

Table 45. *gpt* mutant frequencies in the livers of B6C3F₁ *gpt* delta male mice treated with Furan for 13 weeks

| Dose | Animal No. | Cm ^R Colonies (x 10 ⁵) | 6-TG ^R and Cm ^R Colonies | Mutant Frequency (x 10 ⁻⁵) | Mean ± S.D. |
|----------|------------|---|---|--|-------------|
| 0 mg/kg | 36 | 3.92 | 2 | 0.51 | 0.63 ± 0.32 |
| | 37 | 7.29 | 7 | 0.96 | |
| | 38 | 4.19 | 4 | 0.96 | |
| | 39 | 14.54 | 3 | 0.21 | |
| | 40 | 13.19 | 7 | 0.53 | |
| 2 mg/kg | 46 | 3.06 | 5 | 1.63 | 0.58 ± 0.63 |
| | 47 | 9.72 | 5 | 0.51 | |
| | 48 | 9.72 | 5 | 0.51 | |
| | 49 | 0.27 | N.D. | - | |
| | 50 | 22.50 | 5 | 0.22 | |
| 15 mg/kg | 56 | 2.03 | 5 | 2.47 | 0.68 ± 1.01 |
| | 57 | 17.51 | 1 | 0.06 | |
| | 58 | 7.88 | 4 | 0.51 | |
| | 59 | 14.13 | 2 | 0.14 | |
| | 60 | 21.60 | 5 | 0.23 | |

N.D.: No mutant colonies were detected on the plate, with those data being excluded from the calculation of mutant frequency.

Table 46. Spi⁻ mutant frequencies in the livers of B6C3F1 *gpt* delta male mice treated with Furan for 13 weeks

| Dose | Animal No. | Plaques within XL-1 Blue MRA (x 10 ⁵) | Plaques within WL95 (P2) | Mutant Frequency (x 10 ⁻⁵) | Mean ± S.D. |
|----------|------------|--|-----------------------------|--|-------------|
| 0 mg/kg | 36 | 26.37 | 7 | 0.27 | 0.27 ± 0.11 |
| | 37 | 11.79 | 1 | 0.08 | |
| | 38 | 30.15 | 11 | 0.36 | |
| | 39 | 22.23 | 7 | 0.31 | |
| | 40 | 39.06 | 9 | 0.23 | |
| 2 mg/kg | 46 | 11.43 | 2 | 0.17 | 0.23 ± 0.06 |
| | 47 | 27.72 | 6 | 0.22 | |
| | 48 | 60.12 | 17 | 0.28 | |
| | 49 | 52.11 | 9 | 0.17 | |
| | 50 | 59.13 | 17 | 0.29 | |
| 15 mg/kg | 56 | 9.54 | 2 | 0.21 | 0.20 ± 0.06 |
| | 57 | 58.14 | 6 | 0.10 | |
| | 58 | 60.48 | 15 | 0.25 | |
| | 59 | 21.33 | 5 | 0.23 | |
| | 60 | 52.74 | 12 | 0.23 | |

Table 47. *gpt* mutant frequencies in the livers of B6C3F₁ *gpt* delta female mice treated with Furan for 13 weeks

| Dose | Animal No. | Cm ^R Colonies (x 10 ⁵) | 6-TG ^R and Cm ^R Colonies | Mutant Frequency (x 10 ⁻⁵) | Mean ± S.D. |
|----------|------------|---|---|--|-------------|
| 0 mg/kg | 31 | 2.16 | N.D. | - | 0.33 ± 0.14 |
| | 32 | 7.70 | 3 | 0.39 | |
| | 33 | 14.09 | 5 | 0.35 | |
| | 34 | 23.27 | 3 | 0.13 | |
| | 35 | 6.93 | 3 | 0.43 | |
| 2 mg/kg | 41 | 6.39 | 1 | 0.16 | 0.22 ± 0.08 |
| | 42 | 14.27 | 3 | 0.21 | |
| | 43 | 3.69 | 1 | 0.27 | |
| | 44 | 18.81 | 6 | 0.32 | |
| | 45 | 14.94 | 2 | 0.13 | |
| 15 mg/kg | 51 | 25.25 | 7 | 0.28 | 0.41 ± 0.12 |
| | 52 | 17.51 | 9 | 0.51 | |
| | 53 | 14.40 | 5 | 0.35 | |
| | 54 | 4.68 | N.D. | - | |
| | 55 | 7.97 | 4 | 0.50 | |

N.D.: No mutant colonies were detected on the plate, with those data being excluded from the calculation of mutant frequency.

Table 48. Spi⁻ mutant frequencies in the livers of B6C3F₁ *gpt* delta female mice treated with Furan for 13 weeks

| Dose | Animal No. | Plaques within XL-1 Blue MRA (x 10 ⁵) | Plaques within WL95 (P2) | Mutant Frequency (x 10 ⁻⁵) | Mean ± S.D. |
|----------|------------|--|-----------------------------|--|-------------|
| 0 mg/kg | 31 | 1.68 | 5 | 1.07 | 0.46 ± 0.34 |
| | 32 | 11.79 | 3 | 0.25 | |
| | 33 | 21.96 | 8 | 0.36 | |
| | 34 | 23.13 | 6 | 0.26 | |
| | 35 | 10.62 | 4 | 0.38 | |
| 2 mg/kg | 41 | 10.89 | 7 | 0.64 | 0.39 ± 0.24 |
| | 42 | 16.74 | 3 | 0.18 | |
| | 43 | 3.15 | 1 | 0.32 | |
| | 44 | 20.07 | 3 | 0.15 | |
| | 45 | 17.28 | 11 | 0.64 | |
| 15 mg/kg | 51 | 30.42 | 10 | 0.33 | 0.37 ± 0.11 |
| | 52 | 21.60 | 7 | 0.32 | |
| | 53 | 16.56 | 5 | 0.30 | |
| | 54 | 6.39 | N.D. | 0.00 | |
| | 55 | 12.87 | 7 | 0.54 | |

N.D.: No mutant colonies were detected on the plate, with those data being excluded from the calculation of mutant frequency.

Table 49. Comet assay in the livers of B6C3F₁ *gpt* delta male mice treated with Furan for 13 weeks

| | Male | | | Female | | |
|----------|-------------|--------------|----------------|-------------|--------------|----------------|
| | Tail moment | Tail length | Tail intensity | Tail moment | Tail length | Tail intensity |
| 0 mg/kg | 0.21 ± 0.14 | 17.27 ± 2.48 | 2.02 ± 1.21 | 0.05 ± 0.02 | 15.15 ± 0.54 | 0.54 ± 0.21 |
| 2 mg/kg | 0.19 ± 0.08 | 18.11 ± 1.50 | 1.69 ± 0.67 | 0.07 ± 0.05 | 14.55 ± 1.48 | 0.76 ± 0.43 |
| 15 mg/kg | 0.06 ± 0.03 | 15.76 ± 1.33 | 0.70 ± 0.38 | 0.07 ± 0.05 | 16.14 ± 0.63 | 0.78 ± 0.42 |

Fig. 1. Growth curves for male and female *gpt* delta mice given 1-MN for 13 weeks.

