± 0.20 | 1.08 ± 0.22 | 1.41 ± 0.26 |
| CRE  µmol/L | 17.5 ± 1.1 | 18.5 ± 2.6 | 19.2 ± 1.2 | 20.9 ± 1.8 (3) <DL(1) | 20.4 ± 3.4 | 19.9 ± 2.3 | 22.9 ± 5.4 |
| GLU  mmol/L | 10.8 ± 1.2 | 10.2 ± 0.8 | 10.2 ± 0.3 | 10.6 ± 0.7 | 10.4 ± 0.6 | 10.1 ± 1.1 | 10.9 ± 0.3 |
| K  mmol/L | 4.8 ± 0.1 | 4.8 ± 0.2 | 5.0 ± 0.1 | 4.8 ± 0.2 | 4.9 ± 0.1 | 4.9 ± 0.3 | 4.8 ± 0.3 |
| Ca  mmol/L | 2.58 ± 0.06 | 2.49 ± 0.05 | 2.53 ± 0.05 | 2.70 ± 0.29 | 2.52 ± 0.02 | 2.55 ± 0.06 | 2.54 ± 0.06 |
| Cl  mmol/L | 98 ± 0.7 | 100 ± 1.2 | 99 ± 0.5 | 98 ± 0.8 (3) | 98 ± 1.3 | 98 ± 0.8 | 99 ± 1.0 |
| Fe  µmol/L | 36.3 ± 1.9 | 33.4 ± 2.1 | 35.4 ± 1.4 | 33.8 ± 5.7 | 35.3 ± 2.0 | 36.7 ± 1.4 | 40.1 ± 4.2 |
| Na  mmol/L | 142 ± 0.7 | 141 ± 1.0 | 141 ± 0.6 | 140 ± 0.3 (3) | 141 ± 0.4 | 141 ± 1.3 | 142 ± 1.5 |
| Urea  mmol/l | 6.2 ± 0.2 | 6.1 ± 0.3 | 5.8 ± 0.3 | 6.8 ± 1.7 | 7.1 ± 0.3 | 6.1 ± 0.4 | 6.6 ± 1.6 |
| Uric Acid | 43 ± 10.4 | 39 ± 8.2 | 43 ± 5.3 | 49 ± 19.8 | 41 ± 4.5 | 47 ± 10.0 | 45 ± 7.2 |
| TBAX  µmol/L | 43 ± 5 | 26 ± 17 | 33 ± 13 | 34 ± 17 | 50 ± 13 | 45 ± 18 | 31 ± 12 |
| TBIL  µmol/L | 7.1 ± 1.0 | 8.4 ± 1.0 | 7.8 ± 0.8 | 9.7 ± 3.6 | 7.5 ± 0.7 | 8.3 ± 1.0 | 7.7 ± 0.9 |
|  |  |  |  |  |  |  |  |
| ROM  IU/L | 265 ± 28 | 258 ± 7 | 255 ± 12 | 268 ± 34 | 266 ± 16 | 260 ± 14 | 288 ± 35 |
| SHP  µmol/L | 260 ± 17 | 272 ± 11 | 233 ± 11 | 216 ± 54 | 276 ± 13 | 240 ± 9 | 244 ± 17 |

Values represent mean ± SD. Blood was obtained at day 26 (21 days after the last administration). Abbreviations: ALB, albumin; ALP, alkaline phosphatase, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAP, biological anto-oxidant potential; Ca, calcium; Cl, chloride; ; CHOL, cholesterol; CRE, creatinine; Fe, iron; GGT, gamma glutamyltransferase; GLU, glucose; K, potassium; LDH, lactate dehydrogenase; Na, sodium; ROM, reactive oxygen metabolites; SHP, plasmatic thiol groups; TBAX, total bile acids; TBIL, total bilirubin; TG, triglycerides; TP, total protein; TTT, total thiols. DL, detection limit.

Number of animals n=4 unless indicated otherwise within brackets. Significant differences with non treated control animals \*p<0.05, \*\*p<0.01 (ANOVA).

