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STUDY OF MUTAGENIC AND TOXIC ACTIVITY IN WATERS AND SEWAGE EFFLUENTS WITHIN THE GREAT LAKES BASIN

by

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ABSTRACT

Thirty-three water and effluent samples as well as six mud samples were collected from the central, southern and eastern part of the Ontario Great Lakes basin for mutagen and toxicity screening tests. Six samples had toxic activity and six samples indicated mutagenic activity in microbial tests. Summary of techniques and positive sites are presented.

CONCLUSIONS AND SUMMARY

The only water and effluent samples extracted by liquid/liquid procedures showing mutagenic activity at the concentrations tested were the Elmira Sewage Treatment Plant effluents, Don River, and Red Hill Creek samples. All other sampling sites shown in Figure 1 were negative for mutagenic activity.

The presence of toxicants in the water and effluent samples as indicated by the Microtox and Spirillum volutans acute toxicity tests were only found in Cornwall Sewage Treatment Plant effluent, Elmira Sewage Treatment Plant effluent and Canagagique and Red Hill Creek samples.

The results of these studies indicate that it may be possible to simplify the Salmonella typhimurium microsome test by only using three strains TA98, TA1538 and TA1537 as well as only testing samples with the addition of microsomes (S-9 mix). This modification would cut the work load and cost by approximately 50%.

These data provide a broader picture of where potential mutagenic and toxic chemicals may be found in Ontario waters and thus should provide program managers with a guide for priority setting.

It is recommended that a similar but larger study to the one reported here be carried out to provide a more comprehensive coverage of Ontario and other Canadian waters and effluents.

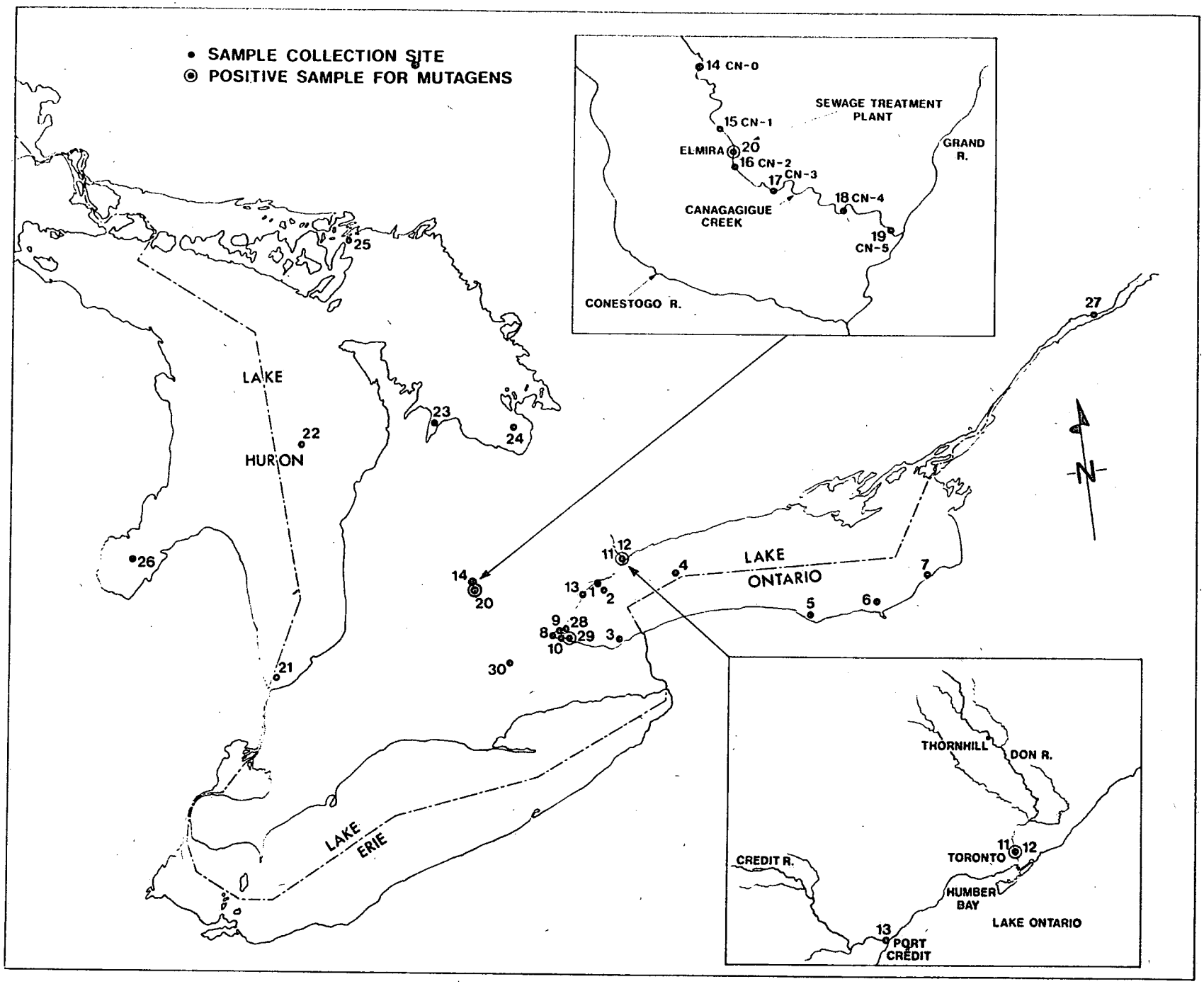
TABLE 1. OFF SHORE SAMPLES TESTED FOR MUTAGENIC ACTICITY