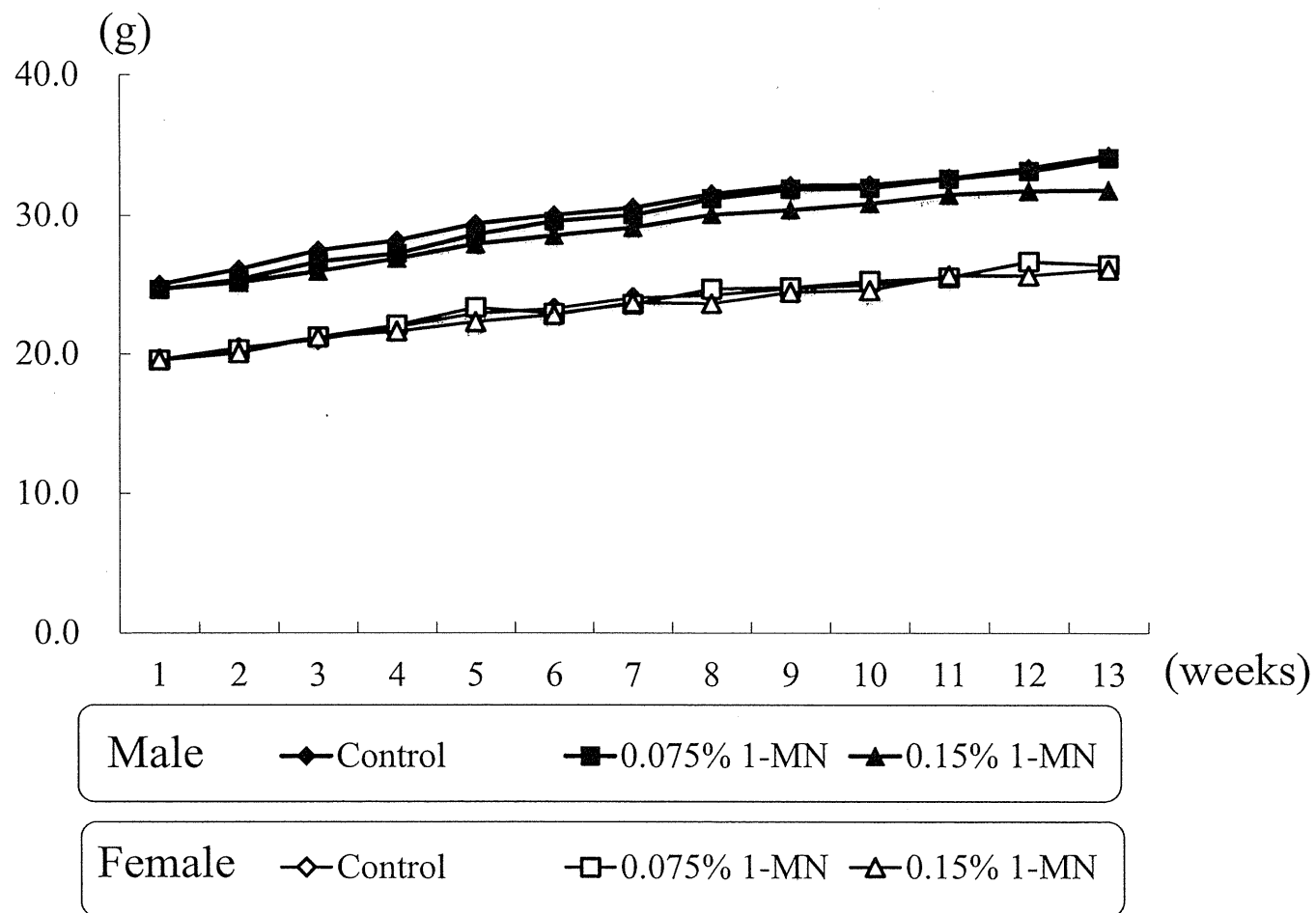


Fig. 2. Food consumption curves for male and female *gpt* delta mice given 1-MN for 13 weeks.

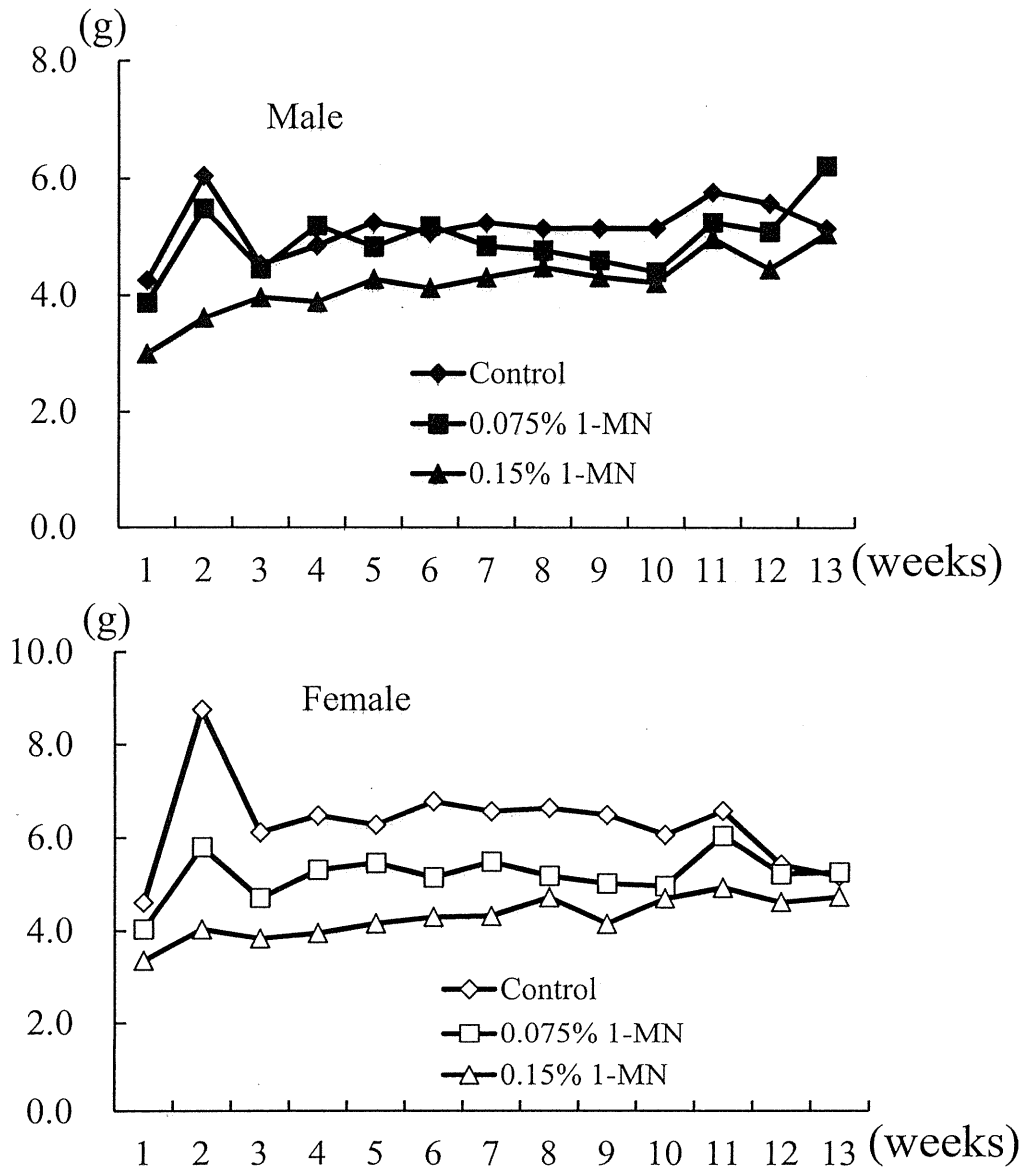


Fig. 3. Immunohistochemical staining of PCNA in lung of *gpt* delta mice given 1-MN for 13 weeks.

