

Study to support the derivation of Environmental Quality Standard for BROMATE

Ecotoxicity of sodium bromate on reproduction of *Ceriodaphnia dubia* and on other freshwater organisms

Report of analyses

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1. Introduction and methodological overview

OEKOTOXZENTRUM, represented by R. KASE, has asked to the lab SOLUVAL SANTIAGO to carry out a bioassay with the microcrustacean *Ceriodaphnia dubia* on bromate, which can be released from wastewater treatment plants as a by-product due to ozonation. In order to propose an *Environmental Quality Standard* (EQS), the purpose is to determine the toxic effects of sodium bromate (CAS n° 7789-38-0) on reproduction of *Ceriodaphnia* (KASE 2015).

The determination of bromate toxicity with long-term tests, as expressed by endpoints *NOEC* (*No Observed Effect Concentration*) or preferably *EC₁₀* (estimated *Effective Concentration* giving rise to 10% inhibition of reproduction with respect to control), will allow to apply an *assessment factor* (= *safety factor*) of 50 for EQS, according to European Guidance Document for EQS derivation (EC 2011).

Within this evaluation and since there is a clear need to provide a wide-ranging set of data for bromate ecotoxicity, with an overview encompassing acute toxicity as well as chronic toxicity on various trophic levels, it has been decided to complete it with additional standard bioassays with freshwater organisms *Daphnia magna*, green algae (*Pseudokirchneriella subcapitata*), rotifera (*Brachionus calyciflorus*) and macrophytes (*Lemna minor*).

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2. Methodological overview

All bioassays are performed according to protocols by ISO (International Organization for Standardization) or OECD (Organisation for Economic Co-operation and Development; for *Lemna* test). When available, useful procedures from other national standardization agencies and giving additional details have also been used. Our laboratory is not certified according to ISO/IEC 17025; nevertheless good laboratory practices and quality assurance take wholly part of performed protocols (see annex).

2.1 Chronic toxicity to *Ceriodaphnia dubia* - Reproduction test procedure

This bioassay determines the effects of chronic toxicity on the freshwater cladoceran *Ceriodaphnia dubia* in 7 days (population growth inhibition test, according to ISO 20665; AFNOR T90-376). Data after 8 days of exposure can be given as well, this exposure time being also approved by the ISO standard and the protocol from Environment Canada (*Env. Protection Series EPS 1/RM/21, 2007*).

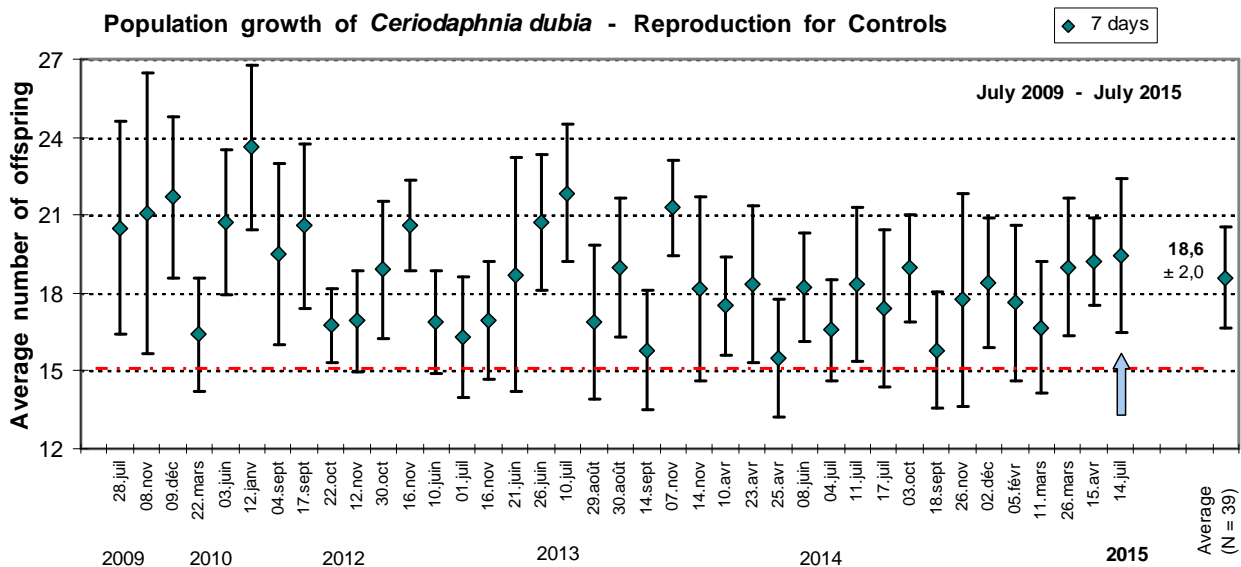
The test is carried out with a slight adaptation of the standards about the medium (as accepted by ISO protocol 20665), in order to get healthy reproduction : the control - dilution medium corresponds to a moderately hard water prepared by mixing $\frac{1}{4}$ of Evian mineral water, $\frac{1}{4}$ of Elendt M4 medium (used also for *Daphnia magna* cultures) and $\frac{1}{2}$ of deionised water, supplemented with selenium and B₁₂ vitamin. Food is composed by a mixture with yeast, digested fish flake suspension (TetraMin[®]) and green algae (*Pseudokirchneriella subcapitata* and *Chlorella* sp.).

Test animals are obtained from our laboratory cultures (origin : CEMAGREF, Lyon, 2007); the young organisms (less than 24 hours old and all those within 8 hours of the same age at the start of the test) are exposed for up to 8 days in static-renewal system to different solutions (one control and minimum 5 concentrations, with 12 replicates per concentration). All tests are carried out at 25 ± 1 °C in temperature controlled chamber; illumination ranging from 300 to 500 lux, with 16h:8h light:dark photoperiod.

At the time of renewal, survival of mothers and offsprings in each vessel are counted every day. Results for the blank controls fulfill with criteria of validity, thus allowing to validate the whole series (on the 7th day, mortality of mothers $\leq 20\%$; proportion of males $\leq 20\%$; at least 60% of mothers alive have produced a minimum of 3 broods; the average number of offspring born per live mother ≥ 15 ; see annex).

Sodium bromate was extra pure quality grade (99+%), from ACROS Organics (Ref. 20887-1000; lot A0358417; purchased at Chemie Brunschwig AG, Basel). Before preparing each daily renewal of test concentrations, the stock solution (1000 mg NaBrO₃ / L in dilution medium) has been renewed every 2 days. All concentrations prepared for tests are nominal.

The reliability as well as relevance of *Ceriodaphnia* test can be evaluated by several criteria as proposed in CRED approach (Criteria for Reporting and Evaluating ecotoxicity Data; R1 = reliable without restrictions, R2 = reliable with restrictions, R3 = not reliable, R4 = not assignable; MOERMOND *et al.* 2015). As example of one validity criterion, the average reproduction in controls has to be greater than or equal to 15 live offsprings born per adult female (ISO 20665; see fig. below).



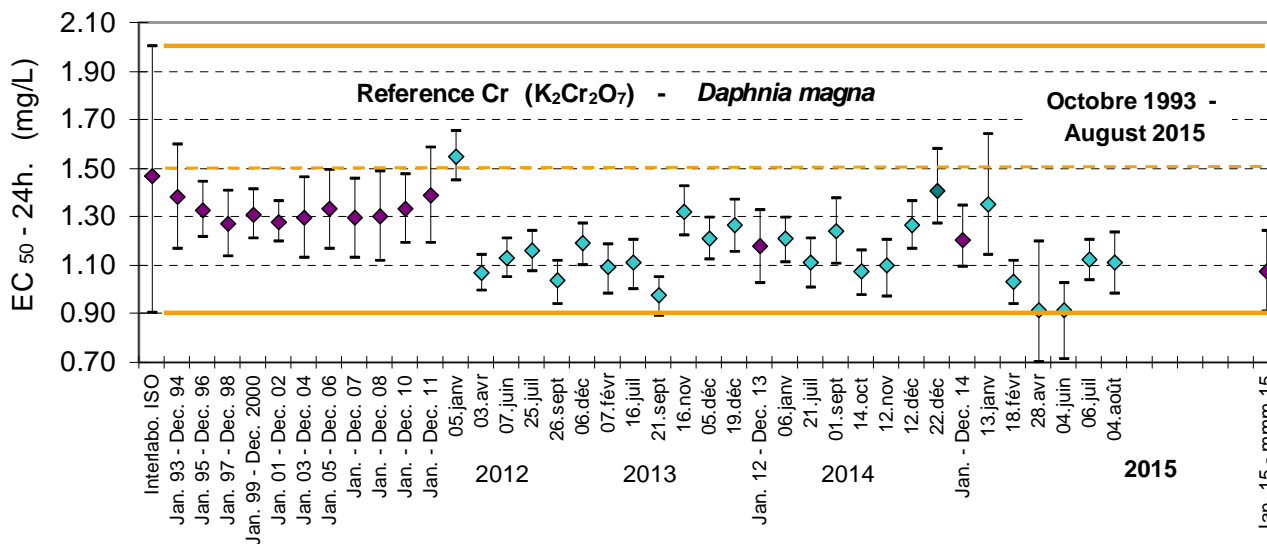
For samples showing toxic effects, bioassay results are integrated and expressed by the estimated concentration of the sample that induces an inhibition of 50% or X% of the organism growth with respect to the control (EC_{50} or EC_x) at the end of the test. Mortality is expressed by the term LC_{50} (for lethal concentration). The lower are the EC_{50} , EC_x or LC_{50} , more toxic is the solution. Values of EC_x are reported with 95% confidence limits (logistic regression with Hill model, calculated with *REGTOX v. 7.0.5* software, VINDIMIAN, 2001).

Particular concentrations for chronic toxicity tests are also determined (*NOEC = no observed effect concentration*, *LOEC = lowest observed effect concentration*; calculated with *TOXSTAT v. 3.4* software; WEST & GULLEY, 1994; including χ^2 test for normality, Bartlett's test for homogeneity of variance, and ANOVA Bonferroni t-test for *NOEC-LOEC* and minimum significant difference).

Acute toxicity of tested solution, expressed in mortality, can also be assessed at 24 and 48 hours after starting the test (LC_{50-24h} ; LC_{50-48h} ; calculated with Probit analysis *TOXDAT* software, USEPA, 1985).

