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Institut für Umwelthygiene und Toxikologie
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Träger: Verein zur Bekämpfung der Volkskrankheiten im Ruhrkohlengebiet e.V.

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Ansprechpartner: Herr Bien

Gelsenkirchen, den 07.01.2015

Bonpet fire-extinguishing liquid
Here: Waste water examination and evaluation

Your order 2014-0052 from 01.10.2014 and our offer A-248765-14-Bi from 30.09.2014

In the framework of the before mentioned order the fire extinguishing agent "**Bonpet fire-extinguishing liquid**" designed by your company has been examined regarding its biodegradability and its impact on higher and lower aquatic organisms.The mentioned product is an aqueous solution based on organic substances which is meant to be used for the extinguishing of fires.

Analytical work was carried out according to the standardized OECD Test Guidelines, as well as the regulations laid down in the German Standard Procedures for examinations of water, waste water and sludge. The examination results will be described below shortly outlining the applied examination procedures and the chosen test conditions. The corresponding examinations have been made in all cases with the concentrate of the fire extinguishing agent.

DAkkS Deutsche Akkreditierungsstelle D-PL-13042-02-00

1. Bacterial toxicity test

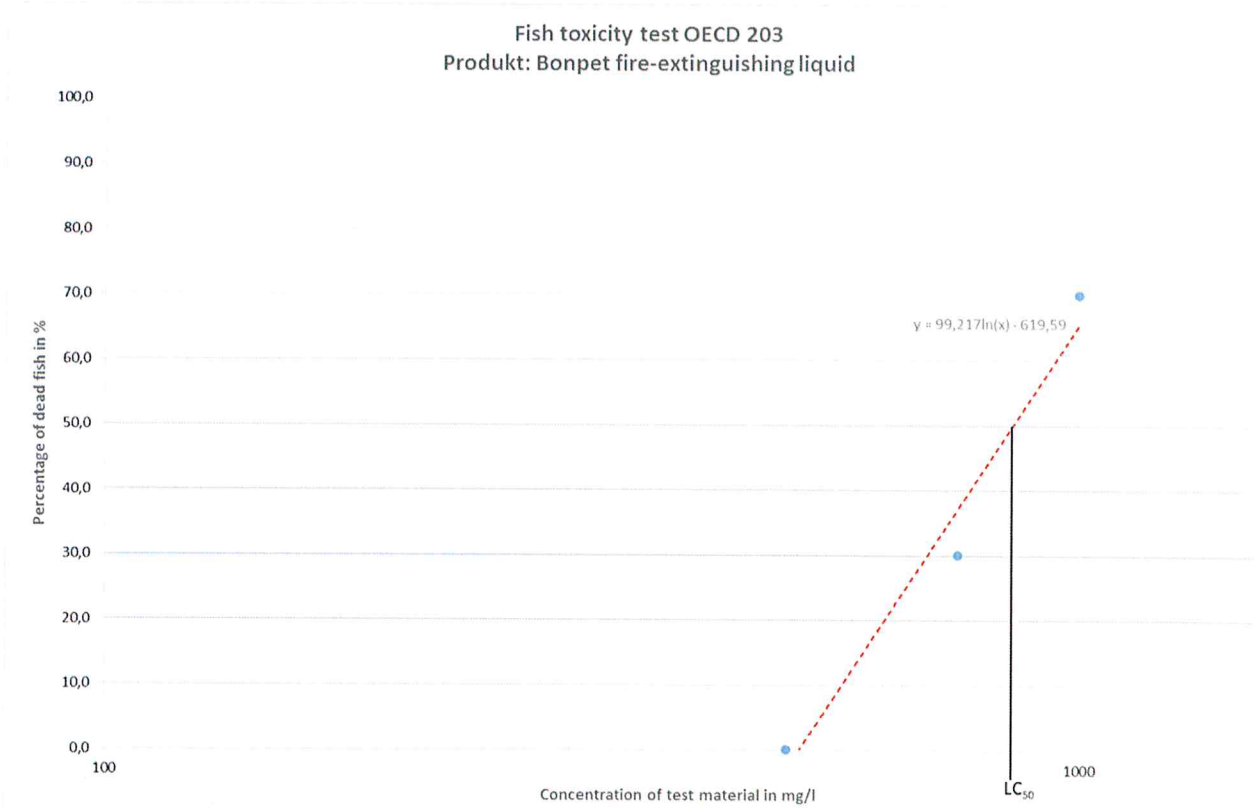
A TTC-test has been carried out in order to be able to make practical statements regarding a possible toxicity of the product **"Bonpet fire-extinguishing liquid"** to be examined with respect to lower aquatic organisms. The method is based on the dehydrogenases of the living cells (activated sludge) reducing 2,3,5- triphenyl tetrazolium chloride (TTC) to red formazan in quantitative dependence on the corresponding active cells. Thus, by means of a quantitative determination of the formed formazan a direct statement can be made about the toxicity of a substance introduced into a defined activated sludge/ TTC suspension on comparing the results with those of a parallel sample which does not contain the substance.