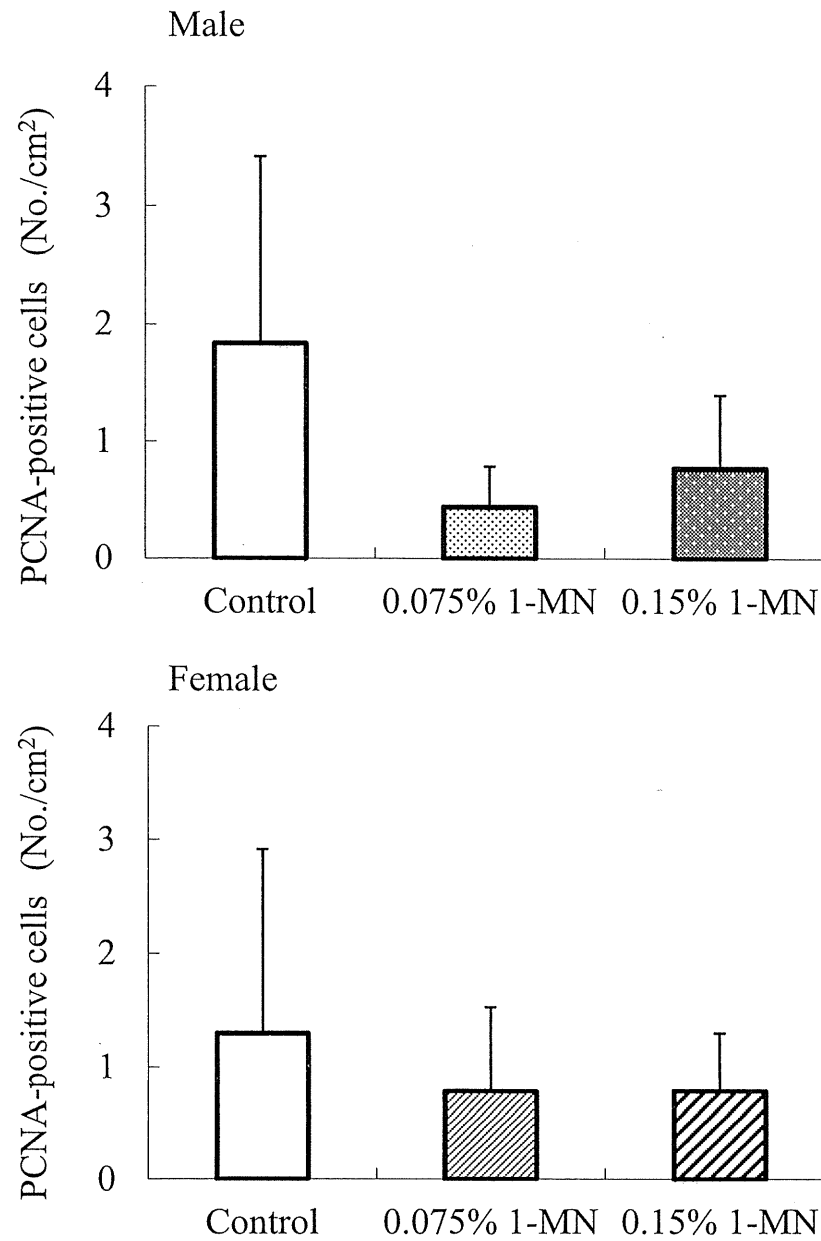


Fig. 4. Venn diagram of up or down-regulated genes in the liver of F344 rat given 600mg/kg estragole or 0.5% safrole for 4weeks.

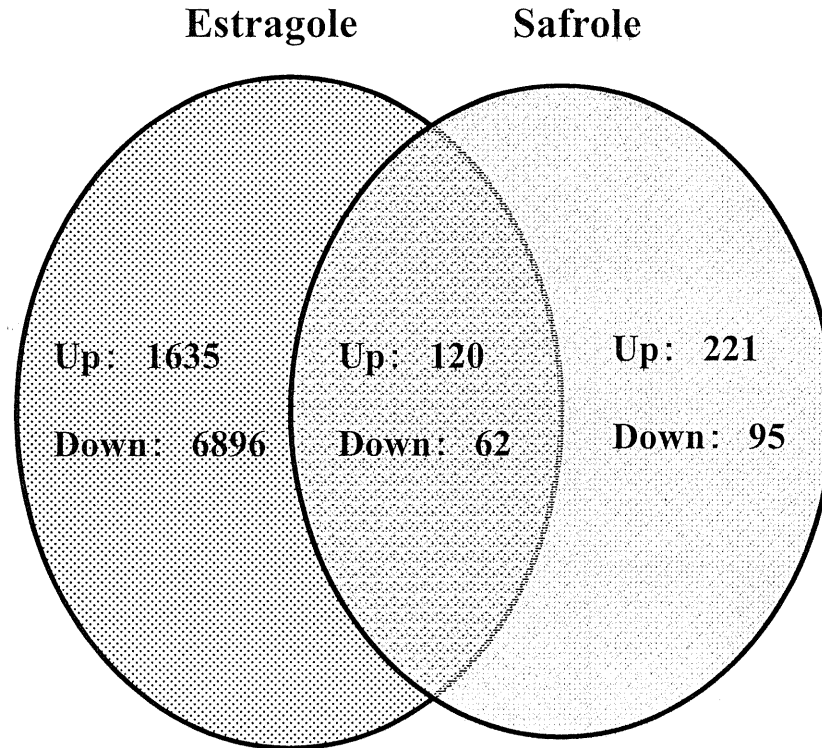
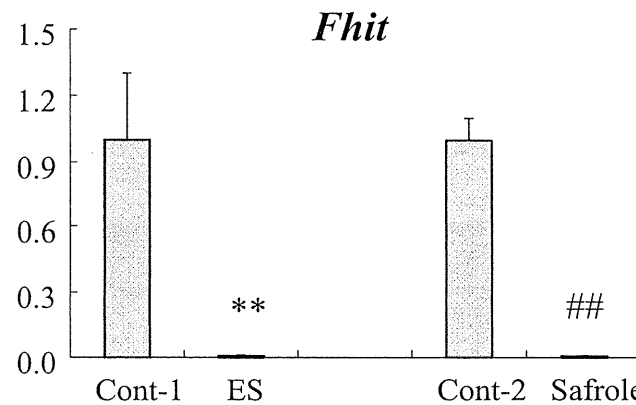
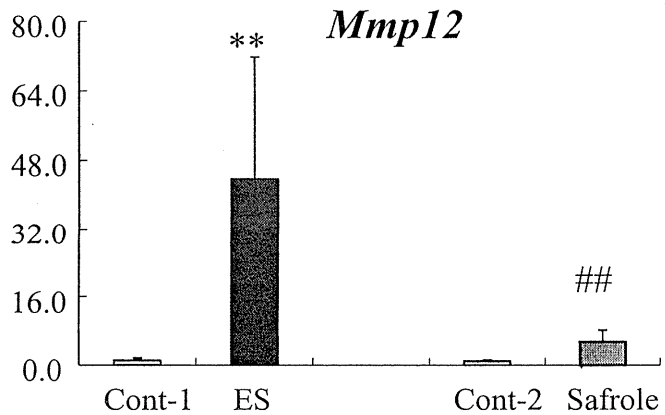
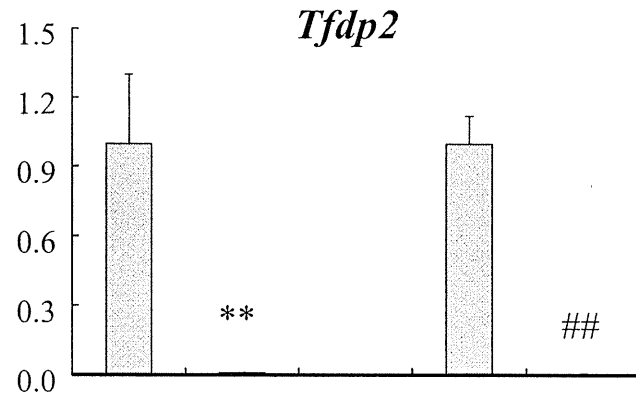
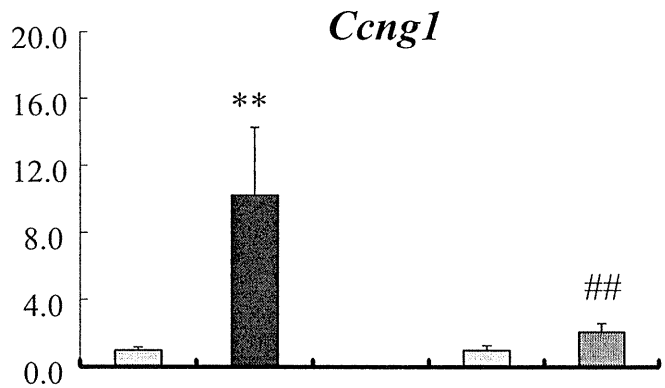
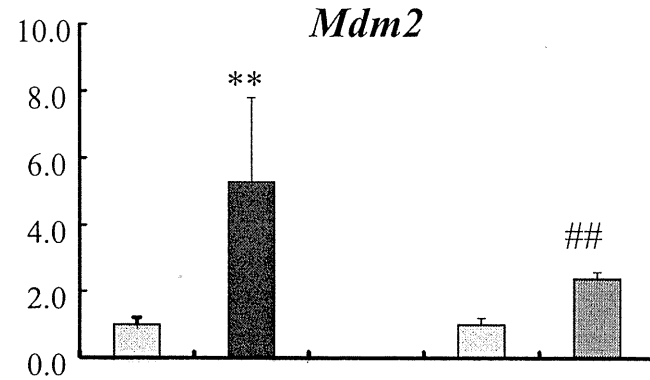
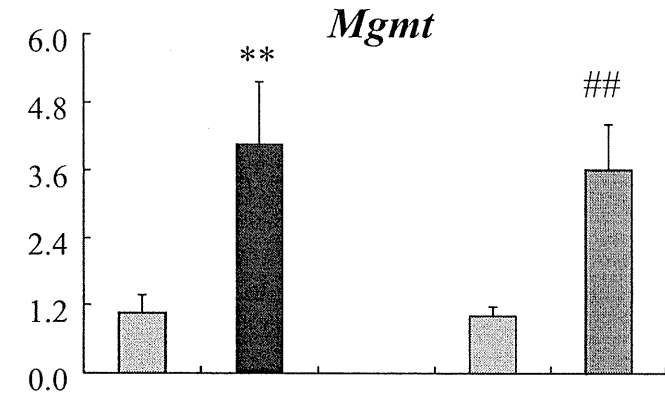