2.2 Acute toxicity test with *Daphnia magna*

This test determines the effects of acute toxicity on the freshwater cladoceran *Daphnia dubia* STRAUS in 24 and 48 hours (inhibition of the mobility, according to standard ISO 6341). Medium : hard synthetic water. N = 20 neonates. As example of validity criterion, the sensitivity of *Daphnia magna* to the reference toxicant potassium dichromate is illustrated below.



2.3 Algal growth inhibition test with *Pseudokirchneriella subcapitata*

This test determines the chronic toxicity in 72 hours on the freshwater green algae *Pseudokirchneriella subcapitata* (formerly named *Selenastrum capricornutum* and *Raphidocelis subcapitata*; growth inhibition test, according to protocol AFNOR T90-375). This norm was originally aimed to test rapidly wastewater samples (not substances); it is performed in PS microplates, with 24 wells. Medium = AAP (USEPA). Continuous light during incubation. Cell density (number of cells per mL) is determined with Coulter® counter. Replicates per concentration : 3 (control = 6). Calculations of EC_x are made on cell density (not on growth rate).

2.4 Chronic toxicity to *Brachionus calyciflorus* in 48 hours

This bioassay determines the effects of chronic toxicity on the freshwater rotifer *Brachionus calyciflorus* in 48 hours (population growth inhibition, according to ISO 20666). Medium : moderately hard synthetic water. Replicates per concentration : from 4 to 6 (control = 11). Juvenile females hatched from cysts are obtained from MicroBioTests Inc (Belgium). In PC microplate (6 + 48 wells).

2.5 Chronic toxicity to *Lemna minor*

This bioassay determines the effects of chronic toxicity on the macrophyte duckweed *Lemna minor* in 7 days (population growth inhibition, according to OECD 221, plus details in ISO 20079). Culture and test are not conducted axenically. Medium : modified SIS (from Swedish Standards Institute). Replicates per concentration : 3 (control = 6). In glass beakers (80 mL). Determination of frond numbers only.

3. Results and discussion

3.1 Bioassays with *Ceriodaphnia dubia*

Chronic toxicity on *Ceriodaphnia dubia* : The following table and figure sum up main results for mortality and reproduction of the cladocerans in the final test after 7 days and also after 8 days of exposure. Population growth is expressed in % relative to controls. All toxicity endpoints (EC_x , $NOEC$) are given in mg NaBrO₃ / L. Detailed data are in annex.

Bioassay (14-07-2015)		Population growth of <i>Ceriodaphnia dubia</i>			
Results at 7 days	Concentration	Mother mortality at 7 days	Average number of offspring	Growth at 7 days (%)	Inhibition (%)
<i>Controls</i> (dilution medium)		0 / 28 = 0 %	19,4 ± 2,9	100 %	0%
<i>Sodium Bromate</i> NaBrO ₃ (CAS 7789-38-0)	30 mg / L	12 / 12 = 100 %	0 ± 0	0 %	100 %
	22 mg / L	12 / 12 = 100 %	0,1 ± 0,3	0,4 %	99,6 %
	16 mg / L	12 / 12 = 100 %	3,3 ± 2,2	17,2 %	82,8 %
	12 mg / L	9 / 12 = 75,0 %	5,8 ± 1,1	30,0 %	70,0 %
	9,0 mg / L	7 / 12 = 58,3 %	10,8 ± 2,2	55,8 %	44,2 %
	6,0 mg / L	1 / 12 = 8,3 %	16,1 ± 1,7	82,8 %	17,2 %
	3,0 mg / L	0 / 12 = 0 %	18,0 ± 3,1	92,6 %	7,4 %
	1,0 mg / L	0 / 12 = 0 %	16,4 ± 1,7	84,5 %	15,5 %
<p>☞ Mortality : $LC_{50} - 7 d = 8,92$ mg NaBrO₃ / L [95% conf. limits : 7,5 – 10,3 mg / L]</p> <p>☞ Reproduction : $EC_{50} - 7 d = 9,90$ mg NaBrO₃ / L [95% conf. limits : 9,3 – 10,5 mg / L] ; $EC_{20} - 7 d = 6,82$ mg NaBrO₃ / L [95% conf. limits : 6,1 – 7,6 mg / L] ; $EC_{10} - 7 d = 5,49$ mg NaBrO₃ / L [95% conf. limits : 4,7 – 6,3 mg / L]</p> <p>$NOEC$ (No observed effect concentration) = 3,0 mg NaBrO₃ / L (see text) ; $LOEC$ (Lowest observed effect concentration) = 6,0 mg NaBrO₃ / L (see text) ; MSD (Minimum statistical difference; % of inhibition) = 10,3 % (see text).</p>					
Bioassay (14-07-2015)		Population growth of <i>Ceriodaphnia dubia</i>			
Results at 8 days	Concentration	Mother mortality at 8 days	Average number of offspring	Growth at 8 days (%)	Inhibition (%)
<i>Controls</i> (dilution medium)		0 / 28 = 0 %	24,2 ± 4,4	100 %	0%
<i>Sodium Bromate</i> NaBrO ₃ (CAS 7789-38-0)	30 mg / L	12 / 12 = 100 %	0 ± 0	0 %	100 %
	22 mg / L	12 / 12 = 100 %	0,1 ± 0,3	0,3 %	99,7 %
	16 mg / L	12 / 12 = 100 %	3,3 ± 2,2	13,8 %	86,3 %
	12 mg / L	10 / 12 = 83,3 %	6,1 ± 1,4	25,1 %	75,0 %
	9,0 mg / L	9 / 12 = 75,0 %	11,6 ± 3,0	47,8 %	52,3 %
	6,0 mg / L	5 / 12 = 41,7 %	18,5 ± 3,3	76,4 %	23,8 %
	3,0 mg / L	0 / 12 = 0 %	22,1 ± 3,1	91,2 %	9,1 %
	1,0 mg / L	0 / 12 = 0 %	22,7 ± 3,9	93,6 %	6,7 %
<p>☞ Mortality : $LC_{50} - 7 d = 7,00$ mg NaBrO₃ / L [95% conf. limits : 5,5 – 8,3 mg / L]</p> <p>☞ Reproduction : $EC_{50} - 8 d = 8,84$ mg NaBrO₃ / L [95% conf. limits : 8,3 – 9,5 mg / L] ; $EC_{20} - 8 d = 5,82$ mg NaBrO₃ / L [95% conf. limits : 5,1 – 6,6 mg / L] ; $EC_{10} - 8 d = 4,56$ mg NaBrO₃ / L [95% conf. limits : 3,8 – 5,5 mg / L]</p> <p>$NOEC$ (No observed effect concentration) = 3,0 mg NaBrO₃ / L ; $LOEC$ (Lowest observed effect concentration) = 6,0 mg NaBrO₃ / L ; MSD (Minimum statistical difference; % of inhibition) = 11,9 % .</p>					

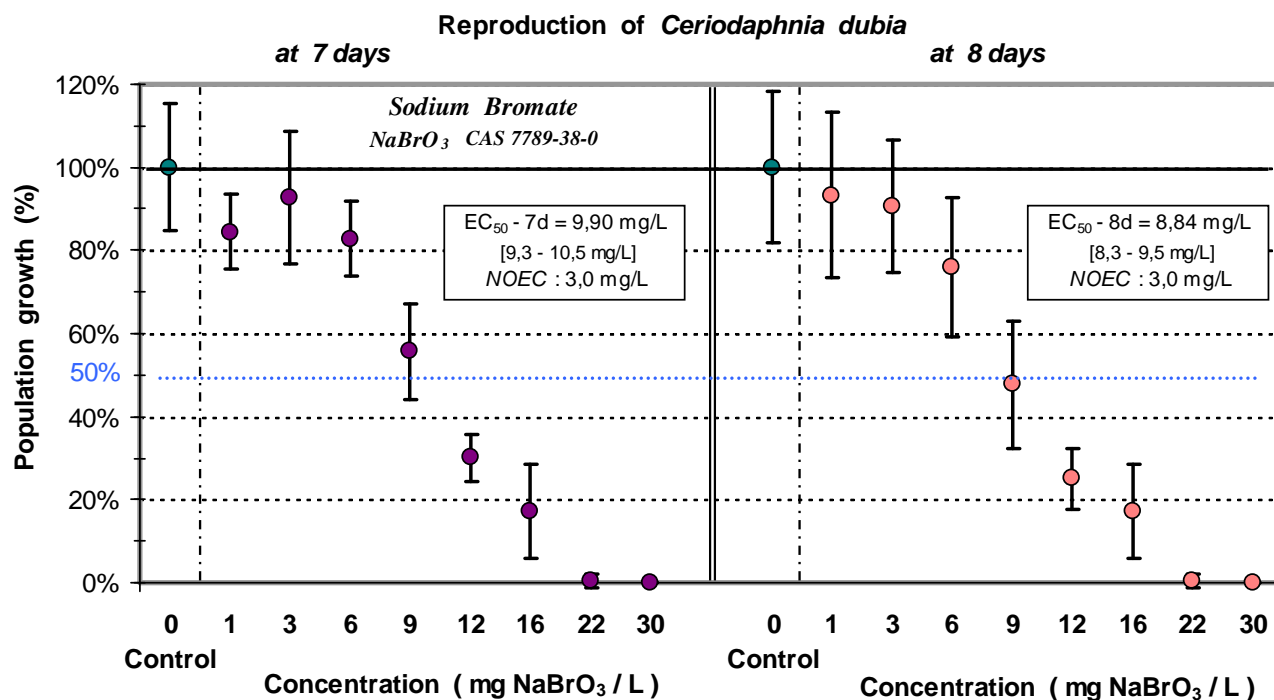


Figure 3.1. Population growth of *Ceriodaphnia dubia* at 7 days and 8 days with NaBrO₃ concentrations (expressed in % relative to controls).

Physico-chemical characteristics of solutions during the test are given in the following table (average of 5 to 7 measurements at the end of each daily exposure). The values of pH, dissolved oxygen and electrical conductivity show great constancy over the concentrations, and they indicate that the parameters in these solutions are well suited for survival and reproduction of the cladocerans.