Sample No.	Date Collected	Water or Mud	Location	Latitude	Longitude
1	Apr 1980	Both	Lake Ontario	43°37'24"	79°27'12"
2	Apr 1980	Water	Lake Ontario	43°35'12"	79°23'42"
3	Apr 1980	Water	Lake Ontario	43°13'30"	79°16'18"
4	Apr 1980	Both	Lake Ontario	43°35'48"	78°48'06"
5	Apr 1980	Water	Lake Ontario	43°16'30"	77°35'30"
6	Apr 1980	Water	Lake Ontario	43°28'36"	76°31'36"
7	Apr 1980	Both	Lake Ontario	43°18'48"	77°00'00"
8	Jun 1980	Mud	Hamilton Harbour	43°16'50"	79°52'20"
8a	Nov 1980	Water	Hamilton Harbour	43°16'50"	79°52'20"
9	Jun 1980	Mud	Hamilton Harbour	43°18'16"	79°49'05"
10	Jun 1980	Mud	Hamilton Harbour	43°17'10"	79°48'13"
21	Sept 1980	Water	Lake Huron	43°05'06"	82°24'36"
22	Sept 1980	Water	Lake Huron	44°44'24"	82°03'35"
23	Sept 1980	Water	Georgian Bay	44°42'54"	80°51'42"
24	Sept 1980	Water	Georgian Bay	44°38'38"	80°09'56"
25	Sept 1980	Water	Georgian Bay	45°54'52"	81°35'48"
26	Sept 1980	Water	Saginaw Bay	43°54'35"	83°31'40"