Fig. 5. The level of mRNA expression of the up or down-regulated genes in common in safrole or estragole-treated groups.



** : $p < 0.01$ vs Cont-1
(Student's *t*-test)
: $p < 0.01$ vs Cont-2
(Student's *t*-test)

Fig. 6. Growth curves for F344 *gpt* delta rats given 0, 10, 30 or 100 mg/kg MEUG for 13 weeks.

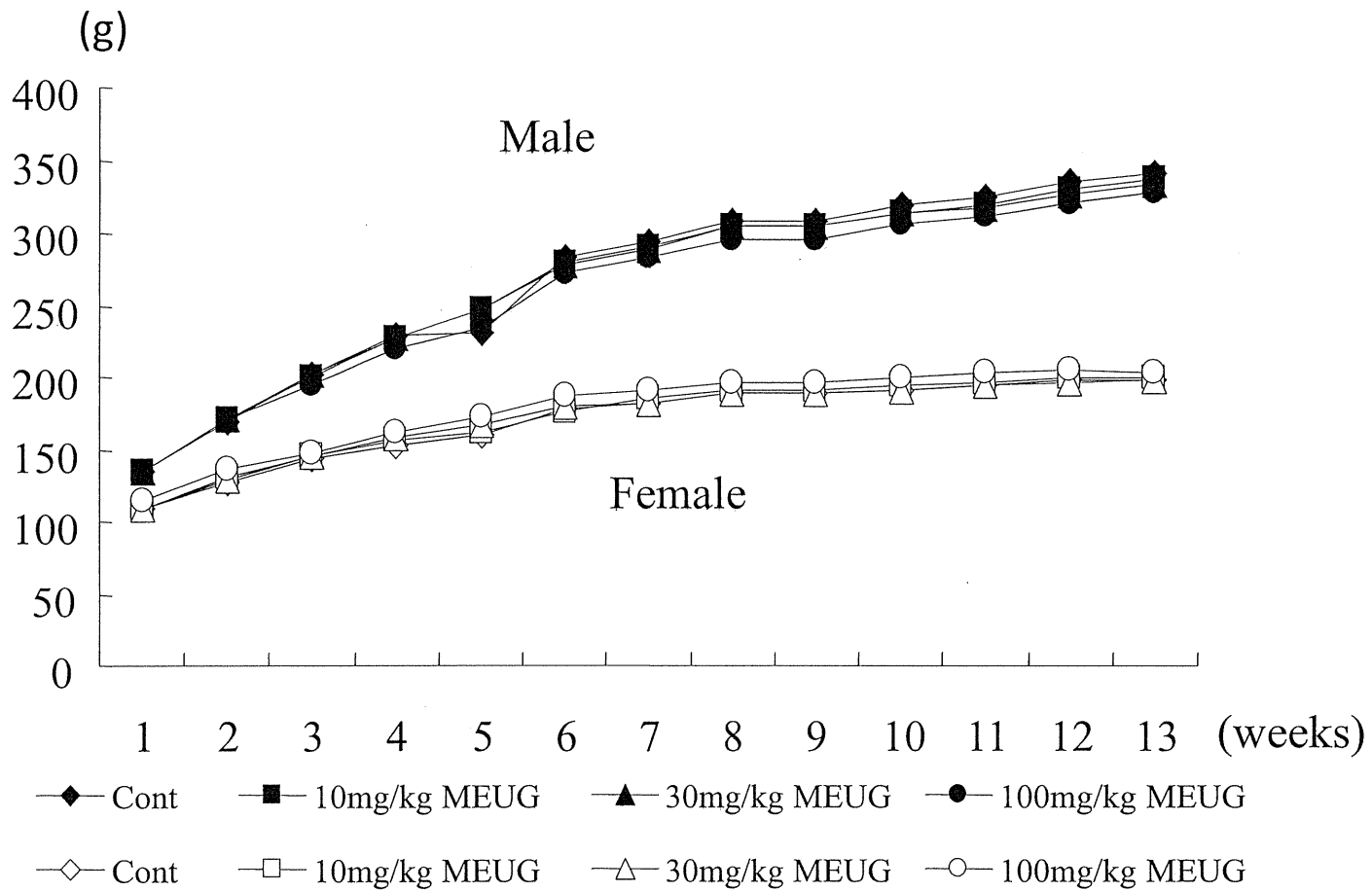


Fig. 7. Food consumption curves for male *gpt* delta rats given 0, 10, 30 or 100 mg/kg MEUG for 13 weeks.