At starting	Control	Stock solution [1000 mg NaBrO ₃ / L]							
pH	7,9	8,0							
Oxygen mg O ₂ / L	7,1	6,2							
Conductivity μS / cm	320	925							
During test	Control	1,0	3,0	6,0	9,0	12,0	16,0	22,0	30,0
<i>Tested concentration</i>		mg / L	mg / L	mg / L	mg / L	mg / L	mg / L	mg / L	mg / L
pH	8,1 ± 0,1	8,2 ± 0,1	8,2 ± 0,1	8,2 ± 0,1	8,2 ± 0,1	8,2 ± 0,1	8,2 ± 0,1	8,2 ± 0,1	8,2 ± 0,1
Oxygen mg O ₂ / L	6,8 ± 0,2	7,0 ± 0,2	7,0 ± 0,2	7,0 ± 0,1	7,0 ± 0,4	7,0 ± 0,4	7,0 ± 0,4	7,0 ± 0,4	7,0 ± 0,4
Conductivity μS / cm	335 ± 5	331 ± 2	332 ± 4	334 ± 4	336 ± 4	338 ± 3	337 ± 2	343 ± 4	347 ± 3

Sodium bromate concentrations have been selected according to results from acute toxicity tests with *Ceriodaphnia dubia* and with *Daphnia magna* (see below), as well as from data in a preliminary chronic test with *Ceriodaphnia*. As expected from this preliminary test, significant mortality is occurring at short-term with concentrations 30 mg NaBrO₃/L and above.

In fact for concentrations from 16 to 6 mg/L, percentages of dead daphnids show higher proportions than inhibition of reproduction at the end of test. So results in terms of lethal concentration LC₅₀ indicate that this endpoint is slightly lower than population growth at the 50% inhibition level (EC₅₀), at 7 days as well as at 8 days. This is due to lethal effects that are occurring all along the test, and even to a kind of collapse at low concentrations at the end (for example 58% mortality at day 7 with 9 mg NaBrO₃/L, and 42% mortality at day 8 with 6 mg/L; but none mortality at 3 nor 1 mg/L; see annex).

One possible explanation for this uncommon pattern (more usually it happens that reproduction is more sensitive than survival) is that, despite a toxic concentration between 10 and 22 mg/L, daphnids invest most of their available energy to reproduce, with some success, before dying very shortly after the last brood.

For endpoints of chronic toxicity EC_{10} and $NOEC$, used for environmental risk assessment and quality standard determination, it should be noticed that the statistical analysis suggests that the exposure time until 8 days provides a useful estimate for $NOEC$ (= 3 mg $NaBrO_3$ /L).

Indeed when having a first look at statistics for 7 days, the response at concentration 3 mg/L is not statistically different from the control, but the response at 1 mg/L can be considered as significantly different (thus $NOEC$ could be even lower than 1 mg/L). But when looking at response after 8 days of exposure, responses seem more logical (both responses at 1 and 3 mg/L are not statistically different from control). This is due on one hand to increased variance in controls, and to another hand to a "relative increase" in reproduction at concentration 1 mg/L from day 7 to day 8. For this reason it is considered that $NOEC$ at 7 days should be also at 3 mg/L.

When taking into account rather EC_{10} values (which should be preferred to $NOEC$ when possible, because interpolation of EC_x is integrating the whole curve concentration versus inhibition), this relative difficulty does not occur.

When comparing these values for chronic toxicity endpoints with other tested organisms, it appears that *Ceriodaphnia* is amongst the most sensitive in this battery, together with *Lemna minor* (see below).

Validity : The reliability of this *Ceriodaphnia* test, as evaluated by *CRED* approach is estimated to **R1** level (all appropriate criteria for reliability are fulfilled other than the use of reference toxicant, which is not run periodically). The relevance of the test is estimated to **C1** level (all appropriate relevance criteria are fulfilled).

Acute toxicity on *Ceriodaphnia dubia* is summarized in the following table. Initially the main purpose of this test was to help in selection of concentrations to be tested for the final 8-d assay. The test was run as for chronic mode (daily renewal; with addition of food), but with increased number of exposed organisms.

The mortality increasing clearly over exposure time from 24 to 72 hours, results indicate that *Ceriodaphnia* is sensitive to sodium bromate, with $LC_{50} - 48 h < 50$ mg/L.

Acute toxicity on <i>Ceriodaphnia dubia</i>		Mortality of juvenile females at					
		24 hours		48 hours		72 hours	
(03-07-2015)	Concentration	N	Mortality (%)	N	Mortality (%)	N	Mortality(%)
Controls (dilution medium)		0 / 28	0 %	0 / 28	0 %	0 / 28	0 %
Sodium Bromate $NaBrO_3$ (CAS 7789-38-0)	60 mg / L	30 / 30	100 %	30 / 30	100 %	30 / 30	100 %
	40 mg / L	0 / 33	0 %	19 / 33	57,8 %	33 / 33	100 %
	25 mg / L	0 / 28	0 %	2 / 28	7,1 %	17 / 28	60,7 %
	15 mg / L	0 / 34	0 %	0 / 34	0 %	0 / 34	0 %
	8,0 mg / L	0 / 30	0 %	0 / 30	0 %	0 / 30	0 %
	2,5 mg / L	0 / 30	0 %	0 / 30	0 %	0 / 30	0 %
☛ Mortality :		$(LC_{50} - 24 h = 49,0 \text{ mg } NaBrO_3 / L \text{ [95\% conf. limits : } 40 - 60 \text{ mg / L]}) ;$ $LC_{50} - 48 h = 37,9 \text{ mg } NaBrO_3 / L \text{ [95\% conf. limits : } 34,2 - 43,9 \text{ mg / L]} ;$ $LC_{50} - 72 h = 23,4 \text{ mg } NaBrO_3 / L \text{ [95\% conf. limits : } 15,0 - 40,0 \text{ mg / L]}.$					

3.2 Acute toxicity on *Daphnia magna*

The following table sums up results for inhibition of mobility and mortality of the cladocerans after 24 hours, 48 and 72 hours of exposure. Detailed data are given in annex.

Acute toxicity on <i>Daphnia magna</i> (03-07-2015) Concentration		Immobilisation / Mortality of neonates at					
		24 hours		48 hours		72 hours	
		N	Inhibition of mobility (%)	N	Inhibition of mobility (%)	N	Inhibition of mobility (%)
<i>Controls</i> (dilution medium)		0 / 20	0 %	0 / 20	0 %	1 / 20	5 %
<i>Sodium Bromate</i> NaBrO ₃ (CAS 7789-38-0)	500 mg / L	14 / 20	70 %	20 / 20	100 %	20 / 20	100 %
	400 mg / L	13 / 20	65 %	17 / 20	85 %	20 / 20	100 %
	300 mg / L	12 / 20	60 %	15 / 20	75 %	18 / 20	90 %
	200 mg / L	0 / 21	0 %	11 / 21	52 %	16 / 21	76 %
	100 mg / L	0 / 20	0 %	3 / 20	15 %	17 / 20	85 %
	50 mg / L	0 / 20	0 %	0 / 20	0 %	6 / 20	30 %
☞ <i>Inhibition of mobility / mortality :</i>		$EC_{50} - 24 h = 285 \text{ mg NaBrO}_3 / \text{L}$ [95% conf. limits : 200 - > 500 mg / L] ; $EC_{50} - 48 h = 194 \text{ mg NaBrO}_3 / \text{L}$ [95% conf. limits : 159 - 230 mg / L] ; $EC_{50} - 72 h = 67,9 \text{ mg NaBrO}_3 / \text{L}$ [95% conf. limits : 40,8 - 92,4 mg / L].					

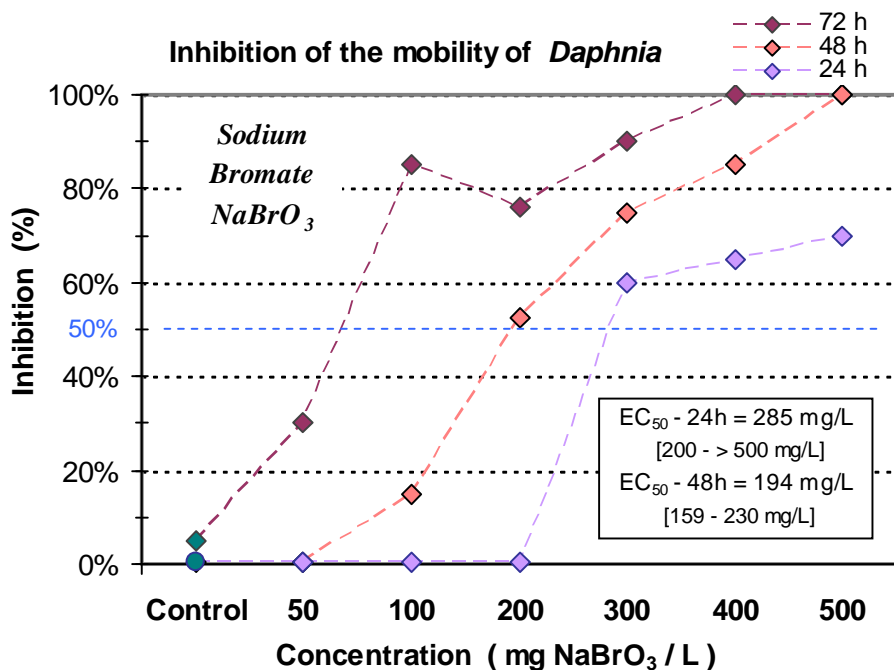


Figure 3.2. Ecotoxicity of sodium bromate on *Daphnia magna*. Inhibition expressed in % relative to control.

Validity : The reliability of this *Daphnia* test, as evaluated by *CRED* approach, is estimated to **R1** level (all appropriate criteria for reliability are fulfilled).

3.3 Chronic toxicity on algal population growth with *Pseudokirchneriella subcapitata*

The following table sums up results for inhibition of freshwater algal growth after 72 hours of exposure. As proposed by AFNOR T90-375 protocol, cell density is measured only at the end of test (not growth rate). Detailed data are given in annex.

Chronic toxicity on Green algae		Algal growth		
(11-07-2015)	Concentration	Average cell density at 72 hours		
		$N \cdot 10^4$ cells / mL	Growth (%)	Inhibition (%)
<i>Controls (dilution medium)</i>		179,5 ± 21,2	100 %	0 %
<i>Sodium Bromate</i> NaBrO ₃ (CAS 7789-38-0)	430 mg / L	89,4 ± 8,0	49,5 %	50,5 %
	286 mg / L	64,8 ± 8,2	35,8 %	64,2 %
	191 mg / L	96,9 ± 11,2	53,7 %	46,3 %
	95,5 mg / L	135,5 ± 24,7	75,3 %	24,7 %
<i>Population growth :</i>		$EC_{50} - 72 h = 204$ mg NaBrO ₃ / L [95% conf. limits : 169 – 256 mg / L] ; $EC_{10} - 72 h = 49,0$ mg NaBrO ₃ / L [95% conf. limits : 19,6 – 84,6 mg / L] * LOEC (Lowest observed effect concentration) = 95,5 mg NaBrO₃ / L ; MSD (Minimum statistical difference; % of inhibition) = 17,4 %		

* $EC_{10} - 72 h$ is given as indicative value (since there was no inhibition less than or equal to 10% and *NOEC* is not available, interpolation may be considered as less robust for this specific endpoint).

