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	Protocol No.	Study Title	Conclusions
1	IET 04-0138	Acute oral toxicity study in rats	Oral LD50 is greater than 2000mg/kg
2	IET 04-0139	Repeated dose 28-day oral toxicity study in rats	No oral toxicity in rats receiving 1000mg/kg/day for 4-weeks
3	IET 04-0140	Bacterial reverse mutation test	Not mutagenic to the bacteria used
4	IET 04-0141	In vitro chromosome aberration test	Did not include either structural or numeric chromosome aberrations
5	IET 04-0142	Micronucleus test in mice	Did not induce micronuclei in bone marrow cells of the mice
6	IET 04-0143	Dermal sensitization study in guinea pigs	Had no skin sensitizing potential in the guinea pig maximization test
7	IET 04-0144	4 week immunotoxicity study in rats	No toxic effects on immuno-toxicological parameters tested at 1000mg/kg body weight/day for 4 weeks

1. Summary

This study was conducted to evaluate the acute oral toxicity of Coenzyme Q10 in females of Specific-pathogen-free Wistar Hannover GALAS (BrlHan: WIST@Jcl) rats using acute toxic class method (OECD Guideline No.423). A dose of 2000 mg/kg was given twice for limit test (2000 mg/kg, step 1 and step 2). Then animals were observed for 14 days and weighed on days 7 and 14. At the end of the observation period, all surviving animals were necropsied after euthanasia by ether gas.

- There were no deaths at the dose of 2000 mg/kg (2000 mg/kg, step 1 and step 2).
There were no abnormal clinical signs that were detected during the observation period.
- All surviving animals gained their body weights on days 7 and 14.
- No macroscopic abnormalities were noted in any animal at the final necropsy after the end of the observation period.
- Based on the results mentioned above, the LD50 value of the test substance was determined according to the flow chart of acute toxic class method (Appendix 1) and LD50 value range was estimated using OECD GHS (Globally Harmonised System) category.

It is determined that oral LD50 value of Coenzyme Q10 in female Wistar Hannover GALAS (BrlHan: WIST@Jcl) rats was greater than 2000 mg/kg, which is equivalent to the GHS category 5 (>2000 mg/kg).

1. Summary

In order to evaluate the oral toxicity of Coenzyme Q10 (CoQ10) in rats, the test substance was administered by gavage to Wistar Hannover GALAS (Br/Han: WIST@Jcl) rats of both sexes at a dose level of 0 or 1000 mg/kg/day for a period of 4 weeks (31 days).

Each dose group consisted of 10 animals per sex. Mortality, clinical signs, body weight, and food consumption were monitored for all animals during the treatment period. Ophthalmological examination was performed on all animals during the acclimatization period and those at 4 weeks of treatment. After 31 days of treatment, all animals were subjected to hematological and blood biochemical examinations followed by necropsy, organ weight management, and histopathological examination. The results are summarized as follows:

Chemical analyses verified that a good homogeneity of the test substance in the test solution was obtained by the preparation method used and that the test substance was stable in test solution kept under the conditions of the present study. In addition, concentrations of the test substance in test solution at each preparation were confirmed to be within acceptable limits.

There were no deaths in any group of either sex. No treatment-related changes were observed in clinical signs, body weight, food consumption, food efficiency, ophthalmology, urinalysis, hematology, blood biochemistry, necropsy, organ weights or histopathology in the treated group of either sex.

Based on the results described above, it was concluded that there was no evidence of oral toxicity of Coenzyme Q10 in male or female Wistar Hannover GALAS rats receiving 1000 mg/kg/day under the conditions of the present study.

1. Summary

Bacterial reverse mutation test by a pre-incubation method was performed on Coenzyme Q10 using four tester strains of *Salmonella typhimurium* TA100, TA1535, TA98, and TA1537 and one strain of *Escherichia coli* WP2 *uvrA*/pKM101. Coenzyme Q10 was suspended in dimethyl sulfoxide for the tests.

On the Basis of the result of the preliminary dose range-finding test, the reverse mutation test was carried out at dose levels of 313-5000 µg/plate and 156-5000 µg/plate in the presence and absence of a metabolic activation system, respectively.

The mean number of revertant colonies in any strain at any dose level of Coenzyme Q10 did not exceed two times above that of revertant colonies in the corresponding solvent control, either in the presence or in the absence of a metabolic activation system.

On the other hand, a two-fold or greater increase above the solvent control in the mean number of revertant colonies was observed in the positive control group of each strain.

It is concluded that Coenzyme Q10 is not mutagenic to the bacteria under the conditions.

1. Summary

The *in vitro* chromosome aberration test using cultured Chinese hamster CHL cells was performed to evaluate the clastogenic potential of Coenzyme Q10. Dimethyl sulfoxide was used as a solvent (vehicle) in the study.

In the preliminary growth inhibition test, Coenzyme Q10 showed almost no cytotoxicity to the cells, but precipitation of the test substance was observed at 125 µg/mL or higher. Accordingly, four concentrations of 31.3, 62.5, 125, and 250 µg/mL were selected for the experiments.

In the experiment by a short-term treatment method, the cells were treated with Coenzyme Q10 for six hours in both the presence and absence of a metabolic activation system and chromosome preparations were made at 18 hours after re-cultivation. In the experiment by a continuous treatment method, on the other hand, the cells were continuously treated with Coenzyme Q10 for 24 hours in the absence of a metabolic activation system and then chromosome preparations were obtained.