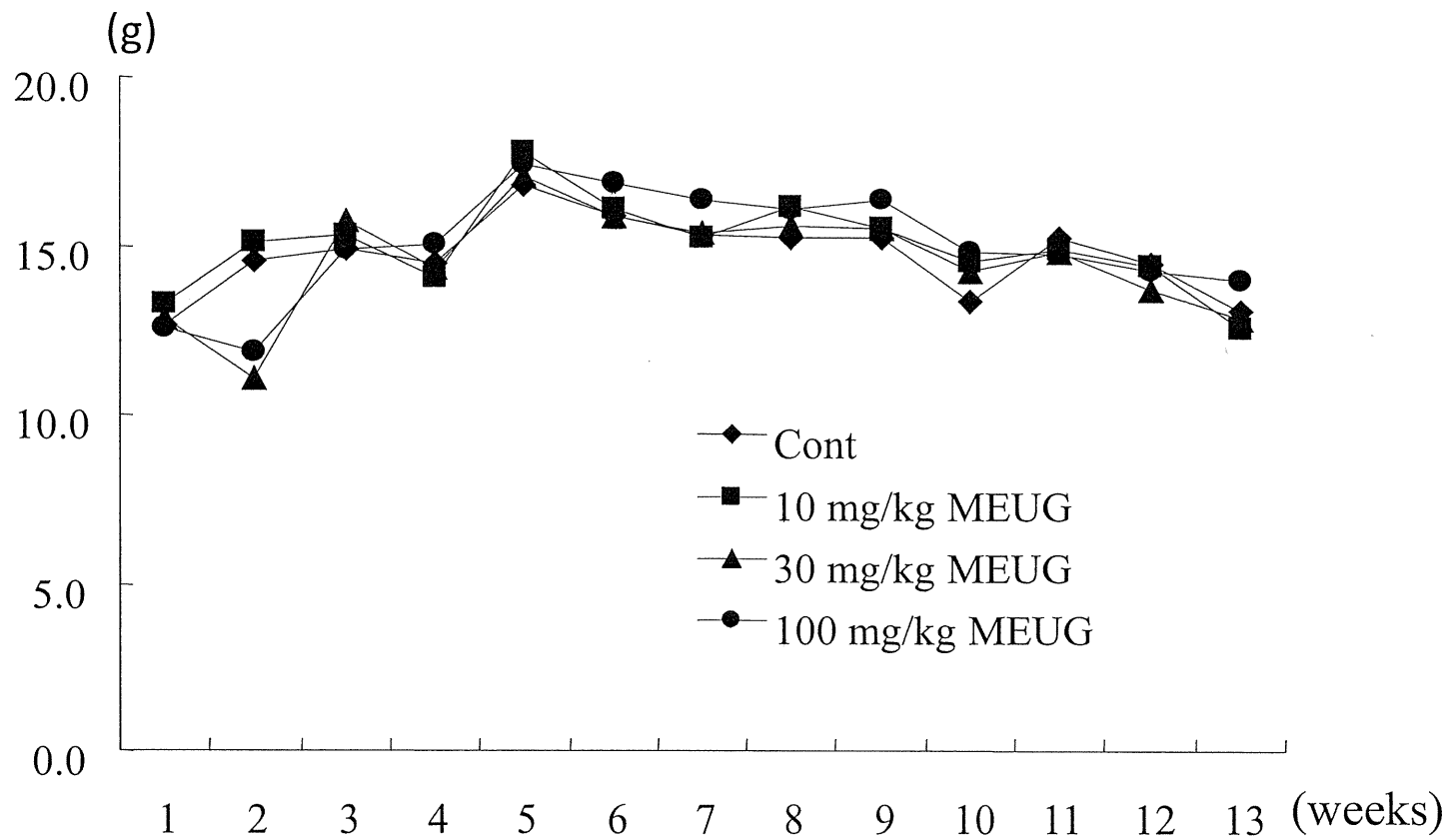


Fig. 8. Food consumption curves for female *gpt* delta rats given 0, 10, 30 or 100 mg/kg MEUG for 13 weeks.

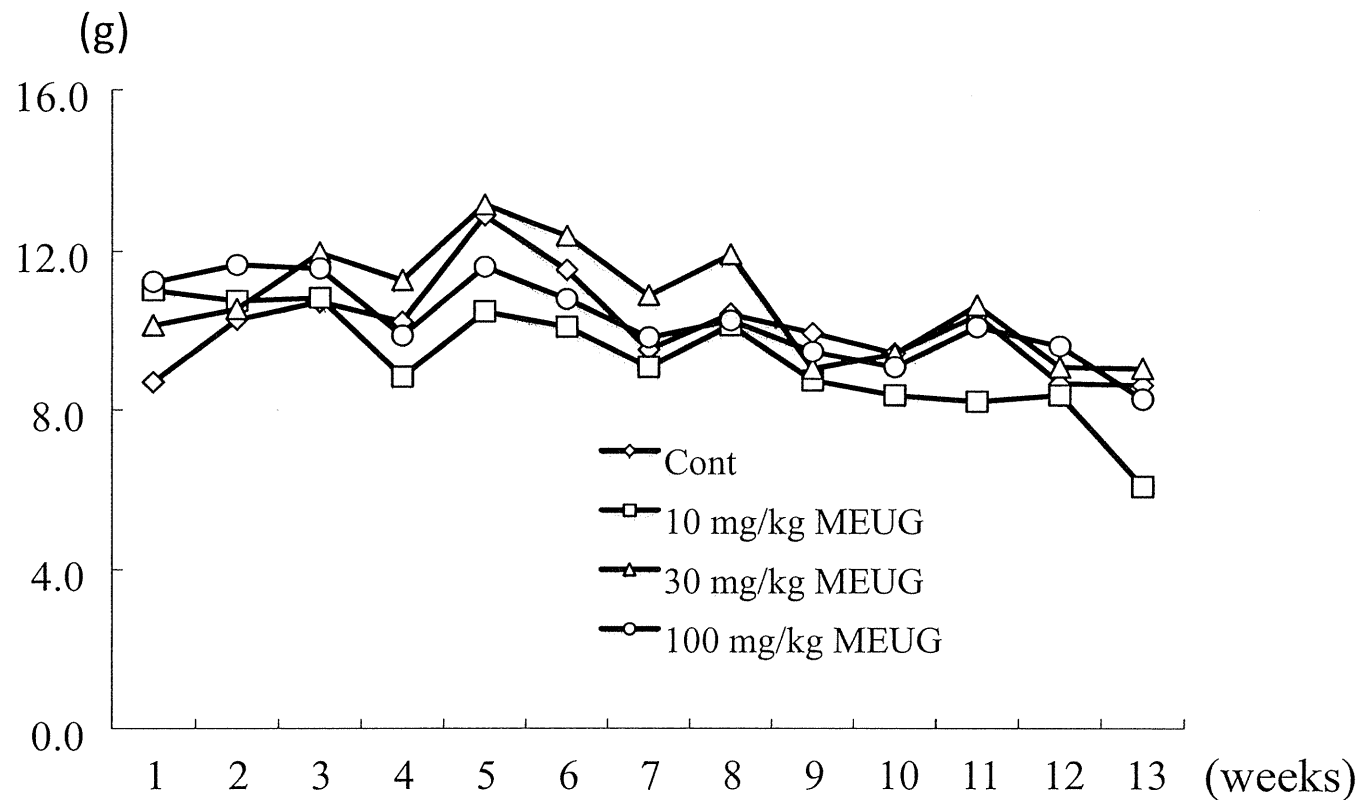
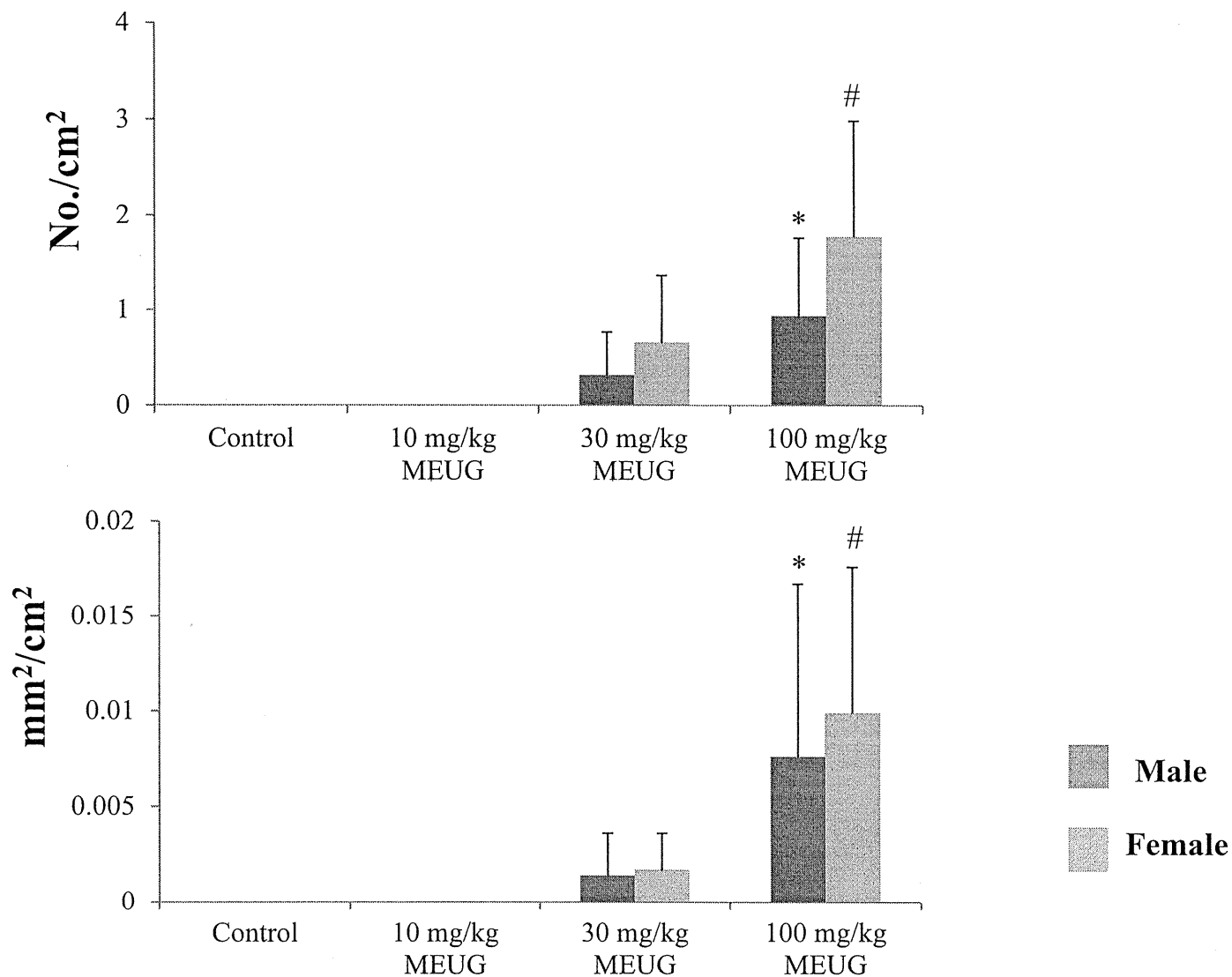