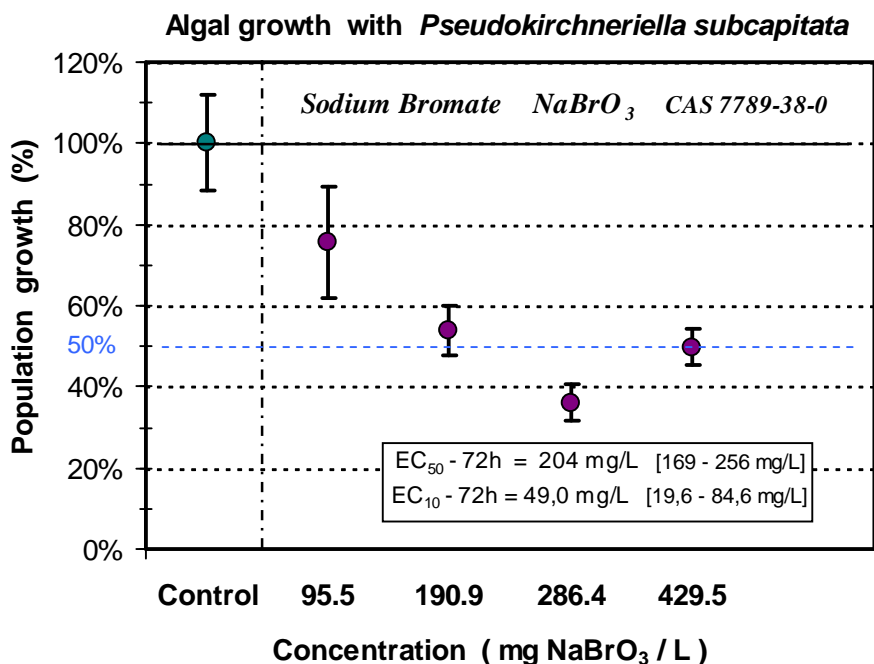


Figure 3.3. Ecotoxicity of sodium bromate on *Green algae*. Growth expressed in % relative to control.

For the calculation of endpoints EC_x and to improve the fit of the relationship, the response for concentration 430 mg/L has been kept away from the linear interpolation (considered as outlier). No *NOEC* can be estimated (only *LOEC*).

Validity : The reliability of this phytoplankton test, as evaluated by *CRED* approach, is estimated to **R2** level (all appropriate criteria for reliability are fulfilled, other than the use of negative control, which is less relevant for testing substances than wastewater samples, and one pair concentration / response considered as outlier, without chemical analysis of concentrations).

3.4 Chronic toxicity on population growth with *Brachionus calyciflorus*

The following table sums up results for inhibition of rotifer growth after 48 hours of exposure. Detailed data are given in annex.

Chronic toxicity on <i>Brachionus calyciflorus</i>		Reproduction of juvenile females		
(05-07-2015)	Concentration	Average number of females at 48 hours		
		N	Growth (%)	Inhibition (%)
<i>Controls</i> (dilution medium)		10,2 ± 3,4	100 %	0 %
<i>Sodium Bromate</i> NaBrO ₃ (CAS 7789-38-0)	400 mg / L	2,3 ± 1,2	14,5 %	85,5 %
	300 mg / L	4,0 ± 1,8	32,7 %	67,3 %
	250 mg / L	7,0 ± 2,0	65,4 %	34,6 %
	200 mg / L	6,3 ± 2,5	58,1 %	41,9 %
	170 mg / L	8,7 ± 1,0	83,5 %	16,5 %
	140 mg / L	10,5 ± 2,3	103 %	(- 3,0 %)
	120 mg / L	8,8 ± 4,6	84,4 %	15,6 %
	100 mg / L	9,0 ± 2,6	87,1 %	12,9 %
	80 mg / L	10,0 ± 1,8	98,0 %	2,0 %
60 mg / L	9,3 ± 2,5	90,8 %	9,2 %	
<i>Population growth :</i>	<i>EC</i> ₅₀ - 48 h = 262 mg NaBrO ₃ / L [95% conf. limits : 221 - 302 mg / L] ; <i>EC</i> ₁₀ - 48 h = 149 mg NaBrO ₃ / L [95% conf. limits : 91,5 - 221 mg / L] ; <i>NOEC</i> (No observed effect concentration) = 170 mg NaBrO ₃ / L ; <i>LOEC</i> (Lowest observed effect concentration) = 200 mg NaBrO ₃ / L ; <i>MSD</i> (Minimum statistical difference; % of inhibition) = 33,0 % .			

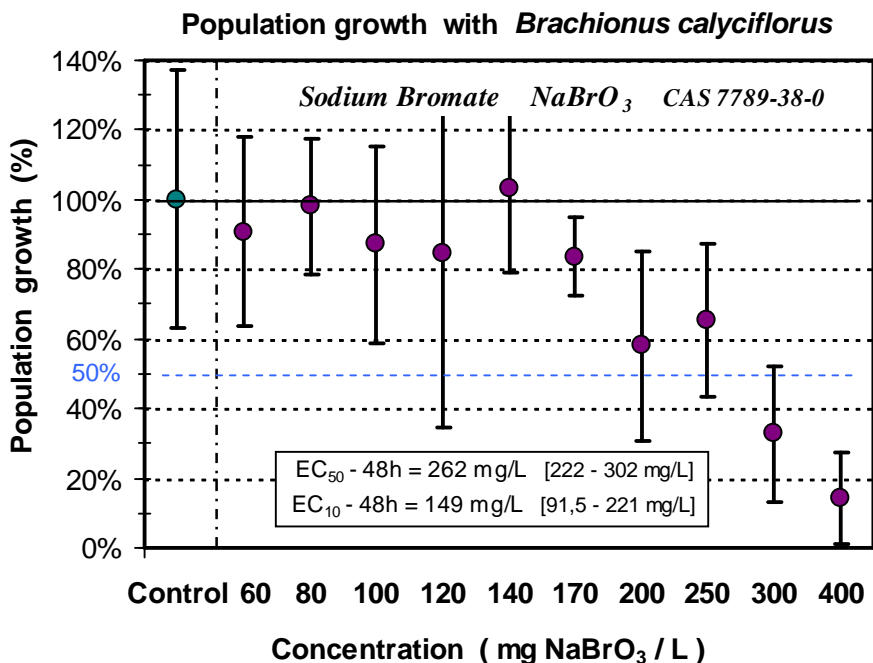


Figure 3.4. Ecotoxicity of sodium bromate on *Rotifer*. Growth expressed in % relative to control.

Validity : The reliability of this rotifer test, as evaluated by *CRED* approach, is estimated to **R2** level (most of reliability criteria are fulfilled; but limited number of replicates for tested concentrations; also high sensitivity to copper sulphate).

3.5 Chronic toxicity on population growth with *Lemna minor*

The following table sums up results for inhibition of duckweed growth after 7 days of exposure. Frond number is measured as basic primary variable (yield), but no other variable (i.e. total frond area or dry weight) was measured. Estimates for EC_x are based upon yield (EyC_x; not upon specific growth rate). Detailed data are given in annex.

Chronic toxicity on <i>Lemna minor</i>		Macrophyte growth		
(05-08-2015)	Concentration	Average number of fronds at 7 days		
		N	Growth (%)	Inhibition (%)
<i>Controls</i> (dilution medium)		90,2 ± 6,4	100 %	0 %
<i>Sodium Bromate</i> NaBrO ₃ (CAS 7789-38-0)	148 mg / L	3,3 ± 1,6	4,2 %	95,8 %
	78,8 mg / L	3,3 ± 1,6	4,2 %	95,8 %
	49,3 mg / L	18,7 ± 6,0	10,8 %	89,2 %
	29,6 mg / L	27,0 ± 1,7	21,2 %	78,8 %
	14,8 mg / L	61,0 ± 8,0	63,6 %	36,4 %
	4,9 mg / L	73,0 ± 5,2	78,6 %	21,3 %
<i>Population growth</i> :		<i>EC</i> ₅₀ - 7 d = 17,3 mg NaBrO ₃ / L [95% conf. limits : 13,9 - 20,4 mg / L] ; <i>EC</i> ₁₀ - 7 d = 5,3 mg NaBrO ₃ / L [95% conf. limits : 3,3 - 7,9 mg / L] * <i>LOEC</i> (Lowest observed effect concentration) = 4,9 mg NaBrO ₃ / L ; <i>MSD</i> (Minimum statistical difference; % of inhibition) = 11,0 % .		

* EC₁₀ - 7 d is given as indicative value (since there was no inhibition less than or equal to 10% and NOEC is not available, interpolation may be considered as less robust for this specific endpoint).

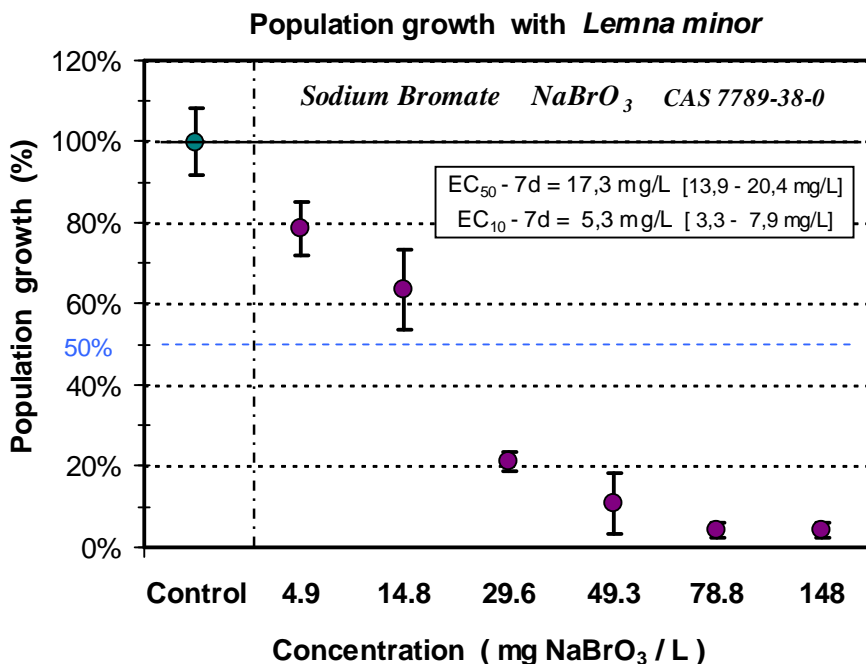


Figure 3.5. Ecotoxicity of sodium bromate on *Macrophyte*. Growth expressed in % relative to control.