**Supplementary Table 5C Clinical chemistry in male rats at day 6 after 5 consecutive days of oral administration of Cu2CO3(OH)2 NPs.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cu2CO3(OH)2 NPs |  |  |  |  |  |  |  |  |  |
| Dose | 0  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg | 64  mg/kg | 128  mg/kg |  | 128  mg/kga |
|  |  |  |  |  |  |  |  |  |  |
| TP  g/L | 55.4 ± 0.6 | 55.9 ± 0.8 | 56.5 ± 1.2 | 54.7 ± 2.2 | 53.5 ± 1.4 | 50.3 ± 2.5\* | 55.8 ± 3.7 (3) |  | 52.1 ± 4.3 (3) |
| ALB g/L | 15.7 ± 0.6 | 16.0 ± 0.5 | 15.9 ± 0.7 | 16.1 ± 0.8 | 15.0 ± 0.7 | 14.2 ± 1.5 | 15.9 ± 1.8 (3) |  | 14.5 ± 0.9 (3) |
| ALP  IU/L | 222 ± 35 | 256 ± 20 | 208 ± 16 | 266 ± 48 | 238 ± 19 | 247 ± 51 | 80 ± 21\*\* |  | 116 ± 18\*\* |
| ALT  IU/L | 54 ± 2 | 59 ± 4 | 57 ± 13 | 58 ± 10 | 60 ± 14 | 310 ± 94\*\* | 409 ± 360 |  | 612 ± 186\*\* |
| AST  IU/L | 93 ± 9 | 96 ± 8 | 97 ± 15 | 101 ± 10 | 113 ± 19 | 438 ± 110\*\* | 1224 ± 545\* |  | 1771 ± 1122\* |
| GGT  U/L | 0.36 ± 0.2 (3) <DL(1) | 0.50 ± 0.1 (2) <DL(2) | 0.35 ± 0.1 (2) <DL(1) | 047 ± 0.3 (2) <DL(2) | 0.55 (1) <DL(2) | 0.32 ± 0.2 (3) <DL(1) | 0.27 ± 0.2 (3) <DL(1) |  | 7.1 (1) |
| LDH  IU/L | 2229 ± 468 | 1796 ± 455 | 2556 ± 656 | 2229 ± 710 | 2754 ± 462 | 3139 ± 1726 | 9406 ± 4903\* |  | 3667 ± 1173 |
| FFA  Mmol/L | 0.17 ± 0.04 | 0.20 ± 0.1 | 0.19 ± 0.03 | 0.19 ± 0.04 | 0.25 ± 0.04 | 0.42 ± 0.2\*\* | 0.65 ± 0.1\*\* |  | 0.62 ± 0.2\*\* |
| CHOL  mmol/L | 1.21 ± 0.1 | 1.40 ± 0.2 | 1.32 ± 0.1 | 1.42 ± 0.2 | 1.23 ± 0.2 | 1.88 ± 0.4\* | 1.58 ± 0.4 |  | 2.44 ± 0.5\*\* |
| TG  mmol/L | 1.70 ± 0.44 | 1.71 ± 0.37 | 2.45 ± 0.47 | 1.97 ± 0.24 | 1.84 ± 0.29 | 1.59 ± 0.6 | 1.23 ± 0.5 |  | 1.06 ± 0.28 |
| CRE  µmol/L | 22.6 ± 2.2 | 21.7 ± 3.5 | 23.7 ± 0.9 | 24.2 ± 2.4 | 25.0 ± 3.1 | 23.0 ± 3.2 | 190 ± 110 (3)\*\* |  | 56.2 ± 49 |
| GLU  mmol/L | 11.8 ± 0.8 | 11.0 ± 0.5 | 11.6 ± 1.2 | 11.6 ± 1.1 | 10.8 ± 0.3 | 10.2 ± 1.3 | 7.1 ± 2.0\*\* |  | 9.6 = 4.0 |
| K  mmol/L | 4.81 ± 0.2 | 4.94 ± 0.4 | 4.90 ± 0.1 | 4.89 ± 0.4 | 5.00 ± 0.2 | 5.24 ± 0.5 | 6.10 ± 0.6 (3)\* |  | 5.59 ± 0.2 (3)\* |
| Ca  mmol/L | 2.49 ± 0.04 | 2.60 ± 0.04\* | 2.57 ± 0.03\* | 2.59 ± 0.06 | 2.55 ± 0.01\* | 2.53 ± 0.02 | 2.56 ± 0.18 |  | 2.53 ± 0.15 |
| Cl  mmol/L | 101 ± 1.5 | 100 ± 0.4 | 101 ± 0.9 | 100 ± 2 | 101 ± 0.7 | 104 ± 0.8\* | 99 ± 0.9 (3) |  | 102 ± 1.5 (3) |
| Fe  µmol/L | 30.9 ± 2 | 36.3 ± 4 | 39.7 ± 6\* | 51.3 ± 11\* | 64.8 ± 19\* | 43.3 ± 24 | 66.5 ± 23\* |  | 51.6 ± 33 |
| Na  mmol/L | 142 ± 0.5 | 141 ± 0.5 | 142 ± 2.0 | 143 ± 3.3 | 142 ± 0.6 | 143 ± 1.8 | 137 ± 3.7 (3) |  | 140 ± 2.4 (3) |
| Urea  mmol/l | 6.06 ± 1.0 | 6.44 ± 0.7 | 5.78 ± 1.2 | 6.48 ± 0.6 | 6.24 ± 0.7 | 5.47 ± 0.6 | 25.8 ± 14.5 |  | 13.3 ± 5.3 |
| Uric Acid | 69 ± 27 | 53 ± 4 | 74 ± 12 | 50 ± 5 | 66 ± 7 | 55 ± 6 | 151 ± 49\* |  | 119 ± 58 |
| TBIL  µmol/L | 5.96 ± 0.19 | 5.33 ± 0.62 | 5.55 ± 0.66 | 5.75 ± 0.5 | 6.38 ± 0.75 | 6.11 ± 0.4 | 13.73 ± 9.66 |  | 12.92 ± 7.65 |
| ROM  IU/L | 292 ± 30 | 280 ± 22 | 256 ± 14 | 261 ± 8 | 246 ±16 | 332 ± 35 | 406 ± 189 |  | 360 ± 47 |
| SHP  µmol/L | 323 ± 41 | 373 ± 27 | 335 ± 16 | 354 ± 20 | 328 ± 13 | 277 ± 36 | 197 ± 160 |  | 148 ± 112\* |