As is made clear by the semi-logarithmic graphical illustration (Annex 1), the formation of formazan expressed in absolute values initially lies above the blank value. The further course of the curve proves that a decrease of the formazan formation takes place with the percentage of **"Bonpet fire-extinguishing liquid"** being increased which reaches the blank value at a concentration of approx. 0,1 ml/10 ml (= 10 ml/l) of the total solution.

2. Fish toxicity test

The examination of the product **"Bonpet fire-extinguishing liquid"** regarding its fish-toxic properties has been carried out with the help of the procedure (fish test) described OECD Directive 203 ("Fish Acute Toxicity Test"). According to this, 1 to 3 cm long Zebra-fish (*Danio rerio*) were introduced into test solutions prepared with the before mentioned product and diluting water and the animals' behaviour monitored for 96 hours. During the duration of the test, the temperature of the aired test water was maintained at 23 ± 2 degrees Celsius. The results have been summarized in the following table:

Concentration of test material in	mg/l	100	250	500	750	1000
Percentage of dead fish in	%	0	0	0	30	70



From the before mentioned data the following LC-50 value can be determined:

LC-0	:	500mg **"Bonpet fire-extinguishing liquid"**/l
LC-50*	:	850mg **"Bonpet fire-extinguishing liquid"**/l
LC-100	:	>1000mg **"Bonpet fire-extinguishing liquid"**/l

3. Daphnia toxicity test

Daphnia toxicity has been determined according to the procedure described in OECD Directive 202 ("Acute Immobilisation Test") with the test organism "Daphnia magna STRAUS". The indication of the dilution level of the test substance, in which a certain percentage of daphnia

* LC 50 = the concentration of the test material to be examined, determined by graphical methods or calculation, with 50 % of the introduced fish dying during the 48-hour-test.

remains buoyant after 48 hours of testing (temperature: 20 degrees C. ± 1 degree C.; no illumination), serves as a measure for the effect of water ingredients.

The effect of the mass concentration of the examined fire extinguishing agent "**Bonpet fire-extinguishing liquid**" on the buoyancy of the small aquatic crustaceans Daphnia magna has been compiled in the following table:

Concentration of test substance in mg/l	5	7,5	10	25	50
Percentage of daphnia incapable of swimming in %	0	0	0	0	0

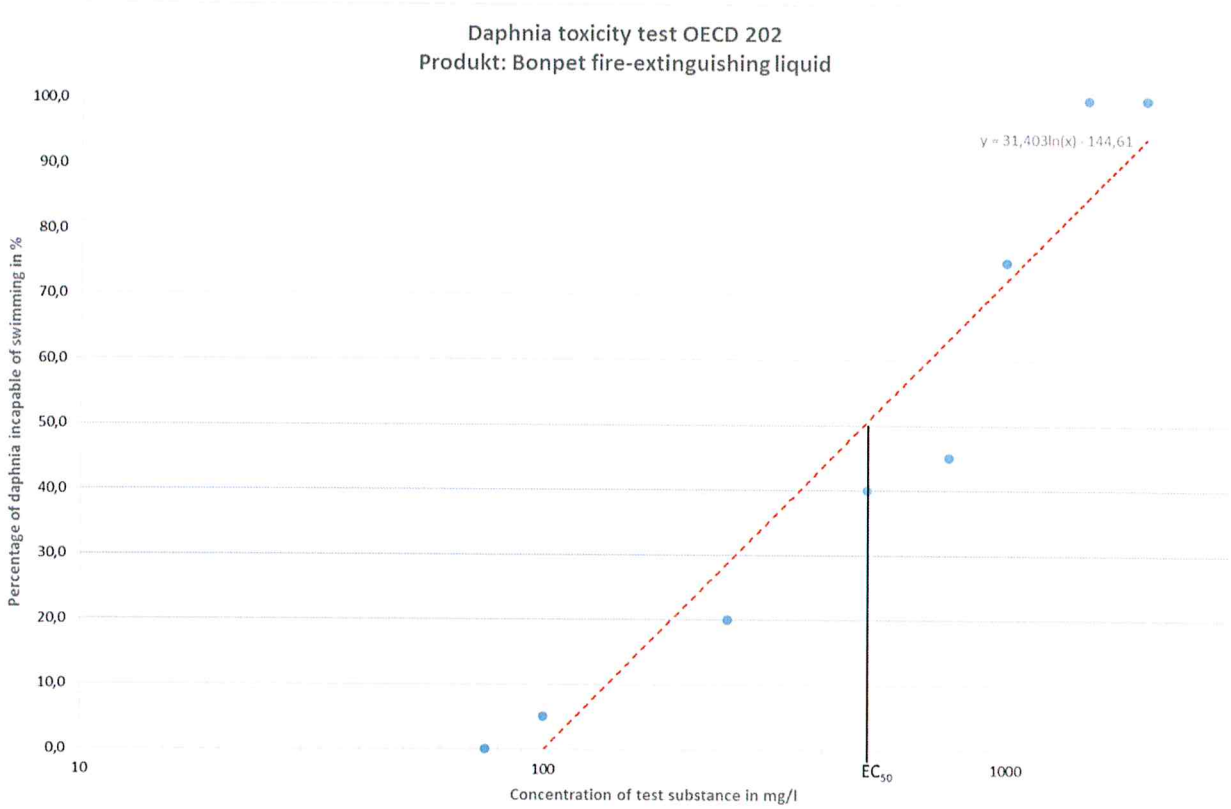
Concentration of test substance in mg/l	75	100	250	500	750
Percentage of daphnia incapable of swimming in %	0	5	20	40	45

