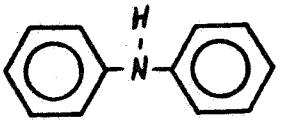
RDCoberly:deg
October 12, 1967

Chemical Name	:	Diphenylamine
Chemical Structure	:	
Empirical Formula	:	$C_{12}H_{11}N$
Molecular Weight	:	169.22
Physical State	:	Crystals
Odor	:	Floral odor
Melting Point	:	53°-54°
Flash Point	:	153°
Solubility	:	Insoluble in water. Soluble in alcohol, benzene, ether.
Reaction	:	Forms salts with strong acids.
Human Toxicity	:	May be irritating to mucous membranes. Methemoglobinemia has been produced experimentally (Merck).
Veterinary Use	:	Treatment of screwworm (Merck)
Label Use	:	Apple storage scald
Company	:	American Cyanamid Co.

Acute Rat Oral	:	Male LD_{50} = 3.2 gm/Kg Surviving animals showed dark spleens.
Acute Rabbit Dermal	:	Dosage levels up to and including 10 gm/Kg produced no deaths or systemic toxicity. Slight erythema and edema were noted.
Acute Rabbit Eye Irritation	:	A moderate degree of conjunctivitis and injection of the sclera and nictitating membrane, accompanied by lacrimation was noted.
Subacute Rat Feeding (30 days)	:	Levels tested were 0.01, 0.10, and 1.13 gm/Kg. The 1.13 gm/Kg level produced paleness of the extremities with 9/10 animals showing darken spleens with roughened surface texture. May be signs of methemoglobinemia.
Methemoglobin Study	:	Dermal - 8 gm/Kg caused a slight increase in methemoglobin. Oral - 2.5 gm/Kg caused a slight increase in methemoglobin.

Summary (Diphenylamine)

The toxicological data shows the chemical to exhibite a low degree of oral and dermal toxity. Both the methemoglobin study and the subacute rat feeding study showed the chemical to have the ability to increase the percentage of methemoglobin in the blood. Both routes used in the methemoglobin study, dermal at 8 gm/Kg and oral at 2.5 mg/Kg, produced a slight to moderate increase in methemoglobin.

It appears logical to know the toxicity of this material by the inhalation route, this includes measurement of methemoglobin. As this data is not available, no comment in this area can be offered.

Due to the fact that the chemical does have the ability to produce methemoglobin, it appears logical to prevent its use in hospitals as an additive to laundry, as a diaper additive or general wash additive (includes any type of material which comes in direct contact with the skin).

RDCoberly:deg
October 12, 1967

Diphenylamine

Acute Rat Oral

Five male rats were tested per dosage level of 1.0 and 2.2 gm/Kg (with a 10% aqueous suspension) and 4.6 and 10 gm/Kg (using a 50% aqueous suspension).

Results

The LD_{50} = 3.2 gm/Kg. All of the animals on the two high levels died and all of the animals on the two lower levels survived. For several hours prior to death the animals were extremely depressed and exhibited lacrimation, bloody discharge around the nose, labored respiration, ataxia and paleness of the extremities.

The surviving animals were sacrificed on day seven whereupon the spleens were noticed to be unusually dark in appearance. No other significant gross pathology was observed.

Acute Rabbit Dermal

Four rabbits were tested per dosage level of 1.0, 2.2, 4.6, and 10 gm/Kg. The dose was made into a paste by means of adding sufficient water. The exposure time was 24 hours.

Results

There were no deaths nor any signs of systemic toxicity at any dosage level, during either the period of exposure or a seven day observation

4

period that followed. In most cases slight erythema of the skin was observed on removal of the cuff, and a slight edema was present in several instances. The edema subsided within 24 hours and the erythema with 48 to 72 hours.

Acute Rabbit Eye Irritation

A 3.0 mg quantity of the dry product was placed in the conjunctival sac of the left eye of each of three rabbits. The lids were held closed for approximately 30 seconds after which the eye was examined immediately and again at intervals over the next seven days.

Results

There was little immediate reaction; however, within four hours there developed a moderate degree of conjunctivitis and injection of the sclera and nictitating membrane, accompanied by lacrimation and the formation of slight amounts of exudate around the margin of the lids. These signs of irritation had cleared in two of the three animals by 24 hours. Toxic signs in this animal cleared the following day.

Subacute Rat Feeding (30 days)

Ten male rats were tested per dosage level of 0.01, 0.1 and 1.0% (10,000 ppm) respectively. These dosage levels were calculated as being equivalent to 0.01, 0.10, and 1.13 gm/Kg.

Results

There were no deaths nor signs of systemic toxicity at the 0.01 and 0.1% levels. At the 1.0% level, mean body weight was only approximately half that of the control group although mean food intake was not significantly different. Paleness of the extremities was observed in most of the animals at the highest level of feeding. This may be indicative of methemoglobinemia.

At autopsy the animals on the levels of 0.01 and 0.1% disclosed no gross pathology which could be attributed to feeding of the test material. One rat of the high level showed a cyst and two very small darkened areas between cortex and medulla. The spleen of this animal was dark in appearance and roughened on the surface. The spleens of eight other animals of this group presented a similar appearance in texture. The kidneys of three rats were hyperemic, and sections showed a very pale medulla with a bluish tinge.

Methemoglobin Study

Study No. 1

A dose of 32 ml/Kg of a 25% solution (8 gm/Kg of active ingredient) was applied to closely clipped skin of rats. Doses were not covered, and the animals were kept under restrain for four hours after dosing. At the end of this time the post-exposure blood sample was collected. Two sets of animals were dosed about one week apart.

Prior to the application of the test material blood was extracted from each of the animals and analyzed for its methemoglobin content.

Study No. 2

A single oral dose of 10 ml/Kg (2.5 gm/Kg of material) or 5 ml/Kg (1.25 gm/Kg of test material) was given to each of two animals. They were returned to their cages after dosing and post-exposure blood samples collected four hours later.

Results

The procedure employed determines methemoglobin as percent of total hemoglobin, and no hemoglobin values were determined separately. However, assuming an average hemoglobin concentration of 14.8 gm/Ml, the maximum amount of methemoglobin found would be less than 1 gram per 100 ml.

The results of the two dermal studies show that at four hours post-exposure from 0.8 to 6.4% methemoglobin was found in the test animals as compared to -1.0 to +0.1% methemoglobin found in the control animals. The reports states that this is not a significant find, nevertheless, it does indicate an increase in the percentage of methemoglobin in the blood. Thus I can only assume that the material does cause an increase in the percent methemoglobin in the blood.

The animals on the oral studies showed a +3.0 and +3.5% value for methemoglobin in the blood. However, the pre-exposure value was not obtained.

In considering this test two points appear obvious (1) the material does produce an increase in methemoglobin, and, (2) this end is accomplished only at relatively high acute dosage levels. These points present another problem - what is the effect of the material on a subacute basis involving lower dosage rates?