Validity : The reliability of this duckweed test, as evaluated by *CRED* approach, is estimated to **R2** level (most of reliability criteria are fulfilled; but no second variable measured, as frond area or weight, and no reference toxicant has been used in parallel to this series).

4. Summary and conclusion

A battery of tests of acute toxicity and chronic toxicity has been performed on freshwater organisms, including the cladocerans *Ceriodaphnia dubia* and *Daphnia magna*, green algae, rotifer and duckweed (macrophyte), in order to provide an enlarged assessment for sodium bromate ecotoxicity. All data are gathered in the following summary table.

Summary table of toxicity endpoints for sodium bromate : Endpoint values as LC₅₀, EC₅₀, EC₁₀ and/or NOEC are given in mg NaBrO₃ / L; corresponding concentrations in bromate ion are deduced and given in (). Assessment for reliability of tests was made according to CRED approach (Criteria for Reporting and Evaluating ecotoxicity Data; R1 = reliable without restrictions, R2 = reliable with restrictions, R3 = not reliable, R4 = not assignable; Moermond *et al.* 2015). All criteria for relevance are assumed to be fulfilled.

Effektdatensammlung für Natriumbromat. Testkonzentrationen wurden grösstenteils auf Bromat umgerechnet und in () angegeben. Eine Bewertung der Validität wurde nach den CRED (Moermond *et al.* 2015) durchgeführt.

SUMMARY OF EFFECT DATA / EFFEKTDATENSAMMLUNG													
Taxonomic group Sammelbezeichnung	Organismu Organismus	Parameter	Duration time Dauer	Dimension	Endpoint Endpunkt	Operator	Value Wert (mg/L)	Chemische Analyse ^a	Testsystem ^b	Substance Grade Reinheit (%)	Remarks Bemerkungen	Reliability Validität	Referenz
Acute toxicity data / Akute Daten													
Water flea Krebstiere	<i>Ceriodaphnia dubia</i>	Mortality Mortalität	48	h	LC50	=	37.9 (32.1)	C	R	≥ 99; extra pure	Test mit NaBrO ₃	(R1)	
Water flea Krebstiere	<i>Daphnia magna</i>	Mortality Mortalität / Immobilisation	48	h	LC50	=	194 (164.4)	C	S	≥ 99; extra pure	Test mit NaBrO ₃ ; nach ISO 6341	R1	
Water flea Krebstiere	<i>Daphnia magna</i>	Mortality Mortalität/ Immobilisation	72	h	LC50	=	67.9 (57.6)	C	S	≥ 99; extra pure	Test mit NaBrO ₃	(R1)	
(Sub)chronic toxicity Data / (Sub)chronische Daten													
Algae Algen	<i>Pseudokirchneriella subcapitata</i>	Algal growth Wachstum	72	h	EC50	=	204 (172.9)	C	S	≥ 99; extra pure	Test mit NaBrO ₃ ; nach AFNOR T90-375 (in Mikrotiterplatten)	R2	
Algae Algen	<i>Pseudokirchneriella subcapitata</i>	Algal growth Wachstum	72	h	EC10 *	=	49.0 * (41.6)	C	S	≥ 99; extra pure	Test mit NaBrO ₃ ; nach AFNOR T90-375 (in Mikrotiterplatten)	R2	
Rotifera	<i>Brachionus calyciflorus</i>	Population growth Reproduktion	48	h	EC50	=	262 (221.8)	C	S	≥ 99; extra pure	Test mit NaBrO ₃ ; nach ISO 20666	R2	
Rotifera	<i>Brachionus calyciflorus</i>	Population growth Reproduktion	48	h	EC10	=	149 (126.3)	C	S	≥ 99; extra pure	Test mit NaBrO ₃ ; nach ISO 20666	R2	
Water flea Krebstiere	<i>Ceriodaphnia dubia</i>	Population growth Reproduktion	7	d	EC50	=	9.9 (8.4)	C	R	≥ 99; extra pure	Test mit NaBrO ₃ ; nach ISO 20665	R1	
Water flea Krebstiere	<i>Ceriodaphnia dubia</i>	Population growth Reproduktion	7	d	EC10	=	5.5 (4.6)	C	R	≥ 99; extra pure	Test mit NaBrO ₃ ; nach ISO 20665	R1	
Water flea Krebstiere	<i>Ceriodaphnia dubia</i>	Population growth Reproduktion	8	d	EC10	=	4.6 (3.9)	C	R	≥ 99; extra pure	Test mit NaBrO ₃ ; nach ISO 20665	R1	
Water flea Krebstiere	<i>Ceriodaphnia dubia</i>	Population growth Reproduktion	8	d	NOEC	=	3.0 (2.5)	C	R	≥ 99; extra pure	Test mit NaBrO ₃ ; nach ISO 20665	R1	
Macrophytes Macrophyten (Wasserlinsen)	<i>Lemna minor</i>	Population growth Reproduktion	7	d	EC50	=	17.3 (14.7)	C	S	≥ 99; extra pure	Test mit NaBrO ₃ ; nach OECD 221	R2	
Macrophytes Macrophyten (Wasserlinsen)	<i>Lemna minor</i>	Population growth Reproduktion	7	d	EC10 *	=	5.3 * (4.5)	C	S	≥ 99; extra pure	Test mit NaBrO ₃ ; nach OECD 221	R2	

^a Substance analysis : C = Nominal test concentration. ^b Test system : F = flow-trough renewal; R = semi-static renewal; S = static.

* EC₁₀ for *Green algae* and *Lemna minor* are given as indicative value, in supporting results for other endpoints.

^a C = Nominale Testkonzentrationen verwendet. ^b F = Durchfluss; R = semi-statisch; S = statisch

Both bioassays with *Ceriodaphnia dubia* and *Lemna minor* show comparable sensitivity, providing close toxicity endpoints for EC₁₀-7d, with values at 5,5 mg NaBrO₃ / L and 5,3 mg / L respectively. The *No observed effect concentration* NOEC for *Ceriodaphnia dubia* at 7 and 8 days is 3,0 mg NaBrO₃ / L.

We wish you a good reception of these results. Please do not hesitate to contact us if you have any question or need for further information.

Dr. Sergio Santiago



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5. Bibliography

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6. Annexes

Annex A. Data of bioassay with *Ceriodaphnia dubia*

Annex B. Data of bioassays with *Daphnia magna* and with *Green Algae*

Annex C. Data of bioassays with *Brachionus calyciflorus* and with *Lemna minor*

Annex A. Data of bioassay with *Ceriodaphnia dubia*



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Bioassays of toxicity


Summary of results

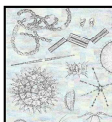
Identification Origin : <i>Sodium Bromate NaBrO₃ CAS 7789-38-0</i> Sample type : <i>Substance (extra pure 99+%)</i> Sampling : <i>ACROS Organics ; Ref. 20887-1000, lot A0358417</i> Date : <i>July 2015</i> Carried out by : <i>Chemie Brunschwig AG, Basel</i> Remarks : <i>Pretest (range finding test) with Daphnia magna</i> <i>Pretest with Ceriodaphnia on July, 3rd 2015</i> <i>Concentrations in tests are nominal.</i>		Recipient : <i>Dr. Robert Kase</i> Society : <i>Oekotoxzentrum</i> Address : <i>CH-8600 Dübendorf</i> Analyse(s) plan : <i>Ceriodaphnia dubia</i> <i>+ other bioassays</i> Reception date : <i>01 - 07 - 2015</i> Registration nr : <i>8747</i> Responsible : <i>S. Santiago</i>
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Ceriodaphnia dubia (ISO 20665 ; AFNOR T90-376 ; Environment Canada SPE 1/RM/21)	Organism : <i>Ceriodaphnia dubia (IFAF-Cemagref)</i> PP beakers (25 ml); 25±1°C; 0,4±0,1 Klux (photoper.16h:8h) Dilution : medium AFNOR T90-376 modified; food A, Y, xt.T	Date : 14-07-2015 Carried out by : SS Controlled by :
--	---	---

Sample nr Concentration	Mortality at 7 days	Chronic toxicity : inhibition of growth population at 7 days							Growth (%)	Inhibition (%)
		Number of offspring ; ♀ = dead mother ; ⊕ = + unhatched egg				Σ offs.	Average	Std. dev.		
		Replicates								
Controls (synthetic medium = dilution medium)	0 / 28 = 0 %	20	15	16	21	544	19.4	2.9 = 15.2%	100%	0%
		20	20	18	14					
		22	21	21	17					
		15	22	23	21					
		19	23	24	18					
		15	20	25	20					
		18	16	22	18					
Sodium Bromate <i>NaBrO₃</i>	30 mg / L 12 / 12 = 100 %	12 * ♀ (all dead after 5 days of exposition)				0	0	0	0%	100%
	22 mg / L 12 / 12 = 100 %	1 offspring ; 12 * ♀ (all dead after 5 days)				1	0.1	0.3	0.4%	99.6%
	16 mg / L 12 / 12 = 100 %	5 ; ♀	3 ; ♀	4 ; ♀	4 ; ♀	40	3.3	2.2	17.2%	82.8%
		0 ; ♀	5 ; ♀	4 ; ♀	3 ; ♀					
		0 ; ♀	6 ; ♀	6 ; ♀	0 ; ♀	(all dead after 6 days)				
	12 mg / L 9 / 12 = 75,0 %	6 ; ♀	6 ; ♀	6 ; ♀	6 ; ♀	70	5.8	1.1	30.0%	70.0%
		6 ; ♀	5 ; ♀	7 ; ♀	3 ; ♀					
	9.0 mg / L 7 / 12 = 58,3 %	12 ; ♀	9	6 ; ♀	12	130	10.8	2.2	55.8%	44.2%
	13 ; ♀	9 ; ♀	13 ; ♀	11 ; ♀						
	13 ; ♀	9	10	13						
6.0 mg / L 1 / 12 = 8,3 %	15	19	16	14	193	16.1	1.7	82.8%	17.2%	
	16	16	15	15						
	19	18	14 ; ♀	16						
3.0 mg / L 0 / 12 = 0 %	22	18	17	16	216	18.0	3.1	92.6%	7.4%	
	22	15	14	20						
	20	14	16	22						
1.0 mg / L 0 / 12 = 0 %	15	16	14	16	197	16.4	1.7	84.5%	15.5%	
	16	18	14	17						
	16	20	18	17						