Concentration of test substance in mg/l	1000	1500	2000
Percentage of daphnia incapable of swimming in %	75	100	100

Based on the before mentioned test results the following effect concentrations (EC values) for the fire extinguishing agent "**Bonpet fire-extinguishing liquid**" can be determined:

EC - 0 (48 hs) = 75 mg /l
EC - 50** (48 hs) = 500 mg /l
EC - 100 (48 hs) = 1500 mg /l

** EC 50 = the concentration of the test material to be examined, determined by graphical methods or calculation, with 50 % of the introduced daphnia are incapable of swimming during the 48-hour-test.



4. Algae toxicity test

The determination of the inhibitation of cell reproduction with green algae was carried out according to OECD Directive 201 ("Growth Inhibition Test"). Under this standard the green algae *Scenedesmus subspicatus* are cultivated in a nutrient medium for 72 hours and under defined conditions (23 degrees C. ± 2 degrees C.; permanent illumination at 8000 lux) and at different sample concentrations; at certain times (24 hs, 48 hs and 72 hs) the toxicity of the test substance is to be checked by the determination of the number of cell count.

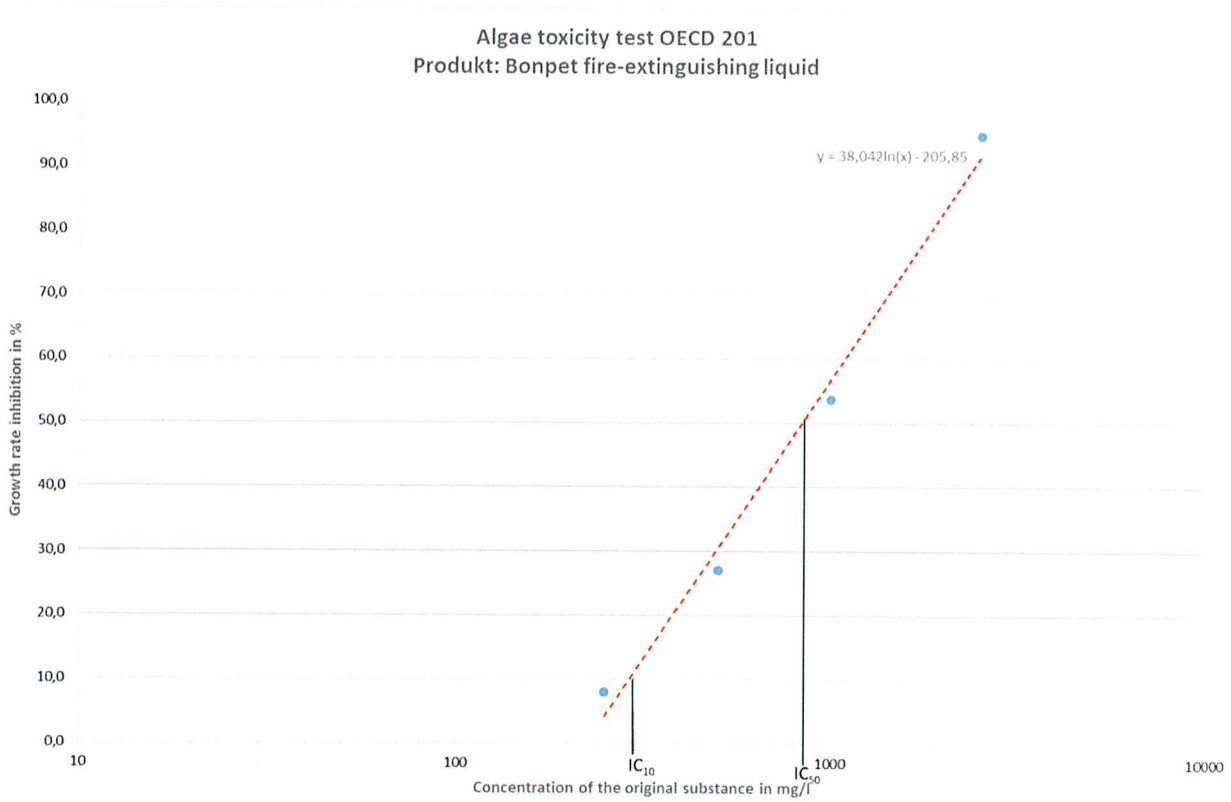
The results of the cell reproduction inhibition test are listed below:

Concentration of the original substance	in mg/l	75	100	250	500	1000
Growth rate inhibition	in %	0	0	8,0	27,0	53,6

Concentration of the original substance	in mg/l	2500	5000
Growth rate inhibition	in %	94,9	97,2

This shows the following effective concentrations (IC values) for the product **"Bonpet fire-extinguishing liquid"** in a graphical assessment (see below) at an inhibition of 10 % and 50 %:

IC_{10}	(0 to 72 h)	=	300	mg/l
IC_{50}	(0 to 72 h)	=	850	mg/l



5. Biodegradation pattern

The biodegradability of the fire-extinguishing agent "**Bonpet fire-extinguishing liquid**" was specified using manometry to determine the biochemical oxygen demand based on OECD-Guideline 301 f (Manometric Respirometry Test). This method does not only state a measurement for the microbiological-oxidative degradation of organic ingredients, but also allows for assessments regarding the kinetics of the degradation. The reference value for the degradation rate is the chemical oxygen demand that is determined experimentally using the dichromate-method. The COD can be seen as a measurement for the complete mineralisation of the organic substance of the test solution.

Because Ammoniumcarbonate and Armoniumhydrogencarbonate are a part of the formulation of the Bonpet fire extinguishing liquid it was necessary to suppress a potential nitrification during the degradation-test, so the nitrification inhibitor N-allylthiourea (ATH) was added to the sample.

Assessing the chemical oxygen demand of the undiluted extinguishing agent "**Bonpet fire-extinguishing liquid**" of 28300 mg O_2/l as the necessary amount of oxygen for the 100 % degradation, the biochemical degradation, expressed as BOD, amounts to 17800 mg O_2/l = 62,9 % after a period of five days.

As can be seen in the chart (Annex 2) regarding the manometrically determined biochemical degradation kinetics, the microbiological degradation of biochemical oxidisable ingredients, under the given test conditions, was completed after a period of approx. 27 to 28 days; it amounts to approx. 83,4 %.

6. Oral mammal toxicity

The limit test in compliance with OECD guideline 420 with a limit concentration of 2000 mg "**Bonpet fire-extinguishing liquid**" per kilogram body weight tested on wistar rats showed no toxic influences on the test animals. The acute oral mammal toxicity can be defined as > 2000 mg per kilogram bodyweight.

Summary

Based on the results of the TTC-test it can be maintained, in view of the disposal of the product through sewage systems to the waste water treatment plants, that no negative effects are to be expected for the biologically operating section of the treatment plant as long as it is made sure that the fire extinguishing agent solution "**Bonpet fire-extinguishing liquid**" is diluted at a ratio of **at least** 1:100 with other waters such as, for instance, domestic waste water.

Furthermore, it must be guaranteed that the sewage treatment plant does not get overloaded with quantities of water containing fire extinguishing agents.

Water containing the product "**Bonpet fire-extinguishing liquid**" should not be disposed of via draining canals as it might lead to adverse changes of the water quality due to its established aquatic toxicity.