Values represent mean ± SD with n=4 unless indicated otherwise within brackets. Blood was obtained at day 6 (24 hours after the last administration).

Abbreviations: ALB, albumin; ALP, alkaline phosphatase, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAP, biological anto-oxidant potential; Ca, calcium; Cl, chloride; ; CHOL, cholesterol; CRE, creatinine; Fe, iron; GGT, gamma glutamyltransferase; GLU, glucose; K, potassium; LDH, lactate dehydrogenase; Na, sodium; ROM, reactive oxygen metabolites; SHP, plasmatic thiol groups; TBAX, total bile acids; TBIL, total bilirubin; TG, triglycerides; TP, total protein; TTT, total thiols. DL, detection limit.

a)Unscheduled autopsy of dose group of 128 mg/kg at days 6/7 after treatment in view of poor condition of the animals.

Significant differences with non treated control animals \*p<0.05, \*\*p<0.01 (ANOVA).

**Supplementary Table 5D Clinical chemistry in male rats at day 26 after 5 consecutive days of oral administration of Cu2CO3(OH)2 NPs.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Cu2CO3(OH)2 NPs |  |  |  |  |  |  |
| Dose | 0  mg/kg | 4  mg/kg | 8  mg/kg | 16  mg/kg | 32  mg/kg | 64  mg/kg |
|  |  |  |  |  |  |  |
| TP  g/L | 58.6 ± 2.4 | 56.9 ± 2.7 | 56.4 ± 3.0 | 59.4 ± 1.7 | 57.5 ± 1.9 | 57.1 ± 1.6 |
| ALB g/L | 16.3 ± 0.7 | 15.5 ± 1.0 | 15.3 ± 1.1 | 16.8 ± 0.2 | 16.6 ± 0.5 | 16.0 ± 1.3 |
| ALP  IU/L | 205 ± 18 | 188 ± 24 | 178 ± 31 | 229 ± 40 | 197 ± 28 | 256 ± 39 |
| ALT  IU/L | 63.7 ± 4.7 | 70.4 ± 9.6 | 48.5 ± 3.8\*\* | 69.3 ± 13.1 | 66.0 ± 8.3 | 87.2 ± 32.3 |
| AST  IU/L | 120 ± 7 | 110 ± 9 | 92 ± 6\*\* | 121 ± 10 | 112 ± 5 | 153 ± 55 |
| GGT  U/L | 0.71 (1) <DL(3) | 0.49 ± 0.0 (2) <DL(1) | 1.11 (1) <DL(3) | 0.44 (1) <DL(3) | 0.67 ± 0.3 (2) <DL(2) | 0.69 ± 0.2 (3) <DL(1) |
| LDH  IU/L | 3271 ± 548 | 2108 ± 333\* | 1878 ± 455\* | 2254 ± 688 | 2217 ± 657 | 2488 ± 636 |
| CHOL  mmol/L | 1.32 ± 0.2 | 1.39 ± 0.1 | 1.47 ± 0.1 | 1.40 ± 0.1 | 1.14 ± 0.1 | 1.28 ± 0.1 |
