Monsanto

= MINING MEDICAL STUDY CENTER =

TOXICITY FIRE RESISTANT FLUIDS USED IN POWER TRANSMISSION

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DETERMINATION OF SYNTHETIC FIRE RESISTANT FLUIDS TOXICITY

(French coal mines reference : form D)

1. Identity control using an I.R. spectrophotometer.

An I.R. spectrum is obtained on a sample of the liquid. The spectra are not analyzed, but serve as a reference for the identification of the product.

2. Oral toxicity.

The determination of oral toxicity (LD 50, i.e. the dose needed to cause of the death of 50 % of the animals used) must be carried out on white male mice and is to be expressed in grams per kilo body weight of the animal.

The administration of the hydraulic liquid to be examined (in that case diluted in water) is effected by oral probe on white male adult mice. The observation time is 14 days.

The oral probe is fixed like a cannula on a seringe for tuberculin. The head of the probe is rounded and larger so as not to injure the animal.

Each mouse is weighed and the quantity of the product introduced into the stomach is related to the body weight of the mouse.

The first approximate determination of the LD 50 is made on a limited number of animals. To four of the animals, a dose P is administrated. To four others, a dose 2P, to four others a dose 4P, etc ... If however the initial dose P proves to be too high, one carries out subsequent experiments with fractions of the initial dose P.

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STLCOPCB4091656

After this first approximate determination, an indication of LD 50 having to a certain been obtained, one recommences then the test on groups of mice the number of which results from the statistical certitude of the values determined (tolerance limit 20 %). Each group receives 0.4, 0.6, 0.8, 1.0, 1.2, etc ... of the LD 50 determined in increasing proportions.

The number of dead animals for each dose level is expressed as a percentage (the number of animals used for each dose level being 100 %). The value is put as an ordinate on a logarithmetical graph of probability as a function of the dose (g/kg) expressed in abscissa. Through the points obtained, one traces the straight line of approximation from which one determines graphically the dose (g/kg) needed to provoke the death of 50 % of the animals.

The results expressed in points are to be evaluated according to the following values :

LD 50	5 g/kg	: 0
LD 50	5 g/kg	: 5
LD 50	0.05 g/kg	: 10

3. Determination of product to act as an irritant.

a) Irritation value -

I. Test for eye irritation :

By using a pipette, a drop of the liquid under test is placed in the conjunctiva of the right eye of 2 male albino rabbits with a maximum weight of 1 kilo. The condition of the right eye and its surrounding area (outside of eyelid not included) in comparison to the left eye is observed ; this inspection is to be done daily during 5 days.

The animals must be isolated during the test period.

STLCOPCB4091657

Apart from any infection, the results are to be coded as follows :

- no reaction observed	: 0
- inflammatory reaction according to rate of appearance, importance and duration	: 1 to 5
- definitive damage with loss of vision	: 6 to 10
- loss of vision	: 10

II. Test for skin irritation :

Using the animals already used for test I., cutaneous irritation must be measured using the "patch test" which is as follows :

24 hours before the test, the skin on the side of the animal is shaved, without damaging the epidermis; the surface of this shaved area must be 8 x 8 cm. The compress itself consists of a piece of cotton wool measuring 4 x 4 cm, which is covered by an inert film and which will not absorb the liquid under test in such a way that the surface in direct contact with the skin in only 2 x 2 cm.

Having placed 2 ml of the solution onto the cotton wool, the compress is applied to the skin and fixed with adhesive tape.

After the compress has been in contact with the skin for 24 hours, it is taken off and the condition of the skin is examined straight away and daily from then on for a period of 14 days.

Aside from all infection, the results are given the following values :

- no reaction observed	: 0
- erythema, following extent and duration	: 1 to 3
- erythema and cutaneous oedema without induration	: 4

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STLCOPCB4091658

- complex damage of the skin, according to the extent and duration : 5 to 10
- death of animal : 10

b) Tests to determine toxicity of aerosols -

I. Aerosol - cold :

The liquid (non-diluted) is placed in an aerosol generator. The temperature of the latter is maintained at 50°C. The generator must discharge 10 to 15 ml/hour of the liquid in the form of mist containing approx. 90 % of the particules of a diameter inferior to 3 microns. The rate of discharge is to be 1 m3/hour.

