ANNEX XVIII

DETERMINATION OF TOXICITY OF FIRE-RESISTANT HYDRAULIC FLUIDS NOT MISCIBLE WITH WATER, GROUP D

I. INFRA-RED SPECTROPHOTOMETRIC CHECK OF IDENTITY

An infra-red spectrum is taken of a sample of fluid comprising extinction values 0 to 1,5 or transparency values of 0 to 100 % over the whole wavelength range from 3 to 15 microns. The spectra are not analyzed but are used as identity references for the subsequent tests.

II. ORAL TOXICITY

The determination of oral toxicity (mean lethal dose LD 50) is performed on male white mice and is expressed in g/kg of body weight.

The hydraulic fluid under test (diluted with olive oil if necessary) is fed to male white mice by way of a tube inserted through the mouth. The observation period is 14 days.

The tube is fitted like a hypodermic needle to a tuberculin syringe. The front end of the tube is blunted and thickened, to avoid injury to the animals.

Each mouse is weighed, and the quantity of fluid introduced into its stomach referred to the weight of the animal.

A first approximation test of LD 50 is carried out on a fairly small number of animals. Four of them receive a dose P. Then the next four receive a dose 2 P; further animals are given 4 P, etc. If the "first arbitrary" dose P is too high, the subsequent experiments should be performed with fractions of P.

After this first approximation test, the range of LD 50 is pretty well marked out. Tests are then carried out on groups of mice, the numbers of which are determined by the statistical certainty (limit of certainty 20 %) of the values obtained each time. Each group now receives 0,4, 0,6, 0,8, 1,0, 1,2, 1,4 times, etc., of the rough LD 50 already determined.

For each individual dose, the number of dead animals is expressed as a percentage, and the results are plotted on a logarithmic curve (probability paper). Then, the dose which causes the death of 50 % of the animals is determined graphically from the curve.

The results are classified as under:

LD 50	$>$	at 5 g/kg	: 0
LD 50	$\leq$	at 5 g/kg and $>$ 50 mg/kg	: 5
LD 50	$\leq$	50 mg/kg	: 10

STLCOPCB4040914

III. DETERMINATION OF TOXIC EFFECT

A. Irritant effect

1. Tests to determine the irritant effect on the eyes

Using a standard eye-dropper, a drop of test fluid is placed in the conjunctival sac of the right eye of two male albino rabbits of at least 1 kg weight. The state of the right eye and its surroundings (except the eye-lid) is examined and compared with the left eye, once a day for 5 days.

During the whole test period the animals must be isolated from the others.

Disregarding any infections, the results are classified as under:

No reaction observed	: 0
Inflammation according to speed of development, extent and duration	: 1-5
Permanent lesions, except loss of vision	: 6-9
Loss of vision in the treated eye	10

2. Test to determine the irritant effect on the skin

Using the same animals as have been employed for the eye tests, the acute cutaneous irritation is investigated, by means of the "patch test", described below.

For the purposes of the tests, 24 hours before the test, the skin on the flank is shaved over an area of 8 x 8 cm, care being taken not to injure the skin surface in any way. The patch itself consists of a piece of cotton wool 4 x 4 cm, covered by a sheet of any impermeable material which will not react with the test fluid, in such a way that direct contact with the skin is possible only over a surface of 2 x 2 cm in the middle.

After the wool has been soaked with 2 ml of the test fluid, the patch is laid on the skin and bandaged into position.

After a contact time of 24 hours, the patch is removed and the condition of the skin is examined immediately, and also daily for the next 14 days.

Disregarding any infections, the results are classified as under:

No reaction observed	: 0
Erythema, according to extent and duration	: 1-3
Erythema and cutaneous oedema, not indurated	: 4
Major skin lesions (blisters, ulcers, necroses), according to extent and duration	: 5-10
Death of the animals during the test	: 10

B. Test to determine the toxicity of aerosols

1. Cold aerosol

The fluid is kept at 50°C in an aerosol generator, which, fed with an air stream at 15 litres/min, emits 10 ml of the fluid per hour in aerosol form. At least 90 % of the aerosol particles must be below 3 microns in size. After passing through a Vigreux tube, this aerosol is fed into a 100-litre cylinder with a rotating diameter of 50 cm, which rotates at 1 rpm. Three male Wistar rats of the same age ± 200 g in weight are weighed and exposed to the aerosol for 3 hours. If they survive they are kept under daily observation for a further 14 days, in separate cages away from draughts; they are re-weighed at the end of the 14 days.