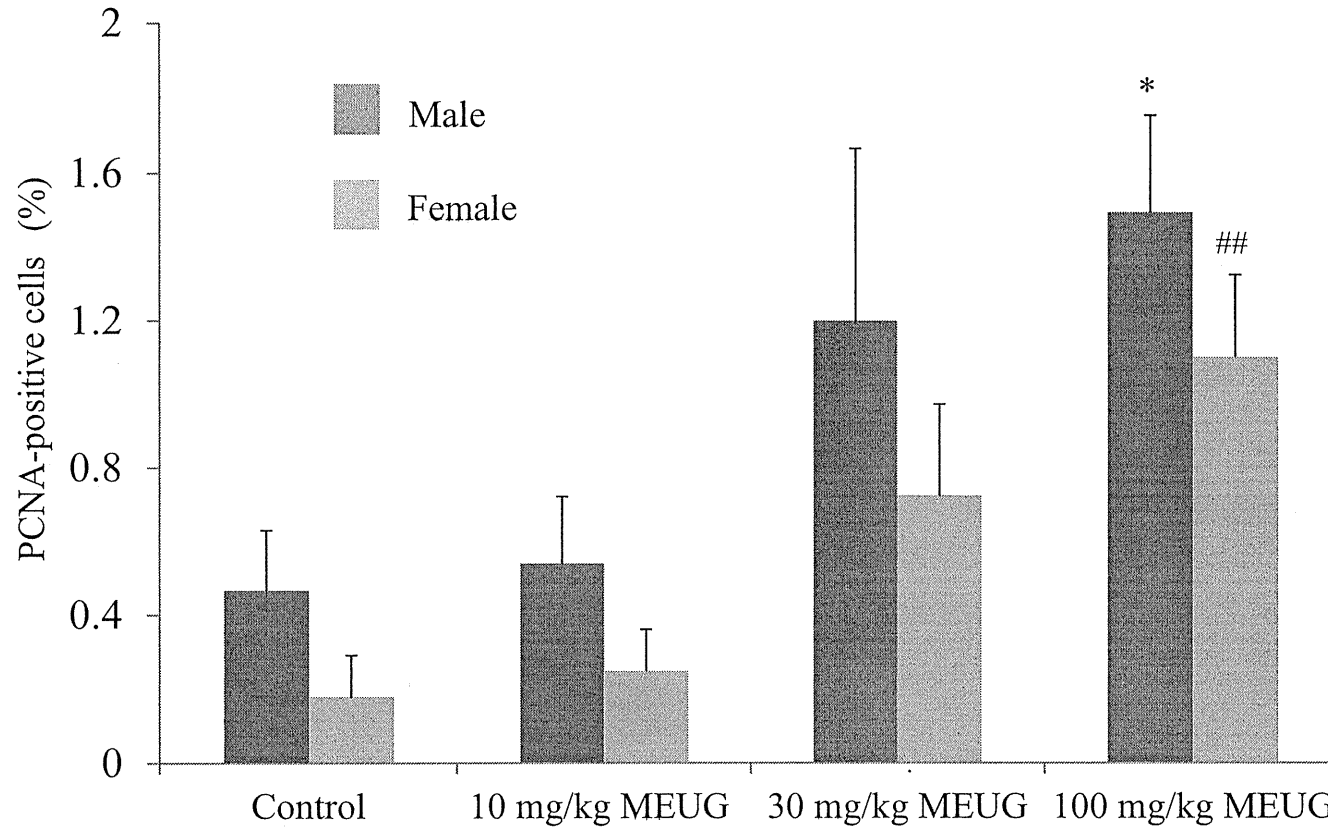
Fig. 9. Number and area of GST-P positive foci (≥ 3 cell) in the livers of F344 *gpt* delta rats given 0, 10, 30 or 100 mg/kg MEUG for 13 weeks



*; $p < 0.05$ vs significantly different from the control group of males (Dunnett's test)

#; $p < 0.05$ vs significantly different from the control group of females (Dunnett's test)

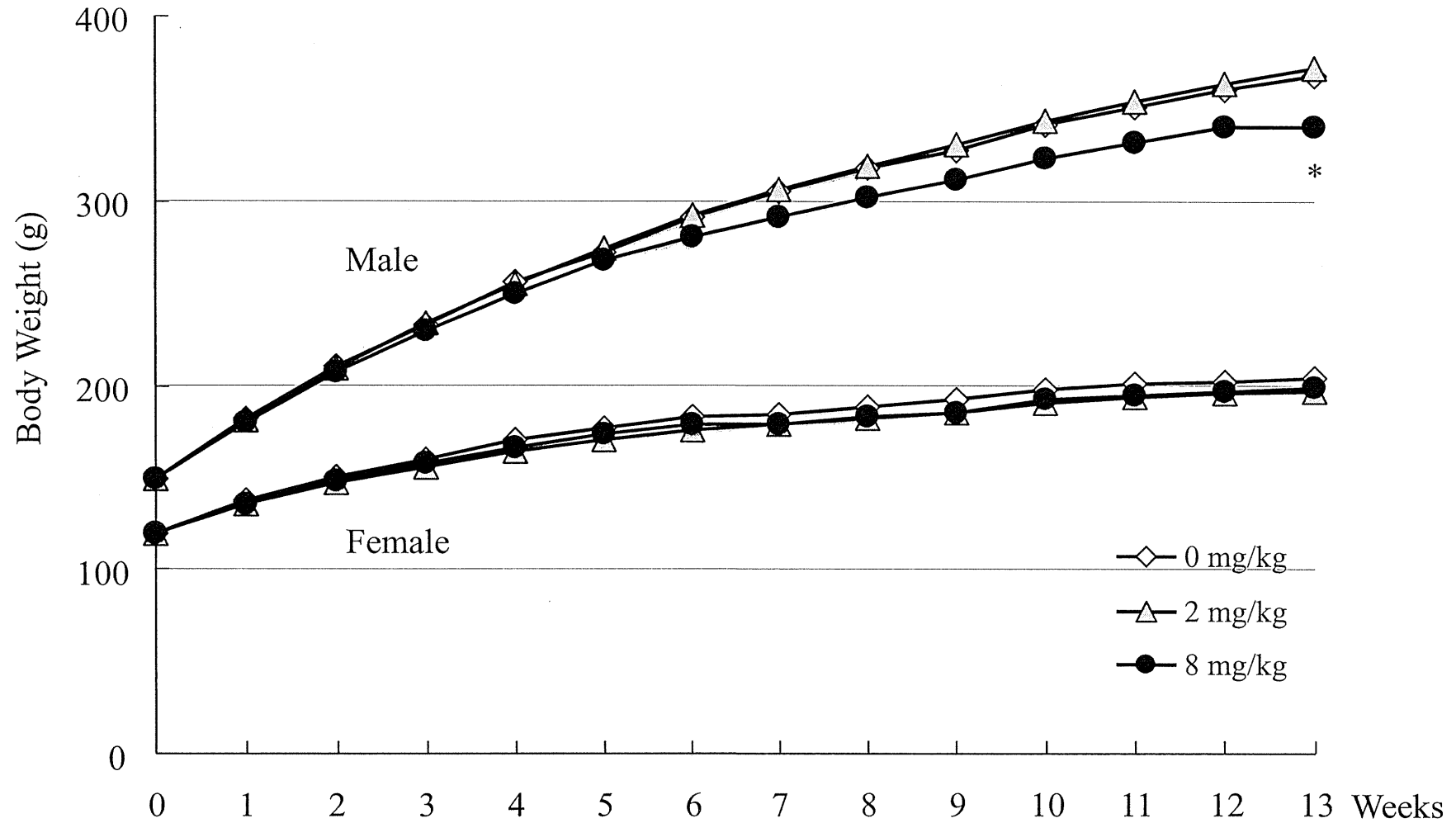
Fig. 10. Immunohistochemical staining of PCNA in the livers of F344 *gpt* delta rats given 0, 10, 30 or 100 mg/kg MEUG for 13 weeks.