| TG  mmol/L | 2.20 ± 0.89 | 2.58 ± 0.80 | 2.27 ± 0.65 | 2.84 ± 1.03 | 2.19 ± 0.91 | 1.80 ± 0.28 |
| CRE  µmol/L | 27.2 ± 2.1 | 28.4 ± 1.8 | 28.0 ± 2.3 | 31.6 ± 2.7 | 29.4 ± 2.3 | 31.2 ± 1.5\* |
| GLU  mmol/L | 11.3 ± 1.8 | 10.8 ± 1.3 | 13.8 ± 2.5 | 11.4 ± 0.8 | 11.1 ± 0.7 | 12.3 ± 1.1 |
| K  mmol/L | 4.84 ± 0.3 | 5.08 ± 0.3 | 4.80 ± 0.3 | 4.94 ± 0.4 | 4.86 ± 0.3 | 4.74 ± 0.1 |
| Ca  mmol/L | 2.50 ± 0.03 | 2.55 ± 0.05 | 2.49 ± 0.07 | 2.50 ± 0.04 | 2.53 ± 0.05 | 2.47 ± 0.06 |
| Cl  mmol/L | 102 ± 1.3 | 102 ± 1.1 | 102 ± 1.4 | 101 ± 1.5 | 102 ± 1.7 | 102 ± 1.0 |
| Fe  µmol/L | 30.5 ± 3.9 | 31.1 ± 7.7 | 29.9 ± 3.0 | 29.5 ± 4.2 | 30.8 ± 5.9 | 29.0 ± 3.8 |
| Na  mmol/L | 144 ± 1.1 | 143 ± 1.9 | 142 ± 1.5 | 142 ± 1.4 | 144 ± 1.0 | 143 ± 1.6 |
| Urea  mmol/l | 7.13 ± 0.7 | 7.28 ± 0.5 | 6.49 ± 0.9 | 7.56 ± 0.6 | 6.59 ± 0.8 | 7.04 ± 0.8 |
| Uric Acid | 48 ± 3 | 45 ±5 (3) <DL(1) | 51 ± 6 | 62 ± 12 | 49 ± 5 | 54 ± 12 |
| TBIL  µmol/L | 5.38 ± 0.28 | 5.30 ± 0.69 | 5.73 ± 0.83 | 6.12 ± 0.17\*\* | 5.22 ± 0.77 | 5.01 ± 0.71 |
| Zn  µg/dL | 119 ± 3 | 136 ± 6 | 124 ± 9 | 125 ± 3 | 132 ± 7 | 114 ± 10 |
| ROM  IU/L | 297 ± 16 | 272 ± 23 | 274 ± 26 | 304 ± 22 | 279 ± 11 | 289 ± 23 |
| SHP  µmol/L | 349 ± 6 | 324 ± 21 | 331 ± 25 | 327 ± 22 | 328 ± 17 | 340 ± 39 |

Values represent mean ± SD with n=4 unless indicated otherwise within brackets. Blood was obtained at day 26 (21 days after the last administration). Abbreviations: ALB, albumin; ALP, alkaline phosphatase, ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAP, biological anti-oxidant potential; Ca, calcium; Cl, chloride; ; CHOL, cholesterol; CRE, creatinine; Fe, iron; GGT, gamma glutamyltransferase; GLU, glucose; K, potassium; LDH, lactate dehydrogenase; Na, sodium; ROM, reactive oxygen metabolites; SHP, plasmatic thiol groups; TBAX, total bile acids; TBIL, total bilirubin; TG, triglycerides; TP, total protein; TTT, total thiols. DL, detection limit.