Remarks : see report

<p>Conclusions - Comments</p> <p><i>Sodium Bromate NaBrO₃ CAS 7789-38-0</i></p> <p>Mortality : <i>LC₅₀ - 7d = 8,92 mg NaBrO₃ / L</i> [95% conf. limits : 7,5 - 10,3 mg/L]</p> <p>Reproduction : <i>EC₅₀ - 7d = 9,90 mg NaBrO₃ / L</i> [95% conf. limits : 9,3 - 10,5 mg/L]</p> <p><i>EC₂₀ - 7d = 6,82 mg NaBrO₃ / L</i> [95% conf. limits : 6,1 - 7,6 mg/L]</p> <p><i>EC₁₀ - 7d = 5,49 mg NaBrO₃ / L</i> [95% conf. limits : 4,7 - 6,3 mg/L]</p> <p><i>NOEC (No observed effect concentration) : 3,0 mg / L *</i></p> <p><i>LOEC (Lowest observed effect concentration) : 6,0 mg / L</i></p> <p><i>MSD (Minimum statistical difference; % of inhibition) : 10,3 %</i></p>	<p>Valid test <input checked="" type="checkbox"/> yes - <input type="checkbox"/> no</p> <p>Controls (at 7 days) : Mortality of the mothers ≤ 20% <input checked="" type="checkbox"/> Proportion of males ≤ 20% <input checked="" type="checkbox"/> Min. 3 broods for ≥ 60% of alive mothers <input checked="" type="checkbox"/> Average nb. offspring born per live mother ≥ 15 <input checked="" type="checkbox"/></p> <p></p> <p>* NOEC : see report</p>
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Analyses environnementales


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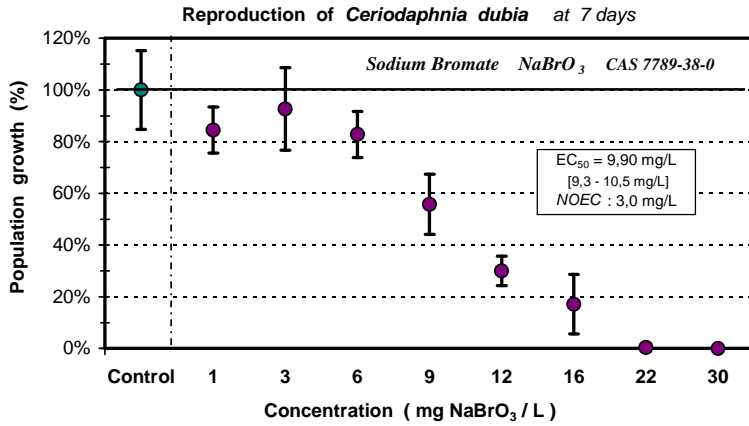
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Bioassays of toxicity

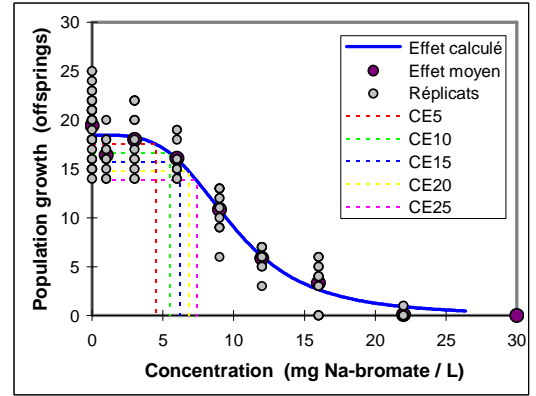
Summary of results

Identification		Organism : <i>Ceriodaphnia dubia</i> (IFAF-Cemagref)		Date : 14-07-2015						
Origin :	Sodium Bromate NaBrO₃ CAS 7789-38-0	PP beakers (25 ml); 25±1°C; 0,4±0,1 Klux (photoper.16h:8h)	Carried out by : SS							
Sample type :	Substance (extra pure 99+%)	Dilution : medium AFNOR T90-376 modified; food A, Y, xt.T	Controlled by :							
Sampling :	ACROS Organics ; Ref. 20887-1000, lot A0358417	Recipient : Dr. Robert Kase								
Date :	July 2015	Society : Oekotoxzentrum								
Carried out by :	Chemie Brunschwig AG, Basel	Address : CH-8600 Dübendorf								
Remarks :	Pretest (range finding test) with <i>Daphnia magna</i>	Analyse(s) plan : <i>Ceriodaphnia dubia</i>								
	Pretest with <i>Ceriodaphnia</i> on July, 3rd 2015	+ other bioassays								
	Concentrations in tests are nominal.	Reception date : 01 - 07 - 2015								
		Registration nr : 8747								
		Responsible : S. Santiago								
Chronic toxicity : inhibition of growth population at 8 days		Mortality at 8 days		Growth (%)		Inhibition (%)				
Sample nr	Concentration	Number of offspring ; ♀ = dead mother ; ♂ = + unhatched egg				Average	Std. dev.			
		Replicates								
					Σ offs.					
Controls (synthetic medium = dilution medium)	0 / 28 = 0 %	27	20	27	29	678	24.2	4.4	100%	0%
		27	29	28	21			=		
		22	21	21	27			18.2%		
		23	30	23	21				100%	0%
		19	23	33	18					
		22	20	34	20					
		18	26	22	27					
Sodium Bromate NaBrO₃	30 mg / L	12 * ♀ (all dead after 5 days of exposition)				0	0	0	0%	100%
	22 mg / L	1 offspring ; 12 * ♀ (all dead after 5 days)				1	0.1	0.3	0.3%	99.7%
	16 mg / L	5 ; ♀	3 ; ♀	4 ; ♀	4 ; ♀	40	3.3	2.2	13.8%	86.2%
		0 ; ♀	5 ; ♀	4 ; ♀	3 ; ♀					
		0 ; ♀	6 ; ♀	6 ; ♀	0 ; ♀					
		(all dead after 6 days)								
	12 mg / L	6 ; ♀	6 ; ♀	6 ; ♀	6 ; ♀	73	6.1	1.4	25.1%	74.9%
		9	5 ; ♀	7 ; ♀	3 ; ♀					
		6 ; ♀	7	5 ; ♀	7 ; ♀					
	9.0 mg / L	12 ; ♀	9	6 ; ♀	12	139	11.6	3.0	47.8%	52.2%
		13 ; ♀	9 ; ♀	13 ; ♀	11 ; ♀					
		13 ; ♀	13 ; ♀	10	18 ; ♀					
	6.0 mg / L	15	19	16	22 ; ♀	222	18.5	3.3	76.4%	23.6%
		16	23 ; ♀	20	24					
		19	18 ; ♀	14 ; ♀	16 ; ♀					
	3.0 mg / L	28	18	17	21	265	22.1	3.1	91.2%	8.8%
		22	23	23	20					
		26	24	21	22					
	1.0 mg / L	24	23	18	22	272	22.7	3.9	93.6%	6.4%
		16	27	19	27					
		26	20	22	28					
Remarks : see report										
Conclusions - Comments						Valid test <input checked="" type="checkbox"/> yes - <input type="checkbox"/> no				
Sodium Bromate NaBrO₃ CAS 7789-38-0						Controls (at 7 days) :				
Mortality :	LC₅₀ - 8d =	7,00 mg NaBrO₃ / L				Mortality of the mothers ≤ 20% <input checked="" type="checkbox"/>				
		[95% conf. limits : 5,5 - 8,3 mg/L]				Proportion of males ≤ 20% <input checked="" type="checkbox"/>				
Reproduction :	EC₅₀ - 8d =	8,84 mg NaBrO₃ / L				Min. 3 broods for ≥ 60% of alive mothers <input checked="" type="checkbox"/>				
		[95% conf. limits : 8,3 - 9,5 mg/L]				Average nb. offspring born per live mother ≥ 15 <input checked="" type="checkbox"/>				
	EC₂₀ - 8d =	5,82 mg NaBrO₃ / L								
		[95% conf. limits : 5,1 - 6,6 mg/L]								
	EC₁₀ - 8d =	4,56 mg NaBrO₃ / L								
		[95% conf. limits : 3,8 - 5,5 mg/L]								
	NOEC (No observed effect concentration) :	3,0 mg / L								
	LOEC (Lowest observed effect concentration) :	6,0 mg / L								
	MSD (Minimum statistical difference; % of inhibition) :	11,9 %								
										

Ecotoxicity of sodium bromate - Reproduction of *Ceriodaphnia dubia* at 7 and at 8 days

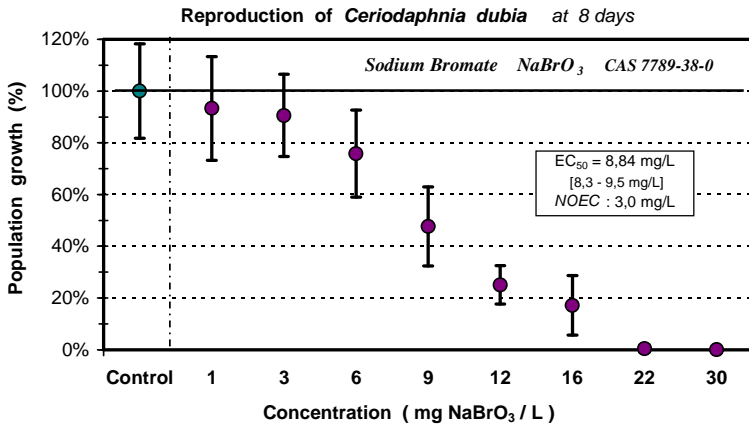


Conc. [mg/L]	Moyenne	Ecart-type	Moyenne	Ecart-type
Control - 0	19.43	2.95	100%	15.2%
1	16.42	1.73	84.5%	8.9%
3	18.00	3.10	92.6%	16.0%
6	16.08	1.73	82.8%	8.9%
9	10.83	2.25	55.8%	11.6%
12	5.83	1.11	30.0%	5.7%
16	3.33	2.23	17.2%	11.5%
22	0.08	0.29	0.4%	1.5%
30	0	0	0.0%	0.0%