STLCOPCB4040915

Three other rats of similar weight and age are kept in the same condition as controls.

Disregarding any infections, the results are classified as under:

No reaction observed	:	0
Symptoms of irritation (circulatory disorders, nervous affections) ceasing after 1 hour	:	1
6 hours	:	2
5 days	:	3
Loss of weight, insufficient gain in weight	:	4
Death of one of the animals within 14 days	:	5
Death of two animals within 14 days	:	6
Death of three animals within 14 days	:	7
Death of one of the animals during the test	:	8
Death of one animal during the test and another within 14 days	:	9
Death of two animals during the test	:	9
Death of one animal during the test and both the others within 14 days	:	10
Death of two animals during the test and the third within 14 days	:	10
Death of all three animals during the test	:	10

2. Hot aerosol

The test described above is then also carried out with fluid kept at 150°C.

Disregarding any infections, the results are classified as under:

No reaction observed	:	0
Symptoms of irritation (circulatory disorders, nervous affections) ceasing after 1 hour	:	1
6 hours	:	2
5 days	:	3
Loss of weight, insufficient gain in weight	:	4
Death of one of the animals within 14 days	:	5
Death of two animals within 14 days	:	6
Death of three animals within 14 days	:	7
Death of one of the animals during the test	:	8
Death of one animal during the test and another within 14 days	:	9
Death of two animals during the test	:	9
Death of one animal during the test and both the others within 14 days	:	10
Death of two animals during the test and the third within 14 days	:	10
Death of all three animals during the test	:	10

C. Thermal-decomposition products

1. Procedure

The formation of thermal-decomposition products by the test fluids is carried out in a metal chamber enclosed in all sides, at one end of which the fluid is sprayed at a pressure of approximately 100 atm by means of a Diesel injector (conical spray nozzle, with a minimum spraying angle of 30 %) on to a hot plate, and at the other end of which the extraction occurs, the air-inlet orifices (approximately 10 l/min) being roughly in the middle of the chamber. A partition

STLCOPCB4040916

between the injector and the air-inlet orifice is intended to prevent the extraction of the injected fluid before it has reached the heating plate.

The injector sprays at a rate of 0.35 to 3.5 ml of fluid/minute.

The temperature of the vaporization plate is checked by means of a thermocouple let in to it, and can be adjusted to values up to 700°C.

A condenser unit is connected to the extractor section of the combustion chamber and, after passing through this, the extracted mixture goes into the device where the rats are subjected to the test.

The test is to be carried out at temperatures of 200°C and 700°C. The output of the injector should be as near as possible to 1 ml per minute. If with this output, the condensate amounts to 1/3 of the injected volume, a further test is carried out with the output reduced to 0,5 ml per minute.

2. Toxicological examination of thermal-decomposition products

A part of the extracted gas (approximately 10 l/minute) is fed into a current of fresh air in the ratio of 1 : 2. This mixture is passed through the rat cage, after it has been cooled to room temperature. The remainder of the procedure follows from the information given in chapter B. 2, except for the duration of the exposure period.

The evaluation scale must apply both to the decomposition products at 200°C and at 700°C.

D. Evaluation of the results and conditions of approval

Each product tested is given a rating between 0 and 170. This figure is obtained from the sum of the rating from each of the individual tests, after multiplication of these rating figures by the coefficients given in the following table.

Oral toxicity	LD 50	1
Irritant effect	Eyes	5
	Skin	5
Aerosols	Cold	2
	Hot	2
Thermal-decomposition products	200°	1
	700°	1

Any product which achieves the rating of 10 before multiplication must be rejected, as should a product reaching the total rating of 50.

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