* ; $p < 0.05$ vs significantly different from the control group of males (Dunnett's test)

##; $p < 0.01$ vs significantly different from the control group of females (Dunnett's test)

Figure 11. Growth curves for F344 *gpt* delta rats treated with Furan for 13 weeks



*: Significantly different from the 0 mg/kg group at $p < 0.05$.

Figure 12. Daily food intakes for F344 *gpt* delta rats treated with Furan for 13 weeks

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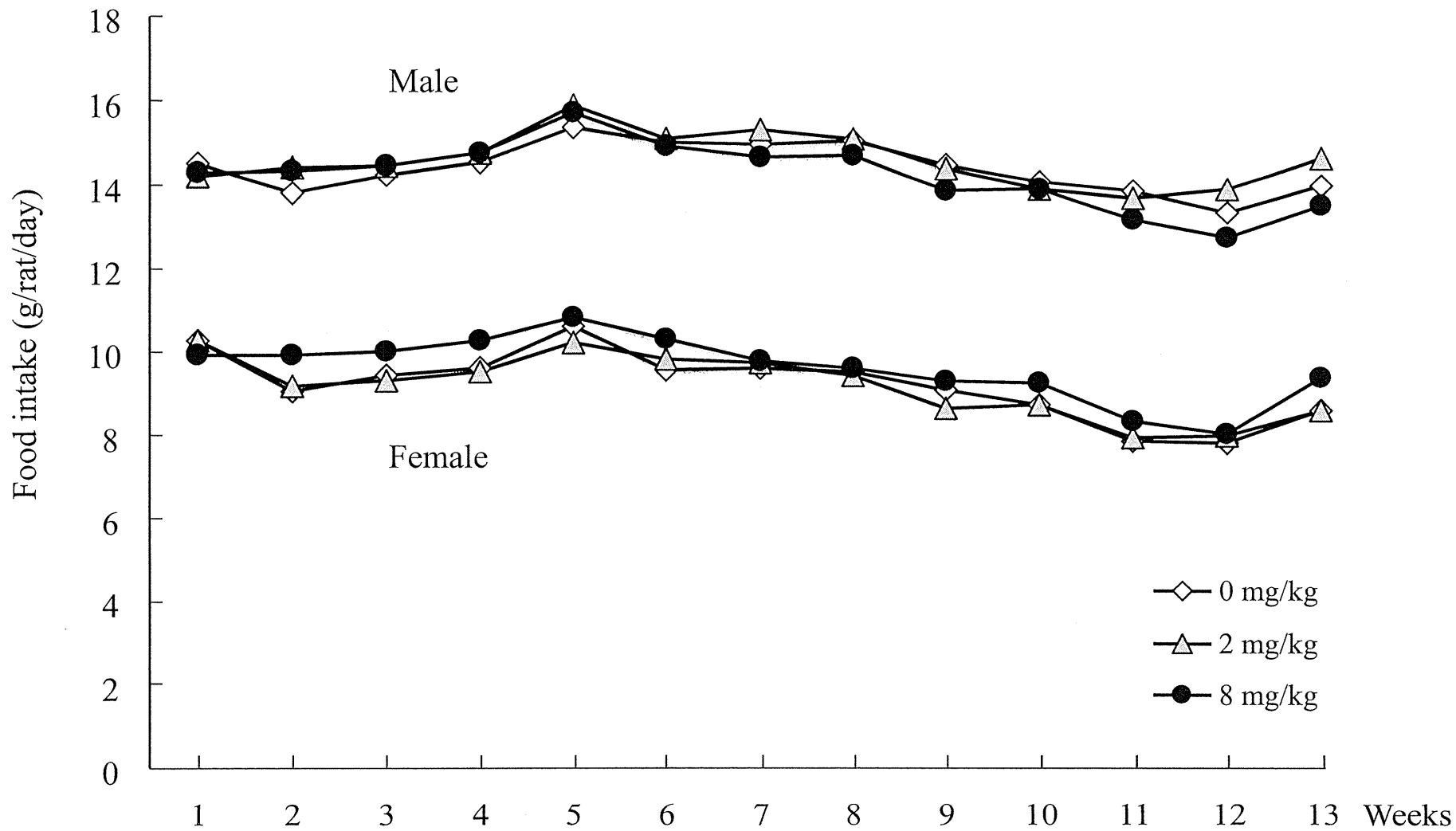
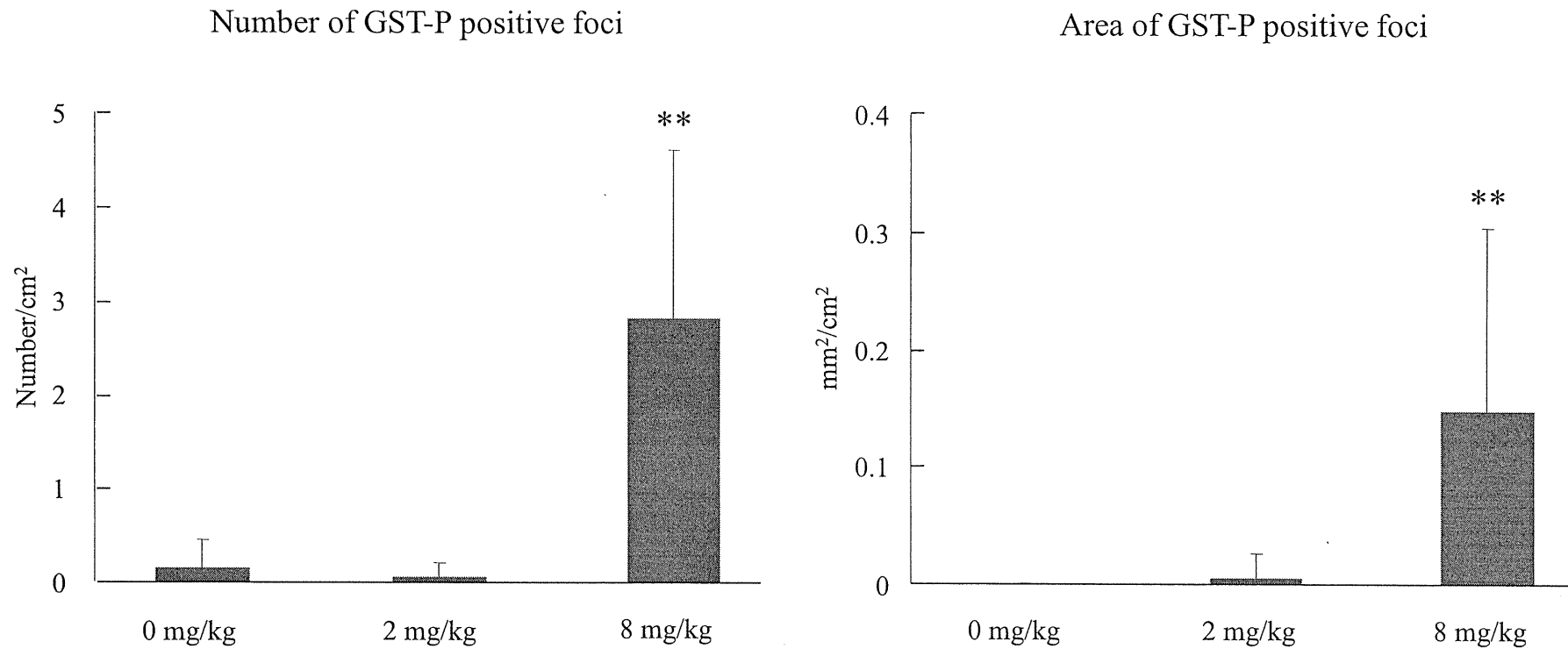


