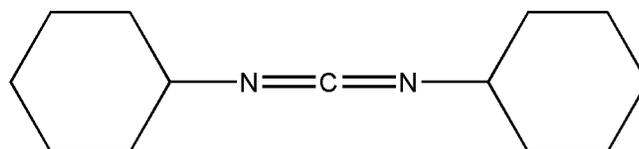




**NTP**  
National Toxicology Program

## Dermal Toxicology and Carcinogenesis Studies of Dicyclohexylcarbodiimide (DCC) in Genetically Modified Mouse Models





# Background Information

- Dicyclohexylcarbodiimide (DCC) and Diisopropylcarbodiimide (DIC) were nominated by the NCI for toxicity and carcinogenicity studies as representatives of the carbodiimide chemical class
- DCC and DIC are used as stabilizing, coupling and condensing agents
- The potential for DCC exposure exists during the synthesis of polypeptides and other chemicals in the chemical, pharmaceutical and recombinant DNA industry
- Results of DIC studies have been reported and were negative for carcinogenicity in traditional bioassay and in GMM models



# Studies Performed by the NTP

- 2- and 13-Week studies in F344 rats and B6C3F1 mice by dermal route of exposure
- Studies in GMM models
  - Female Tg.AC Hemizygous Mice
  - Female p53 Haploinsufficient Mice



## 2-Week Study Results

### Rats

- Doses used: 0, 0.6, 1.8, 5.1, 15 and 45 mg/animal in 0.3 ml ethanol
- DCC was lethal in the top three dose groups
- Of surviving animals body weights were comparable in all groups
- Epidermal hyperplasia, necrosis and inflammation at site of application
- *Doses selected for 13-week studies: 0, 0.75, 1.5, 3.0, 6.0 and 12 mg/kg*

### Mice

- Doses used: 0, 0.2, 0.6, 1.7, 5.0 and 15 mg/animal in 0.1ml ethanol
- One 0.6 mg female and all in the top three groups died
- Body weights were about 10% lower in 0.6 mg group
- Epidermal hyperplasia, necrosis and inflammation at the site of application.
- *Doses selected for 13-week studies: 0, 1.5, 3.0, 6.0, 12.0 and 24 mg/kg*



## **13-Week Study Results in F344 Rats and B6C3F1 Mice**

- High dose male and female groups died
- Body weight decreases mostly less than 10%
- Clinical pathology parameter changes secondary to inflammation
- Reproductive parameters not affected in rats. In mice, slight decreases in epididymis weight and sperm motility in high dose groups
- At the site of application, dose related irritation, inflammation, hyperplasia of epidermis and sebaceous glands; necrosis mostly in the high dose groups
- *0, 0.75, 1.5, 3.0, 6.0 and 12.0 mg/kg dose levels were selected for both GMM model studies*



## **26-Week Study in Female p53 Haploinsufficient Mice**

- 0, 0.75, 1.5, 3, 6 or 12 mg/kg of DCC in ethanol administered to group of 15 mice, 5 days per week for 26 weeks
- Dosing of 6 and 12 mg/kg group was discontinued after 11 and 8 days, respectively, because of severe skin lesions
- Mean body weights were comparable to controls
- No dose related effects on survival
- No neoplasm incidences
- At the site of application, dose related incidences of epidermal hyperplasia and focal chronic active inflammation were observed. In the high dose group there were incidences of focal ulcers and chronic active inflammation



## **20-Week Study in Female Tg.AC Hemizygous Mice**

- Groups of 10 mice were dermally administered 0, 0.75, 1.5, 3, 6 or 12 mg DCC/kg B.W. in ethanol for 5 days per week for 20 weeks
- Dosing of 12 mg dose group was discontinued after eight applications because of severe skin lesions
- Overall, survival was within the range known for this model
- Mean body weights were similar to those of controls
- At the site of application , the incidences of squamous cell papilloma were increased in dose-related manner
- The incidences of chronic active inflammation and epidermal hyperplasia were increased in 3 and 6 mg groups



## Neoplastic and Nonneoplastic Skin Lesions at the Site of Application in DCC Treated Female Tg.AC Mice

|  | Control | 0.75 mg | 1.5 mg | 3 mg | 6 mg | 12 mg <sup>a</sup> |
|--|---------|---------|--------|------|------|--------------------|
| Inflammation   | 0       | 0       | 3      | 5*   | 8**  | 0                  |
| Hyperplasia  | 0       | 0       | 0      | 6**  | 7**  | 1                  |
| Squamous Cell Papilloma, multiple  | 0       | 0       | 1      | 0    | 3    | 5*                 |
| Squamous Cell Papilloma (includes multiple)  | 0       | 0       | 1      | 3    | 6**  | 8**                |
| Squamous Cell Carcinoma  | 0       | 0       | 0      | 0    | 1    | 0                  |
| * p≤0.05    ** p≤0.01 <sup>a</sup> treatment discontinued after eight applications |         |         |        |      |      |                    |



## Genetic Toxicology

- DCC is not mutagenic in a number of *Salmonella typhimurium* strains with or without rat or hamster liver S9 activation enzymes
- A small but significant increase in the frequency of micronucleated normochromatic erythrocytes in male B6C3F1 mice
- Negative results in an acute three-injection micronucleus study in bone marrow of male F344 rats



## Conclusions

- Under the conditions of this 26-week dermal study, there was *no evidence of carcinogenic activity* of dicyclohexylcarbodiimide in female p53 haploinsufficient mice administered 0.75, 1.5, 3.0, 6.0 or 12 mg/kg in ethanol.
- Female Tg.AC hemizygous mice dosed with dicyclohexylcarbodiimide for 20 weeks had significantly increased incidences of squamous cell papilloma of the skin at the site of dermal application.
- Nonneoplastic lesions noted at the site of application included hyperkeratosis, chronic active inflammation, and epidermal hyperplasia in p53 haploinsufficient and Tg.AC hemizygous mice.