Significant differences with non treated control animals \*p<0.05, \*\*p<0.01 (ANOVA).

**Supplementary Table 6 Histopathology in male rats at day 6 and day 26 after 5 consecutive days of oral administration of CuO-NP and Cu2CO3(OH)2 NPs .**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gastro-intestinal system |  | Cu2CO3(OH)2-NP | | | | | | | | | | CuO-NP |
|  |  |  | |  | |  | |  | |  | |  |
| Dose level (mg/kg): | 0 | | 64 | | 128 | | 128 | | 0 | | 64 | 512 |
| Autopsy day | Day 6 | | Day 6 | | Day 6 | | Day 7 | | Day 26 | | Day 26 | Day 6 |
| STOMACH a | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Inflammation* |  | |  | |  | |  | |  | |  |  |
| Minimal | 2 | | - | | 1 | | 1 | | 1 | | - | - |
| Slight | - | | - | | 3 | | 3 | | - | | 2 | 1 |
| Moderate | - | | 4 | | - | | - | | - | | 2 | 3 |
|  |  | |  | |  | |  | |  | |  |  |
| DUODENUM a | 4 | | 3 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Apoptosis* |  | |  | |  | |  | |  | |  |  |
| Slight | - | | - | | - | | 2 | | - | | - | - |
| Moderate | - | | - | | - | | 1 | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| ILEUM a | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Apoptosis* |  | |  | |  | |  | |  | |  |  |
| Moderate | - | | - | | - | | 1 | | - | | - | 4 |
|  |  | |  | |  | |  | |  | |  |  |
| CECUM | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Ulceration/erosion* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | - | | 1 | | - | | - | - |
| *Apoptosis* |  | |  | |  | |  | |  | |  |  |
| Moderate | - | | - | | - | | 1 | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| COLON | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Ulceration/erosion* |  | |  | |  | |  | |  | |  |  |
| Slight | - | | - | | 2 | | 1 | | - | | - | 4 |
| *Edema* |  | |  | |  | |  | |  | |  |  |
| Slight | - | | - | | 1 | | 1 | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| RECTUM a | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Inflammation* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | 1 | | - | | - | | - | - |
| Slight | - | | - | | - | | 2 | | - | | - | - |
| *Edema* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | 1 | | - | | - | | - | - |
| Slight | - | | - | | - | | 1 | | - | | - | - |
| *Ulceration/erosion* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | - | | 1 | | - | | - | - |
| Slight | - | | - | | - | | 1 | | - | | - | - |
| Moderate | - | | - | | - | | 1 | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |

a = Number of tissues examined from each group.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Other organs |  | Cu2CO3(OH)2-NP | | | | | | | | | | CuO-NP |
|  |  |  | |  | |  | |  | |  | |  |
| Dose level (mg/kg): | 0 | | 64 | | 128 | | 128 | | 0 | | 64 | 512 |
| Autopsy day | Day 6 | | Day 6 | | Day 6 | | Day 7 | | Day 26 | | Day 26 | Day 6 |
| LIVER a | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Inflammation* |  | |  | |  | |  | |  | |  |  |
| Slight | - | | 2 | | - | | 1 | | - | | - | - |
| Moderate | - | | 1 | | 1 | | 2 | | - | | - | 1 |
| Marked | - | | - | | - | | - | | - | | - | 1 |
| *Kupffer cell hypertrophy /hyperplasia* |  | |  | |  | |  | |  | |  |  |
| Slight | - | | - | | 1 | | - | | - | | - | 2 |
| Moderate | - | | - | | 1 | | 2 | | - | | - | - |
| *Hypertrophy* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | - | | 1 | | - | | - | 2 |
| Slight | - | | 1 | | 2 | | 2 | | - | | - | - |
| Moderate | - | | 1 | | - | | 1 | | - | | - | 1 |
| *Necrosis* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | 1 | | - | | - | | - | | - | - |
| Slight | - | | - | | 1 | | - | | - | | - | 1 |
| Moderate | - | | - | | - | | - | | - | | - | 1 |
| Marked | - | | - | | - | | - | | - | | - | 1 |
| *Single cell necrosis* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | 1 | | 1 | | - | | - | | 1 | 1 |
| Slight | - | | 2 | | 1 | | 1 | | - | | - | - |
| Moderate | - | | 1 | | 2 | | 1 | | - | | - | 1 |
| Marked | - | | - | | - | | 1 | | - | | - | 1 |
| *Vacuolation* |  | |  | |  | |  | |  | |  |  |
| Slight | - | | - | | 3 | | 1 | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| SPLEEN a | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Lymphoid atrophy* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | 1 | | 2 | | - | | - | - |
| Slight | - | | - | | 1 | | - | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| THYMUS | 4 | | 4 | | 4 | | 2 | | 4 | | 4 | 4 |
| *Lymphoid atrophy* |  | |  | |  | |  | |  | |  |  |
| Moderate | - | | - | | - | | 2 | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| KIDNEYa | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Necrosis* |  | |  | |  | |  | |  | |  |  |
| Marked | - | | - | | 2 | | - | | - | | - | - |
| *Hyaline casts* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | - | | - | | - | | 1 | - |
| Slight | - | | - | | - | | 1 | | - | | - | - |
| Marked | - | | - | | 2 | | - | | - | | - | - |
| *Degeneration/regeneration* |  | |  | |  | |  | |  | |  |  |
| Moderate | - | | - | | - | | 2 | | - | | - | - |
| *Inclusions* |  | |  | |  | |  | |  | |  |  |
| Moderate | - | | - | | - | | 2 | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| TESTIS LEFTa | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 4 |
| *Multinucleated giant cells* |  | |  | |  | |  | |  | |  |  |
| Slight | - | | - | | 1 | | - | | - | | - | - |
| *Degeneration germ cells* |  | |  | |  | |  | |  | |  |  |
| Moderate | - | | - | | 1 | | - | | - | | - | - |
| *Retention* |  | |  | |  | |  | |  | |  |  |
| Marked | - | | - | | 1 | | - | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| SEMINAL VESICLESa | 1 | | - | | 4 | | 4 | | - | | - | - |
| *Decreased fluid* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | - | | 1 | | - | | - | - |
| Slight | - | | - | | 4 | | 2 | | - | | - | -- |
| Moderate | - | | - | | - | | 1 | | - | | - | - |
|  |  | |  | |  | |  | |  | |  |  |
| BONE MARROW FEMURa | 4 | | 4 | | 4 | | 4 | | 4 | | 4 | 3 |
| *Increased myeloid/*  *decreased erythroid* |  | |  | |  | |  | |  | |  |  |
| Minimal | - | | - | | 2 | | - | | - | | - | - |
| Slight | - | | - | | - | | 3 | | - | | - | 1 |
| Moderate | - | | - | | 1 | | - | | - | | - | - |

a = Number of tissues examined from each group.

**Supplementary Table 7 Bench mark dose calculations for effects after oral CuO nanoparticles exposure for 5 consecutive days.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | BMDexp | BMDhill | BMDavg | BMDlow | BMDhigh | ratio |
|  |  |  |  |  |  |  |
| Body weight | 58a | 58 | 58 | 28 | infinitive | No value |
| Liver weight | 26.4 | 26.4 | 26.4 | 1.2 | 30.1 | 25.1 |
| AST | 26.2 | 26.2 | 26.2 | 16.1 | 27.0 | 1.7 |
| ALP05b | 0.41 | 0.41 | 0.41 | 0 | 27.7 | No value |
| ALP10 | 6.11 | 5.97 | 6.04 | 0.0006 | 419.9 | 699833 |
| LDP05 | 26.6 | 26.9 | 26.8 | 0.49 | infinitive | No value |
| LDP10 | 28.5 | 28.5 | 28.5 | 1.98 | infinitive | No value |
| Fe | 7.6 | 7.6 | 7.6 | 1.9 | 20.09 | 10.6 |
| Uric acid | 16.2 | 15.9 | 16.05 | 3.5 | 29.6 | 8.4 |
| SHP | 5 | 5 | 5 | 0.0005 | 10508 | 21016000 |
| WBC | 6.1 | 6.1 | 6.1 | 0 | infinitive | No value |
| RBC | 35.7 | 35.7 | 35.7 | 23.8 | 115.7 | 4.9 |
| Hgb | 32.9 | 32.9 | 32.9 | 24 | 81.5 | 3.4 |
| Hct | 33.2 | 33.2 | 33.2 | 28.8 | 132 | 4.6 |
| Lymph Absol | 2.87 | 2.83 | 2.9 | 0 | infinitive | No value |