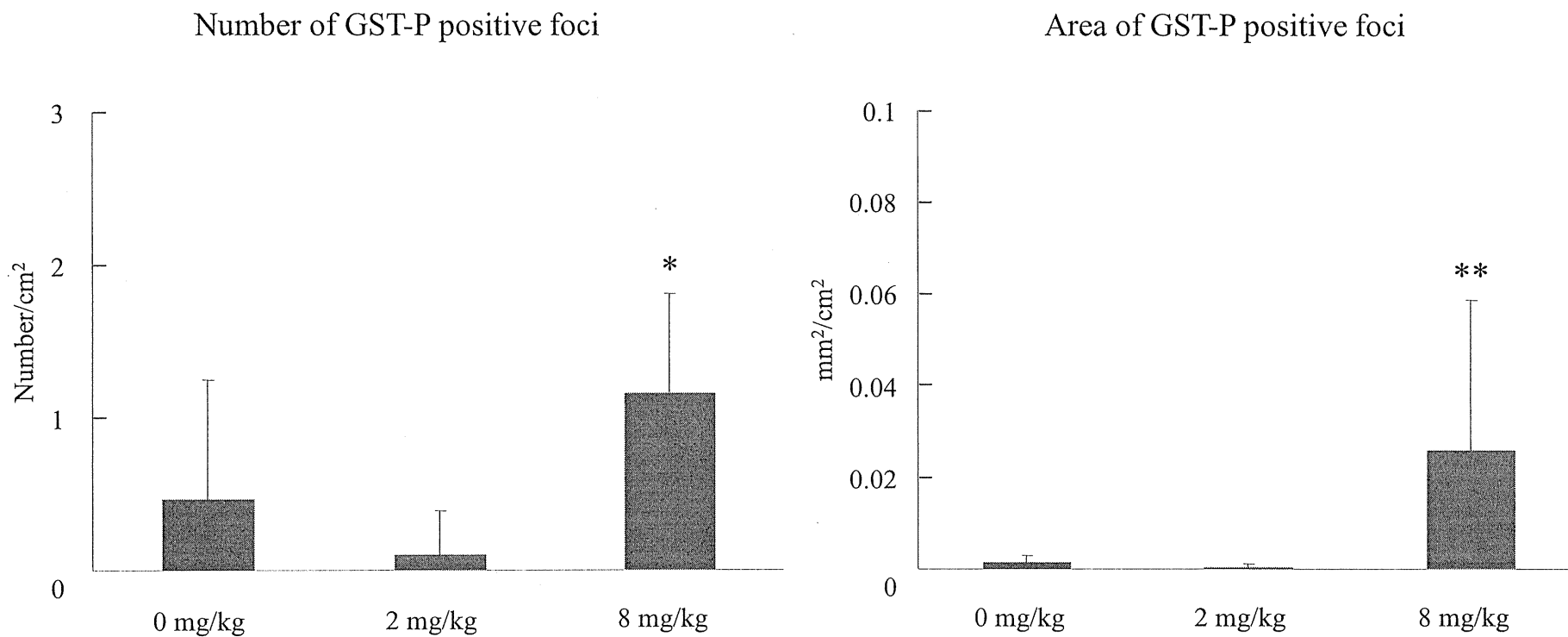
Figure 13. Quantitative analysis of GST-P positive foci in the livers of male F344 *gpt* delta rats treated with Furan for 13 weeks



** : Significantly different from the 0 mg/kg group at $p < 0.01$.

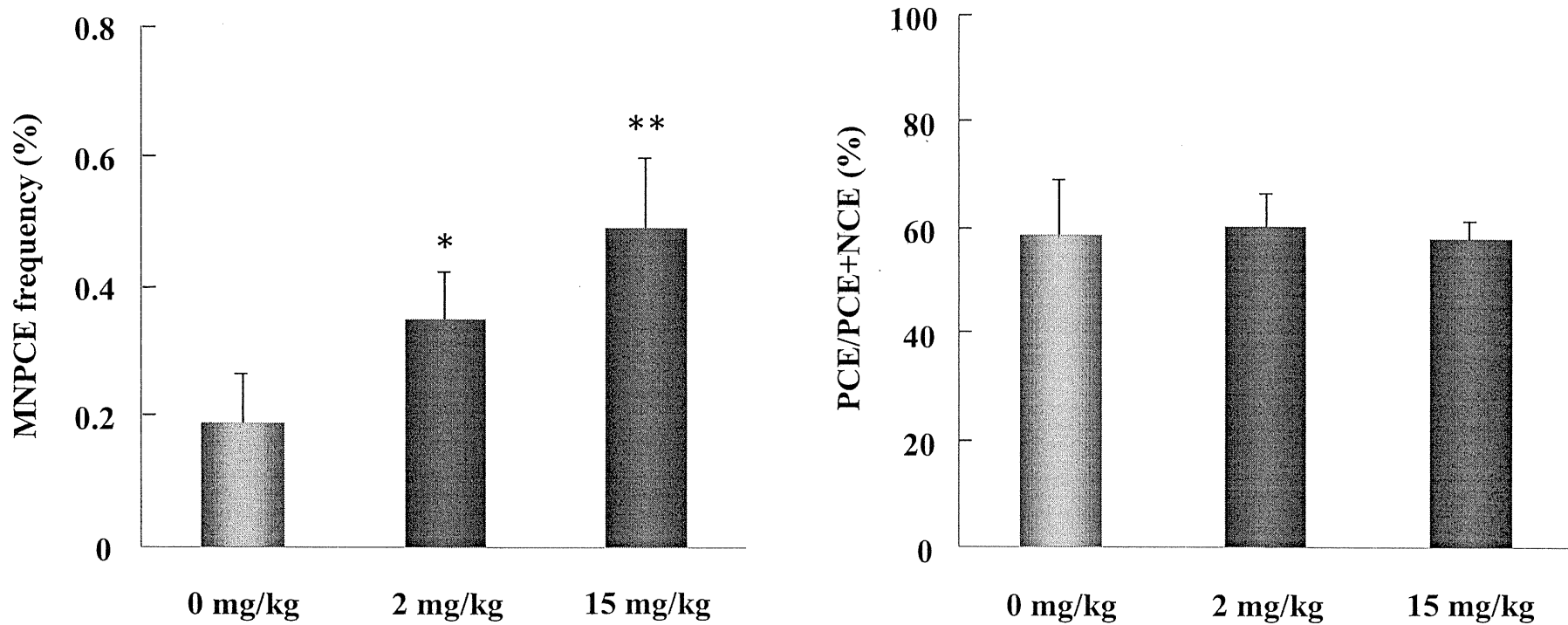
Figure 14. Quantitative analysis of GST-P positive foci in the livers of female F344 *gpt* delta rats treated with Furan for 13 weeks

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***: Significantly different from the 0 mg/kg group at $p < 0.05$ and 0.01 , respectively.

Figure 15. Micronucleus test with bone marrow in B6C3F₁gpt delta male mice treated with Furan for 4 weeks



MNPCE : Micronucleated polychromatic erythrocytes, PCE : Polychromatic erythrocytes, NCE : Normochromatic erythrocytes

*,** : Significantly different from the 0 mg/kg group at $p < 0.05$ and 0.01 , respectively.