TABLE 2. RIVER AND EFFLUENT SAMPLES TESTED FOR MUTAGENIC ACTIVITY

Sample No.	Date Collected	Sample Type	Location
11	Jul 1980	River Water	Don River upstream from Domtar Paper
12	Jul 1980	River Water	Don River downstream from Domtar Paper
13	Jul 1980	River Water	Credit River above Lakeshore Road Bridge
14	Jul 1980	Creek Water	Canagagique Creek, Elmira
15	Jul 1980	Creek Water	Canagagique Creek, Elmira
16	Jul 1980	Creek Water	Canagagique Creek, Elmira
17a	Jul 1980	Creek Water	Canagagique Creek, Elmira
17b	Jun 1981	Creek Water	Canagagique Creek, Elmira
18	Jul 1980	Creek Water	Canagagique Creek, Elmira
19	Jul 1980	Creek Water	Canagagique Creek, Elmira
20a	Jul 1980	Final Effluent	Elmira Sewage Treatment Plant after chlorination
20b	Jan 1981	Final Effluent	Elmira Sewage Treatment Plant before chlorination
20c	Jan 1981	Final Effluent	Elmira Sewage Treatment Plant after chlorination
27a	Nov 1980	Final Effluent	Cornwall area industry
27b	Nov 1980	Final Effluent	Cornwall area industry
27c	Nov 1980	Final Effluent	Cornwall Sewage Treatment Plant after chlorination
28	Nov 1980	Final Effluent	Burlington Skyway Sewage Treatment Plant after chlorination
29	Dec 1980	Creek Water	Red Hill Creek at mouth
30	Dec 1980	River Water	Grand River at Brantford

Figure 1. Sample collection sites for this study.



SITES OF SAMPLES COLLECTED FOR MUTAGEN TESTS

METHODS

Sample Collection

A total of thirty-three water and six mud samples were collected for mutagen content assesment. The sample evaluation sites are shown in Figure 1 and are listed in Tables 1 and 2. All water samples were collected in 20-L amounts in acid cleaned amber bottles. Sediment samples were collected by shipek sampler and 200 grams (approximately) of surface mud were placed in whirl-pak bags.

Sample Extraction and Concentrations

Sediments

Two hundred grams of sediment were extracted with a 1:1 hexane:acetone mixture in an ultrasonic bath. A first extraction using 400 mL of acetone:hexane was followed by a second extraction using 200 mL. Each extraction was passed through a prewashed celite column. The celite column was washed with 20 mL hexane and and the combined extract and washings were placed in a 1 L separatory funnel. The extract was partitioned with distilled water and back extracted with benzene. The combined organic extracts were dried through sodium sulfate, evaporated to dryness and taken up into 25 mL of DMSO.

Water Samples, Liquid/Liquid Extraction

Base/Neutral

Each 10 L sample was extracted in 2 L increments. The sample (2 L) was poured into a separatory funnel and the pH adjusted to 11 or slightly higher. Each sample was extracted with 60 mL methylene chloride, three times. The combined extracts were dried through a column of anhydrous sodium sulfate and evaporated to dryness on a rotary evaporator at 40°C. The extract was dissolved in DMSO and made up to a final volume of 25 mL.

Acid (Phenols)

Each 10 L sample was extracted in 2 L increments. The water from the base/neutral extraction was pH adjusted with H_2SO_4 to 2. Three 60 mL portions of methylene chloride were used for the extraction and then were dried by passing through a column of anhydrous sodium sulfate. The flask originally containing the methylene chloride extracts was rinsed with methylene chloride and also dried. The combined extracts were evaporated to dryness at 40°C on a rotary evaporator. The extract was dissolved in DMSO, transferred to a volumetric flask and made up to a final volume of 25 mL DMSO.

Flash Evaporation

A rotary evaporator with a 45°C water bath was used to concentrate 200 mL of water sample to 20 mL for toxicity testing via the Microtox and Spirillum volutans tests.

Sample Testing

Mutagen Screening

The Salmonella-microsome procedure for mutagen screening outlined in "Methods for Micrbiological Analysis of Waters, Wastewaters and Sediments" (1978) was used in this survey. S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 were used with positive and negative controls, with and without the addition of rat liver microsomes (S-9 mix). A minimum of three sample volumes (taken from 25 mL DMSO concentrate) were tested, 10, 20 and 200 µL, with the 10 and 20 µL volumes being made up to 200 µL with DMSO. All plating was done in triplicate.