As a result of microscopic analysis on the chromosome preparations, there were no significant increases in the frequency of the metaphases with structural and numerical chromosome aberration of Coenzyme Q10 in any treatment method. Aberrations were observed at a significantly high frequency in the positive controls treated with Mitomycin C or Benzo(a)pyrene.

It is concluded that, under the conditions used in the study, Coenzyme Q10 did not induce either structural or numeric chromosome aberrations in the presence of the metabolic activation system.

1. Summary

The bone marrow micronucleus test was performed with Coenzyme Q10 in ICR (Crj:CD1) male mice. On the basis of the result of the preliminary toxicity test, the limit dose of 2000 mg/kg/day was chosen in the micronucleus test.

Coenzyme Q10 was suspended in olive oil and orally administered twice with 24-hour interval to five mice per group by intragastric gavage. Bone marrow smears were obtained from the mice 24 hours after the double administration.

As a result of microscopic examination, no significant increase in the frequency of micronucleated polychromatic erythrocytes was observed at 2000 mg/kg/day of Coenzyme Q10, when compared with the vehicle control group. In the positive control group treated with mitomycin C, on the other hand, marked increase in the frequency of micronucleated polychromatic erythrocytes was observed.

It was concluded from these results that Coenzyme Q10 did not induce micronuclei in bone marrow cells of the mice under the conditions of this study.

1. Summary

This study was conducted to evaluate the dermal sensitization potential of Coenzyme Q10 (CoQ10) by the guinea pig maximization test. CoQ10 was applied to the skin of female Hartley strain guinea pigs. DNCB (2,4-dinitrochlorobenzene) was used as the positive control substance. Animals were assigned to the following 4 groups: Twenty animals to the test substance treatment group (Co Q10 treatment group), exposed to CoQ10 both at the induction and challenge; 10 animals to the negative control group for the test substance (Co Q10), exposed to Co Q10 only at the challenge; and 5 animals to the negative control group for DNCB, exposed to DNCB only at the challenge. Concentrations of 5%, 50% and 50% of CoQ10, and concentrations of 0.1%, 1% and 2% of DNCB were selected as the dose for intradermal induction, topical induction, and challenge, respectively. Skin reaction to the challenge was observed 24 to 48 hrs after the patch removal and skin sensitization rates were calculated.

All 20 animals in Co Q10 treatment group exhibited the reaction of score 0 (no reaction). All 10 animals in the negative control group for Co Q10 also exhibited score 0. Thus, the sensitizing rate, i.e., [(No. of animals positively sensitized)/(No. of animals examined) x 100], was 0% in Co Q10 treatment group, and was classified into Classification I (weak skin sensitization).

In the DNCB treatment group, all 10 animals exhibited the reaction of score 3 (intense redness and swelling). On the other hand, all 5 animals in the negative control group for DNCB exhibited score 0. Thus, the sensitizing rate of DNCB was 100%, and was classified into Classification V (extreme skin sensitization). This was considered to sufficiently assure the reliability of this study.

Based on the results mentioned above, it was concluded that Coenzyme Q10 had no skin sensitizing potential in the guinea pig maximization test.

1. Summary

A 4-week immunotoxicity study was conducted in rats to evaluate the immunotoxicity of Coenzyme Q10. The test substance was administered to specific pathogen-free Wistar male rats (16 males/group) by oral gavage at dose levels of 0 and 1000 mg/kg body weight/day for a period of 4 weeks. Additionally, positive control substance (Cyclophosphamide, CP) was given orally by gavage at a dose of 3 mg/kg body weight/day (16 males/group) for 4 weeks. In each group, 8 males out of 16 males were set for flow cytometry analysis of lymphocyte and remaining 8 males for anti-SRBC immunoglobulin M (Ig M) measurement. All animals were observed daily for clinical signs during the study. Body weight and food consumption were recorded. After 4 weeks of treatment, animals were subjected to necropsy, organ weigh analysis, flow cytometric analysis of lymphocyte, and anti-SRBC Ig M measurement.

There were no toxic effects in the clinical signs, body weights, food consumption, gross findings and organ weights in the CoQ10 treatment group.

In the immunological examinations, no significant immuno-suppressive changes were noted in any parameters of the cellularity analysis of the thymus and spleen, lymphocyte subsets analysis of thymic and splenic cells, and SRBC specific Ig M antibody titers. Whereas, in the splenic lymphocyte subsets analysis, both of Pan-T and Pan-B cell population was increased in the 1000 mg/kg of CoQ10 treatment group, which may be due to the immuno-modulating activity of CoQ10.

In the rats receiving positive control substance of CP, there were immunosuppressive changes in the organ weights and cellularity of the spleen; lymphocyte subsets including T cells and B cells in the splenic lymphocyte subsets; and SRBC specific Ig M antibody titers. These immunosuppressive evidences of CP carried out in this study showed good reliability of the method and procedure used.

In conclusion, there were no toxic effects on immuno-toxicological parameters of lymphocyte subsets of thymic and splenic lymphocytes and SRBC specific Ig M antibody titers in rats following oral administration of Coenzyme Q10 for 4 weeks.