Based on the results of the toxicity testing and the determination of biodegradability and in consideration of the regulations on hazardous substances (Commission Regulation (EU) No 286/2011) the **"Bonpet fire-extinguishing liquid"** has not be declared as hazardous to the aquatic environment.

The Director of the Institute
p.p.

Dipl.-Ing. Michael Sauerwald
Leiter der Abteilung
Abwasser-, Boden- und Lufthygiene

Dipl.-Umweltwiss. Sebastian Bien
Sachgebietsleiter
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Attachments

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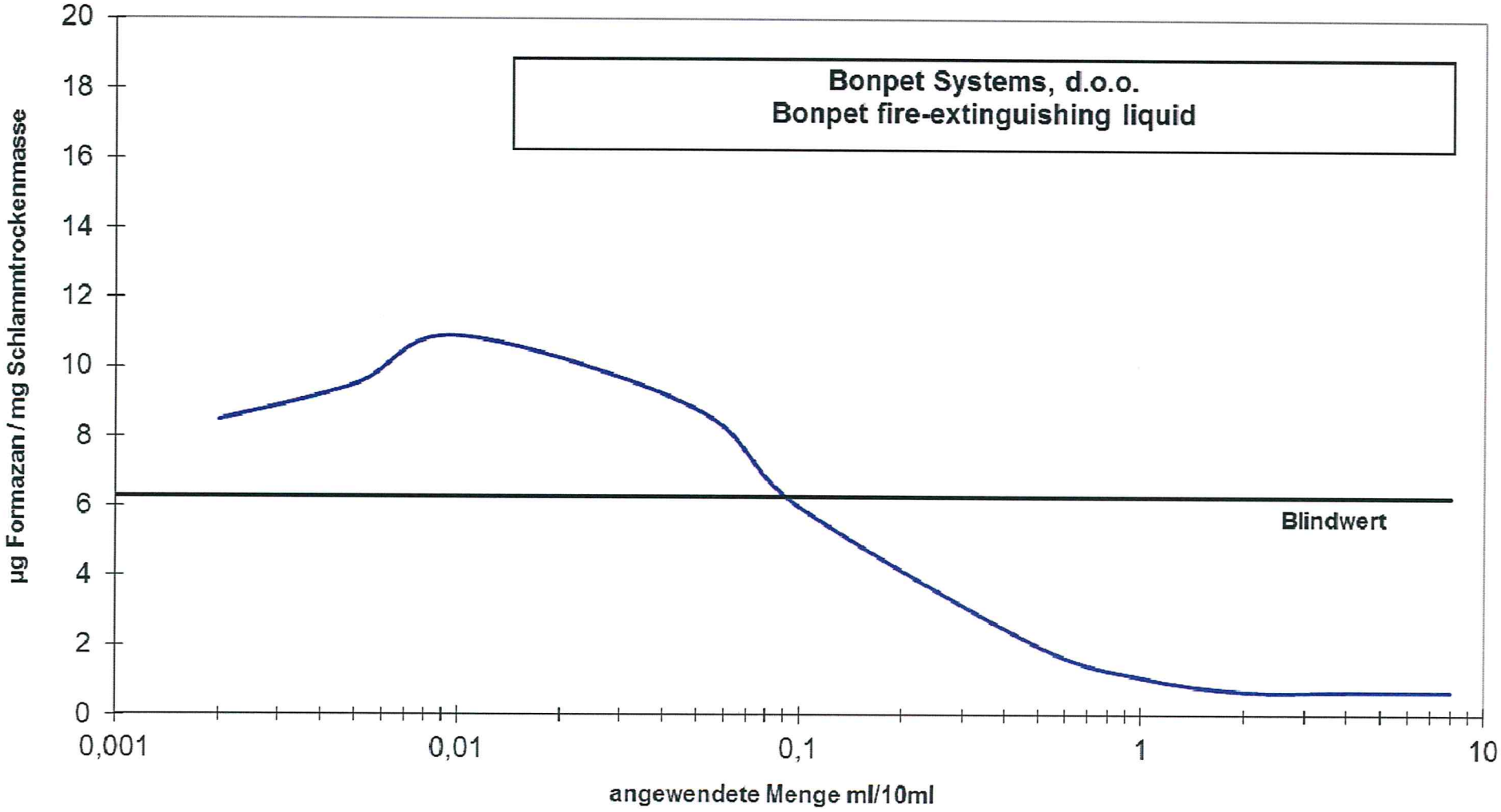
Toxizitätsbestimmung mit 2,3,5-Triphenyltetrazoliumchlorid (TTC)



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Biochemical degradation kinetics
Produkt: Bonpet fire-extinguishing liquid