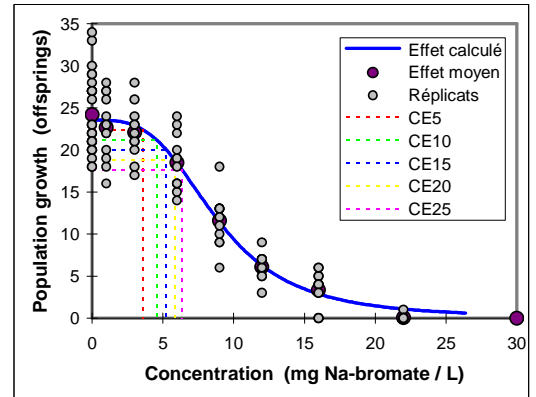


Régression logistique REGTOX (modèle de Hill)

Paramètre	Valeur des paramètres calculés			Intervalles de confiance		
	HILL	Optimale	Moyenne	Médiane	< alpha =5	> alpha =5
Témoin		18.468	18.431	18.432	17.846	19.078
N de Hill		3.723	3.766	3.728	3.094	4.603
CE50		9.898	9.883	9.890	9.311	10.507
CE10		5.486	5.492	5.483	4.653	6.328
CE20		6.821	6.819	6.805	6.053	7.612



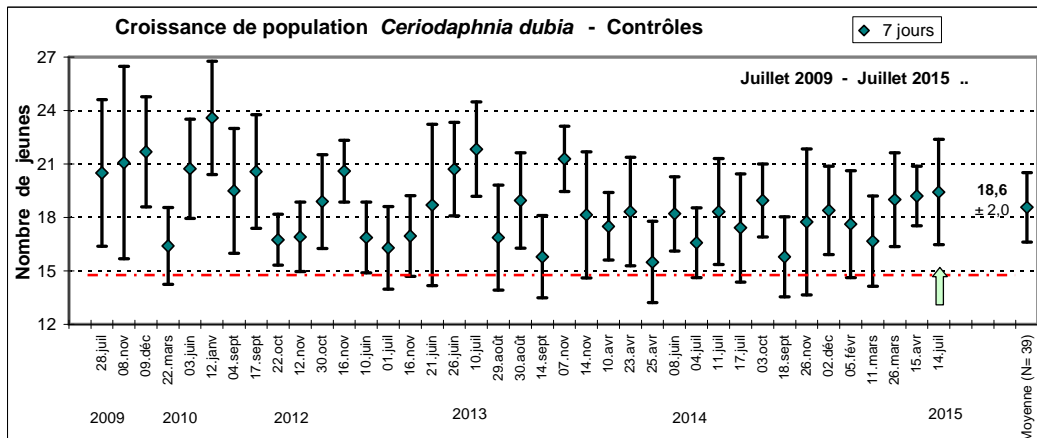
Conc. [mg/L]	Moyenne	Ecart-type	Moyenne	Ecart-type
Control - 0	24.21	4.42	100%	18.2%
1	22.67	3.89	93.3%	20.0%
3	22.08	3.09	90.6%	15.9%
6	18.50	3.26	75.8%	16.8%
9	11.58	2.97	47.7%	15.3%
12	6.08	1.44	25.0%	7.4%
16	3.33	2.23	17.2%	11.5%
22	0.08	0.29	0.4%	1.5%
30	0	0	0.0%	0.0%



Régression logistique REGTOX (modèle de Hill)

Paramètre	Valeur des paramètres calculés			Intervalles de confiance		
	HILL	Optimale	Moyenne	Médiane	< alpha =5	> alpha =5
Témoin		23.541	23.476	23.448	22.547	24.433
N de Hill		3.321	3.395	3.363	2.734	4.280
CE50		8.838	8.849	8.840	8.257	9.455
CE10		4.560	4.611	4.612	3.816	5.453
CE20		5.821	5.862	5.868	5.120	6.614

Quality control - Reproduction of *Ceriodaphnia dubia* at 7 days in controls



Ceriodaphnia dubia - Determination of chronic toxicity to Ceriodaphnia dubia

CRED evaluation method for reliability to be used together with the accompanying guidance in (Moermond et al., submitted)

Mark your evaluation per criterion with an 'x' in the respective box. If a criterion is not fulfilled, the reason for this should be stated in the comments box.

Number	General information	Criterion fulfilled	Criterion not fulfilled applicable	Criterion is not reported	Comments
	Before evaluating the test, please check the physico-chemical characteristics of your compound (handbooks/general sources). What is the solubility, log K _{ow} , pKa, is the compound volatile, does it hydrolyse, photolyse, etc.?				
1	Is a standard method (e.g., OECD/ISO) or modified standard used?*	X			ISO 20665 : 2008
2	Is the test performed under GLP conditions?*		X		
3	If applicable, are validity criteria fulfilled (e.g. control survival, growth)?	X			Controls : mortality of mothers ≤ 20%; proportion of males ≤ 20% at least 60% of mothers alive have produced a minimum of 3 broods; the average number of offspring born per live mother ≥ 15
4	Are appropriate controls performed (e.g. solvent control, negative and positive control)?	(X)			Negative controls and population growth parameters fulfill the validity criteria. No reference toxicant included.
*These criteria are of minor importance for study reliability, but may support study evaluation					
Test compound					
5	Is the test substance identified clearly with name or CAS-number? Are test results reported for the appropriate compound?	X			CAS 7789-38-0
6	Is the purity of the test substance reported? Or, is the source of the test substance trustworthy?	X			Extra pure quality grade; 99%+ ; Source : Acros Organics
7	If a formulation is used or if impurities are present: Do other ingredients in the formulation exert an effect? Is the amount of test substance in the formulation known?	X	X		
Test organism					
8	Are the organisms well described (e.g. scientific name, weight, length, growth, age/life stage, strain/clône, gender if appropriate)?	X			
9	Are the test organisms from a trustworthy source and acclimatized to test conditions? Have the organisms not been pre-exposed to test compound or other unintended stressors?	X			Source : Cemagref, Lyon (2007); since then, continuous culture in own lab.
Exposure conditions					
10	Is the experimental system appropriate for the test substance, taking into account its physico-chemical characteristics?	X			Cups in PP (30 mL); Na-bromate not supposed to adsorb on plastic
11	Is the experimental system appropriate for the test organism (e.g., choice of medium or test water, feeding, water characteristics, temperature, light/dark conditions, pH, oxygen content)? Have conditions been stable during the test?	X			pH, O2 and conductivity measured after each renewal
12	Were exposure concentrations below the limit of water solubility (taking the use of a solvent into account)? If a solvent is used, is the solvent within the appropriate range and is a solvent control included?	X			Max. tested concentration = 30 mg NaBrO ₃ / L ; stock solution : 1 g / L of dilution medium solubility (20°C) : 364 - 420 g/L
13	Is a correct spacing between exposure concentrations applied?	X			Whole curve concentration (ranging from 30 to 1 mg NaBrO ₃ /L) / inhibition (ranging from 100% to <10%)
14	Is the exposure duration defined?	X			7 days; both ISO 20665 and Environment Canada (Env. Protection Series EPS 1/RM/21) allow to increase duration to 8 days to achieve 3 broods produced per female
15	Are chemical analyses adequate to verify substance concentrations over the duration of the study?	X	(X)		No analyses performed;
16	Is the biomass loading of the organisms in the test system within the appropriate range (e.g. < 1 g/L)?	X			concentrations for determination of toxicity endpoints are nominal
Statistical Design and Biological Response					
17	Is a sufficient number of replicates used? Is a sufficient number of organisms per replicate used for all controls and test concentrations?	X			ISO 20665 : at least 10 replicates for each concentration. Test : controls = 28 repl.; conc. = 12 repl.
18	Are appropriate statistical methods used?	X			EC50, EC10 : Reptox (logistic regression; Hill model) Toxstat : Chi-square (normality), Bartlett's test (homogeneity of variance)
19	Is a dose-response curve observed? Is the response statistically significant?	X			ANOVA Bonferroni t-test (NOEC - LOEC)
20	Is sufficient data available to check the calculation of endpoints and (if applicable) validity criteria (e.g., control data, dose-response curves)?	X			Whole curve concentration / inhibition (ranging from 100% to <10%), with min. 4 data between 90 and 10%
Final score (R1 = Reliable, R2 = Reliable with restrictions, R3 = Not reliable, R4 = Not assignable)		R1			

An explanatory guidance on how to interpret these criteria can be found in Moermond et al. 2015.

Please note that most criteria are not per se critical for the reliability of a study, and that this depends strongly on the compound and/or species tested.

Annex B. Data of bioassays with *Daphnia magna* and with Green Algae



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Toxicity Bioassays

Results summary

Identification		Recipient : <i>Dr. Robert Kase</i>
Origin :	<i>Sodium Bromate NaBrO₃ CAS 7789-38-0</i>	Society : <i>Oekotoxzentrum</i>
Sample Type :	<i>Substance (extra pure 99+%)</i>	Address : <i>CH-8600 Dübendorf</i>
Sampling :	<i>ACROS Organics ; Ref: 20887-1000, lot A0358417</i>	Plan of analyse(s) : <i>Daphnia, algae, rotifer, macrophyte</i>
Date :	<i>July 2015</i>	Reception date : <i>01 - 07 - 2015</i>
Carried out by :	<i>Chemie Brunschwig AG, Basel</i>	Registration nr : <i>8747</i>
Remark : <i>Concentrations in tests are nominal.</i>		Responsible : <i>S. Santiago</i>