BMDexp and BMD hill are the two models applied in the PROAST program for calculations of the BMD. BMDlow and BMDhigh indicate the lower and upper end of the 90% confidence interval. The ratio calculated between the BMDlow and BMDhigh gives an indication of the range of the confidence interval and the reliability of the data.

1. BMD is presented in mg/kg of orally administered CuO NPs for 5 consecutive days. The bench mark dose (BMD) determination is performed at a 5% deviation of levels in vehicle control treated animals as bench mark.
2. ALP05. The figure 05 indicates a deviation due to treatment compared to vehicle control with more than 5%. ALP10. Similarly for a deviation of more than 10%.

**Supplementary Table 8 Bench mark dose calculations for effects after oral Cu2CO3(OH)2 NPs exposure for 5 consecutive days.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | BMDexp | BMDhill | BMDavg | BMDlow | BMDhigh | ratio |
| Day 6/7 |  |  |  |  |  |  |
| Body weight | 92.9a | 92.6 | 92.7 | 43.7 | 153.9 | 3.5 |
| Liver weight | 31.5 | 33 | 32.2 | 9.5 | 75.5 | 7.9 |
| Adrenal weight | 53.7 | 53.7 | 53.7 | 5.2 | 106.3 | 20.4 |
| Thymus weight | 69.6 | 68.5 | 69.1 | 43.1 | 97.2 | 2.3 |
| Spleen weight | 90.6 | 88.8 | 89.7 | 50.2 | 99.9 | 2.0 |
| AST | 30.7 | 30.9 | 30.8 | 18.8 | 42.9 | 2.3 |
| ALP05b | 94.1 | 88.8 | 91.5 | 65.6 | 95.6 | 1.5 |
| ALP10 | 101.1 | 95.7 | 98.4 | 75.2 | 102.8 | 1.4 |
| Fe | 101.5 | 101.5 | 101.5 | 34.9 | 122.7 | 3.5 |
| Uric acid | 90.5 | 90.5 | 90.5 | 36.1 | 93.7 | 2.6 |
| SHP | 97.0 | 93.1 | 95.1 | 77.6 | 100.8 | 1.3 |
| WBC | 27.6 | 27.1 | 27.4 | 0.9 | 55.4 | 56.8 |
| RBC | 108.6 | 108.7 | 108.6 | 70.8 | 112.5 | 1.6 |
| Hgb | 106.4 | 94.6 | 100.5 | 67.7 | 115.1 | 1.7 |
| Hct | 113.1 | 113.1 | 113.1 | 74.6 | 117.2 | 1.6 |
| Lymph rel | 96.5 | 92.3 | 94.4 | 65.2 | 97.9 | 1.5 |
| Lymph Absol | 101.0 | 98.4 | 99.7 | 45.4 | 106.1 | 2.3 |
| LDP05 | 61.8 | 61.8 | 61.8 | 32.2 | 91.1 | 2.8 |
| LDP10 | 70.3 | 70.3 | 70.3 | 41.2 | 97.9 | 2.4 |
|  |  |  |  |  |  |  |
| Day 26 |  |  |  |  |  |  |
| AST | 45.5 | 46.1 | 45.8 | 33.6 | 57.0 | 1.7 |

BMDexp and BMD hill are the two models applied in the PROAST program for calculations of the BMD. BMDlow and BMDhigh indicate the lower and upper end of the 90% confidence interval. The ratio calculated between the BMDlow and BMDhigh gives an indication of the range of the confidence interval.

a) BMD is presented in mg/kg of orally administered Cu2CO3(OH)2 NPs for 5 consecutive days. The bench mark dose (BMD) determination is performed at a 5% deviation of levels in vehicle control treated animals as bench mark.

b) ALP05. The figure 05 indicates a deviation due to treatment compared to vehicle control with more than 5%. ALP10. Similarly for a deviation of more than 10%.