Having been passed through a Vigreux tube, the mist is introduced into a cabinet made from Plexiglas of a capacity of 140 1. The dimensions recommended for this apparatus are : length 800 mm, width 350 mm, depth 500 mm. The attachments for the tubes (Ø 20 mm) are found at the front part of the apparatus in order to effect the discharge and evacuation of the mist. The interior of the apparatus can be reached by means of a door.

A barred cage in Plexiglas 300 x 200 mm, heigth 200 mm, is used to harbour to the animals. The base of the cage should have a stand so that the cage can be put exactly in the centre of the apparatus.

Three male Wistar rats of a weight of approx. 200 gms are placed in the cage in order to be exposed to the mist during three hours. Before starting the experiment as well as for 14 days, the weight of the animals is determined.

Three other rats of the same body weight and same age are kept as a control under the same conditions.

Aside from any infection, the results are given the following values :

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STLCOPCB4091659

- no reaction observed : 0
- Symptoms of irritation such as circulatory disturbance, seizure, disappearing after :
  - 1 hour : 1
  - 6 hours : 2
  - 5 days : 3
- Loss of weight or insufficient weight gain : 4
- death of one of the animals within 14 days : 5
- death of two of the animals within 14 days : 6
- death of three of the animals within 14 days : 7
- death of one of the animals during the experiment : 8
- death of one of the animals during the experiment and an other within 14 days : 9
- death of two of the animals during the experiment : 9
- death of one of the animals during the experiment and two others within 14 days : 10
- death of two of the animals during the experiment and an other within 14 days : 10
- death of three of the animals during the experiment : 10

II. Aerosol - hot :

The experiment described under b)I. is repeated with a new group of animals with the temperature of the liquid generator at 150°C. The mist produced will be cooled by passing through the tube in a way that the temperature of the cabinet be maintained at room temperature.

Aside from any infection, the results are given the following values :

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STLCOPCB4091660

- no reaction observed : 0
- symptoms of irritation such as circulatory disturbance, seizure, disappearing after :
  - 1 hour : 1
  - 6 hours : 2
  - 5 days : 3
- weight loss or insufficient weight gain : 4
- death of one of the animals within 14 days : 5
- death of two of the animals within 14 days : 6
- death of three of the animals within 14 days : 7
- death of one of the animals during the experiment : 8
- death of one of the animals during the experiment and an other within 14 days : 9
- death of one of the animals during the experiment and two others within 14 days : 10
- death of two of the animals during the experiment and an other within 14 days : 10
- death of three of the animals during the experiment : 10

c) Product of thermal decomposition -

I. Procedure.

The thermal decomposition of the fluids under test is effected in a closed metallic chamber. At one end of this chamber, the fluid is discharged under a pressure of approx. 100 atm on to a heat plate using a Diesel injector (conical nozzle with angle of pulverization 30° minimum). At the other end of the apparatus, a mixture of the vapor and air is evacuated. The air intake (approx. 10 1/mn) is effected through orifices found in the middle of the chamber.

STLCOPCB4091661

A sheet separating the injector and the air intake avoids an evacuation of the injected fluid before it reaches the heated plate.

The injector enables the injection of 0.35 to 3.5 ml/mn.

The temperature of the evaporation plate is controlled by means of a thermocouple and can be fixed at any temperature up to 700°C.

After the combustion chamber, a condensation unit is installed in the evacuation channel. After passing through this, the gas mixture is given in a test device to the rats.

The tests must be carried out at a temperature of 200°C. The tests are carried out at an intake rate of 1 ml/mn. If the condensate exceeds a third of the volume injected, the test is continued with reduction to 0.5 ml/mn.

II. Toxicological examination of thermal decomposition products.

The evacuated gases (approx. 10 l/mn) are introduced into a stream of fresh air in a ratio of 1 part gas and 2 parts air. This mixture is passed through the cage containing the rats (see b-II.) having been cooled until reaching ambient temperature. The procedure regarding values is also mentioned in b-II.

The levels of values apply for the test at 200°C as well as at 700°C.

d) Conditions of acceptance -

Each product tested is given a rating of 0 to 170 determined by adding the ratings obtained for each test described above, being given a coefficient which will be the following :

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STLCOPCB4091662

Oral toxicity	LD 50	1
Irritation	Eye	5
	Skin	5
Aerosol	Cold	2
	Hot	2
Thermal decomposition	200°	1
	700°	1

A product which obtains before weighting a rating of 10 in any of the tests, or the rating of 50 after weighting, is rejected.

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