<i>Daphnia magna</i> (ISO 6341 ; OECD 202)		Organism : <i>Daphnia magna</i> IPL-96, < 24 h. Ind./repl. = 5; repl./concent. = 4; vol./repl. = 15 ml. Dilution water : medium daphnids ISO 6341						Date : 03 - 07 - 2015 Done by : SS Controlled by :			
Sample	Concentration [mg NaBrO ₃ / L]	Inhibition of mobility - mortality						Physico - chemical parameters at 72 hr.			
		at 24 hr.		at 48 hr.		at 72 hr.		T (°C)	pH	O ₂ (mg/L)	K (µS/cm)
Control		0 / 20	0 %	0 / 20	0 %	1 / 20	5 %	21.3	7.7	6.7	600
<i>Sodium Bromate NaBrO₃</i>	500	14 / 20	70 %	20 / 20	100 %	20 / 20	100 %	21.4	7.9	6.4	780
	400	13 / 20	65 %	17 / 20	85 %	20 / 20	100 %	21.5	7.8	6.5	725
	300	12 / 20	60 %	15 / 20	75 %	18 / 20	90 %	21.4	7.7	6.6	685
	200	0 / 21	0 %	11 / 21	52,4 %	16 / 21	76,2 %	21.3	7.7	6.7	655
	100	0 / 20	0 %	3 / 20	15 %	17 / 20	85 %	21.3	7.7	6.8	625
	50	0 / 20	0 %	0 / 20	0 %	6 / 20	30 %	21.4	7.7	6.8	610

Remarks :

Conclusions - Comments		Valid assay <input checked="" type="checkbox"/> yes - <input type="checkbox"/> no
<i>Sodium Bromate NaBrO₃ CAS 7789-38-0</i>		Control : < 10% immobilized <input checked="" type="checkbox"/>
<i>Inhibition of mobility : EC₅₀ - 24 hr = 285 mg NaBrO₃ / L</i> [95% conf. limits : 200 - > 500 mg/L]		T : 20 ± 2°C <input checked="" type="checkbox"/> Oxyg. > 2,0 mg/l <input checked="" type="checkbox"/>
<i>EC₅₀ - 48 hr = 194 mg NaBrO₃ / L</i> [95% conf. limits : 159 - 223 mg/L]		pH : between 6,8 and 8,5 <input checked="" type="checkbox"/>
<i>EC₅₀ - 72 hr = 67,9 mg NaBrO₃ / L</i> [95% conf. limits : 40,8 - 92,4 mg/L]		Ref. K ₂ Cr ₂ O ₇ [0,9 - 1,5 mg/l]
		Date : 04-06-2015 <input checked="" type="checkbox"/>
		EC ₅₀ -24h. = 1,12 mg/l [1,04 - 1,21 mg/l]
		Date : 06-07-2015 <input checked="" type="checkbox"/>
		EC ₅₀ -24h. = 1,11 mg/l [0,98 - 1,23 mg/l]

<i>Green Algae Pseudokirchneriella subcapitata</i> (AFNOR T90-375; ISO 8692)		Organism : <i>P. subcapitata (S. capricornutum)</i> UTEX1648 Microplate (2,2 ml); 3 replicates; 23 ± 2°C; 5 Klux; 0 t/m Dilution : medium AAP (USEPA); Coulter® Counter						Date : 11 - 07 - 2015 Done by : SS Controlled by :		
Sample	Concentration [mg NaBrO ₃ / L]	Growth of algae				Cell density at start = 1.00E+04		Growth (%)	Inhibition (%)	
		Cell concentration at 72 hr. (cells * 10E+04 / mL)				Mean	Coef.var.			
Controls (dilution medium)		173.1	188.5	158.5			179.5	11.8%	100%	0%
		174.4	217.6	165.1						
<i>Sodium Bromate NaBrO₃</i>	429.5	81.5	89.1	97.5			89.4	9.0%	49.5%	50.5%
	286.4	73.5	63.9	57.2			64.8	12.7%	35.8%	64.2%
	190.9	106.3	84.5	100.0			96.9	11.5%	53.7%	46.3%
	95.5	159.5	136.7	110.2			135.5	18.2%	75.3%	24.7%

Remarks :

Conclusions - Comments		Valid assay <input checked="" type="checkbox"/> yes - <input type="checkbox"/> no
<i>Sodium Bromate NaBrO₃ CAS 7789-38-0</i>		Control : N _{end} ≥ 32 x N _{init.} <input checked="" type="checkbox"/>
<i>Inhibition of growth : EC₅₀ - 72 hr = 203,8 mg NaBrO₃ / L *</i> [95% conf. limits : 169 - 256 mg/L]		pH initial = 8,0 ; pH final = 9,2 variation pH ≤ 1,5 <input checked="" type="checkbox"/>
<i>EC₁₀ - 72 hr = 49,0 mg NaBrO₃ / L **</i> [95% conf. limits : 19,6 - 84,6 mg/L]		Temperature (23 ± 2 °C) : 24,4 ± 0,5 °C <input checked="" type="checkbox"/>
<i>LOEC (Lowest observed effect concentration) : 95,5 mg / L</i>		Ref. K ₂ Cr ₂ O ₇ [0,25 - 0,80 mg/l]
<i>* EC₅₀ - 72 hr calculate without taking into account 429.5 mg/L (outlier)</i>		Date : 05-06-2015 <input checked="" type="checkbox"/>
<i>** EC₁₀ is given as indicative value (since there was no inhibition less than or equal to 10% and NOEC is not available, interpolation may be considered as less robust for this specific endpoint).</i>		EC ₅₀ -72h. = 0,34 mg/l [0,29 - 0,41 mg/l]
		Date : 24-07-2015 <input checked="" type="checkbox"/>
		EC ₅₀ -72h. = 0,56 mg/l [0,51 - 0,62 mg/l]

Dr. S. Santiago

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Annex C. Data of bioassays with *Brachionus calyciflorus* and with *Lemna minor*



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**Toxicity Bioassays
Results summary**

Rotifer <i>Brachionus calyciflorus</i> (draft ISO 20666)		Organism : <i>Brachionus calyciflorus</i> (Rotokit F) Microplate (1,0 ml); 4-6 replicates; 25 ±1°C; darkness Dilution : MHSW (pH=7,6); food : <i>Pseudokir.sub.</i> + <i>Chlor. sp.</i>						Date : 05-07-2015 Done by : SS Controlled by :	
Sample	Concentration [mg NaBrO ₃ / L]	Growth of rotifer population						Growth (%)	Inhibition (%)
		Number of alive rotifera at 48 hr. (N initial = 1)							
		Repl. a ; e	Repl. b ; f	Repl. c ; g	Repl. d ; h	Mean	Coef.var.		
Controls (dilution medium)		9 5 15	13 10 11	15 12 6	7 9	10.2	33.4%	100%	0%
Sodium Bromate <i>NaBrO₃</i>	400	1 3	1 4	2	3	2.3	51.9%	14.5%	85.5%
	300	3 2	5 4	7	3	4.0	44.7%	32.7%	67.3%
	250	8 6	10	6	5	7.0	28.6%	65.3%	34.7%
	200	7 5	6 4	11	5	6.3	39.5%	58.1%	41.9%
	170	9 8	7 9	10	9	8.7	11.9%	83.5%	16.5%
	140	12 11	6 12	11	11	10.5	21.5%	103%	-3%
	120	6	4	14	11	8.8	52.3%	84.4%	15.6%
	100	12	10	8	6	9.0	28.7%	87.1%	12.9%
	80	10 10	12 8	12	8	10.0	17.9%	98.0%	2.0%
	60	10 8	11 13	6	8	9.3	26.8%	90.8%	9.2%

Remarks :

Conclusions - Comments		Valid assay <input checked="" type="checkbox"/> yes - <input type="checkbox"/> no	
<p>Sodium Bromate NaBrO₃ CAS 7789-38-0 Inhibition of growth : EC₅₀ - 48 hr = 261,7 mg NaBrO₃ / L [95% conf. limits : 221 - 302 mg/L] EC₁₀ - 48 hr = 149,4 mg NaBrO₃ / L [95% conf. limits : 91,5 - 221 mg/L] NOEC (No observed effect concentration) : 170 mg/L LOEC (Lowest observed effect concentration) : 200 mg/L MSD (Minimum statistical difference; % of inhibition) : 33,0 %</p>		<p>Control : (N_{48h} / N_{0h}) ≥ 3 <input checked="" type="checkbox"/> Reproduction for min. 7/8 repl. <input checked="" type="checkbox"/> Ref. CuSO₄.5H₂O [30 - 80 ug/l] <input checked="" type="checkbox"/> Date : 05-07-2015 (<input type="checkbox"/>) EC₅₀-48h. = 27,4 ug/l [18,7 - 39,1 ug/l]</p>	

Macrophyte <i>Lemna minor</i> (OCDE 221; ISO 20079)		Organism : <i>Lemna minor</i> (Ökotox. Inst., Stuttgart; via IFAF) Beaker (80 ml); 3 replicates; 24 ±2°C; 5 Klux Dilution : OECD 221 (modified SIS medium)						Date : 05-08-2015 Done by : SS Controlled by :		
Sample	Concentration [mg NaBrO ₃ / L]	Growth of duckweed population at 7 days				Growth (%)	Dry weight [mg]			Growth (%)
		Number of fronds					Mean			
		Repl. a ; e	Repl. b ; f	Repl. c ; g	Mean	Coef.var.	a	b	c	Mean
Controls (dilution medium)		85 88	83 98	98 89	90.2	7.1%	100%			
Sodium Bromate <i>NaBrO₃</i>	147.8	13	15	12	13.3	8.7%	4.2%			
	78.8	15	12	13	13.3	32.8%	4.2%			
	49.3	18	25	13	18.7	29.9%	10.8%			
	29.6	28	25	28	27.0	69.2%	21.2%			
	14.8	61	53	69	61.0	13.4%	63.6%			
4.93	76	76	67	73.0	5.8%	78.6%				

Remarks : Second variable for growth (dry weight) not measured.

Conclusions - Comments		Valid assay <input checked="" type="checkbox"/> yes - <input type="checkbox"/> no	
<p>Sodium Bromate NaBrO₃ CAS 7789-38-0 Inhibition of growth : EC₅₀ - 7 d = 17,3 mg NaBrO₃ / L [95% conf. limits : 13,9 - 20,4 mg/L] EC₁₀ - 7 d = 5,34 mg NaBrO₃ / L [95% conf. limits : 3,27 - 7,92 mg/L] LOEC (Lowest observed effect concentration) : 4,9 mg/L MSD (Minimum statistical difference; % of inhibition) : 11,0 %</p>		<p>Control : growth N_{final} ≥ 7 x N_{initial} <input checked="" type="checkbox"/> (doubling time T_d ≤ 2,5 j.) Temperature (24 ± 2 °C) : <input checked="" type="checkbox"/> 24,7 ± 0,9 °C pH initial = 6,9 ; pH final = 8,4 variation pH ≤ 1,5 <input checked="" type="checkbox"/></p>	