



INSTITUT PASTEUR DE LILLE

Laboratoire de Toxicologie

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STUDY REPORT

CONFIDENTIAL

STUDY TITLE

**MUTAGENICITY STUDY USING THE MICRONUCLEUS TEST IN MICE
WITH PB 100**

AUTHOR

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PERFORMED BY

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SUBMITTED TO

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D / COMMENTS AND CONCLUSION

The potential clastogenic activity of **PB 100** from **C.R.I.D.** was tested using the micronucleus test in mice by the oral route using three dose levels with a treatment schedule of 3 daily administrations with a 24-hour interval, followed by one sampling at 24 hours after the last treatment.

Preliminary toxicity assay showed that the maximum tolerated dose was 125 mg/kg/day (x3). But in the confirmatory study 2 males out of 5 died after 1 and 3 days respectively at this dose. The 80 mg/kg (x3) dose which provoked no death both in the preliminary and confirmatory assays was retained as the high dose for the micronucleus test. Two lower doses were chosen according to a ratio of 2, i.e., 40 and 20 mg/kg/day (x3).

The weight homogeneity of the animals used in this test after random-distribution, was verified for males and females separately, by comparing the weight mean of the treatment groups with the one of the control group. There was no statistically significant difference between the weights of animals treated with the test compound and control mice.

No symptomatology was noted after administration in animals treated at 3 dose levels.

The results obtained on negative control animals and those treated with the reference product were similar to those generally obtained in the laboratory. The marked increase in the frequency of micronuclei in animals treated with cyclophosphamide (25.3 per 1000 PCE in males and 13.0 per 1000 PCE in females combined) attested the sensitivity of the strain of mice used. The validity criteria for the test were fulfilled. Males were more sensitive to the clastogenic activity of cyclophosphamide used as positive control.

The ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) was established at each dose level for each group. A strong and statistically significant decrease ($p < 0.001$) in the ratio PCE to NCE was noted at the highest dose tested of 80 mg/kg when compared with the control group. That depression in the proportion of PCE to NCE which reflects a toxic effect in bone marrow provoked by one of the doses administered is in compliance with the OECD guideline and proved the bone marrow exposure. A slight but statistically significant increase ($p < 0.05$) in the ratio PCE to NCE was noted at the lowest dose tested of 20 mg/kg in males when compared with the control group but this variation was not observed in females and in pooled males and females. This increase is in aid of a stimulation of erythropoiesis in males at this dose.

Regarding the frequency of micronuclei, no statistically significant increase in the number of micronucleated polychromatic erythrocytes was noted in the animals treated with PB100 at any dose tested, two sexes combined or males and females separately, in comparison with the control group.

In conclusion, under these experimental conditions, the test compound **PB 100** from **C.R.I.D.** induced no clastogenic activity that can be demonstrated through the micronucleus test in mice.