Spirillum volutans Test

Spirillum volutans, a large aquatic bacterium with a rotating fascicle of flagella at each pole, was used to test the samples for toxicity, following a modification of the procedure developed in 1974 by Bowdre and Krieg (Dutka, 1978). The procedure involved pipetting 0.1 mL of Defined Test Medium into 13 x 100 mm tubes, and adding 0.8 mL of the sample plus 0.1 mL of healthy bacteria from an overnight culture. The tube is swirled, a drop removed via Pasteur pipette and placed on a slide and quickly examined under a darkfield or phase contrast microscope (125X). This is the 0-minute reading. Samples from the tube were examined at 5, 10, 30, 60, 90 and 120-minute intervals. If, during any one examination, the reversing motility had been eliminated in more than 90% of the cells, a positive toxic effect was recorded. Negative controls in distilled water were routinely used to ensure inhibition was due to the sample being tested. Positive controls were used only to verify percentages of lost motility in doubtful reactions. Samples tested were 10X flash evaporated concentrate and normal water samples.

Microtox Test

Beckman Instruments, Inc. have devised a test for acute toxicants in water in which specialized strains of luminescent

bacteria (Photobacterium phosphoreum) are used as the bioassay organism. This test is functional because the metabolism of the luminescent bacteria is influenced by low levels of toxicants and, occasionally, stimulants. Any alteration of metabolism affects the intensity of the organisms' light output. By sensing these changes in light output, the presence and relative concentration of toxicants can be obtained by establishing the EC_{50} levels from graphed data; EC_{50} being, in this case, that concentration of toxicant (or dilution of unknown) causing a 50% reduction in light from the base level (Beckman Instruments, Inc., Product Development Bulletin 6964). Bascially, the test involves the addition of luminescent bacteria into a vial of precooled dilutent solution (15°C) and allowed to stabilize. Approxiamtely 15 minutes later, a measured amount of sample or sample dilution is added to this vial, which is then transferred to a light-tight turret where it is exposed to a photomultiplier tube. Total light output is read, usually over a 5, 10 or 15-minute period, from a digital panel meter attached to an accessory recorder. By testing several concentrations of sample, an EC_{50} concentration can be established. Microtox reagent lot No. M006009 was used almost exclusively in this study.

In an attempt to confirm a dose response, the 10X rotary evaporated sample was tested in the following final concentrations: 4.5X, 2.5X and 1X.

RESULTS AND DISCUSSION

In the Salmonella typhimurium microsome test for mutagen activity, two basic criteria can be used to indicate a positive effect, i.e., increase in reversion rate. One of these is that the difference between the control reversion rate and the sample reversion rate must be statistically significant at the 99 percent level. The other, and more frequently used criterion, is that the test sample must show at least 2X the reversions compared to the controls. In this report the latter criterion was used.

Positive results were obtained from the following samples: No. 11, Don River, upstream from the Domtar Paper Co., No. 12, Don River, downstream from the Domtar paper Co., No. 29, mouth of the Red Hill Creek in Hamilton Harbour, No. 20a, final effluent, after chlorination from the Elmira Sewage Treatment Plant collected July 1981, No. 20b, final effluent, before chlorination from the Elmira Sewage Treatment Plant collected January 1981, No. 20c, final effluent, after chlorination from the Elmira Sewage Treatment Plant collected January 1981 (Tables 3a and 3b).

With the exception of Sample No. 11, only Salmonella typhimurium strains TA98 and TA1538 (which is a more sensitive version of TA98) were sensitive to the mutagen effects of the chemicals in the samples tested and all the samples required the addition of microsomes

to the produce the mutagenic effects. The requirement for the microsomes (S-9) could be either to activate the procarcinogens present or to detoxify the sample, allowing the viable cells to react with any primary carcinogens present. Based on the results seen in Sample No. 11, it is possible that either or both processes were taking place.

Sample No. 11 was the only sample where strain TA1537 indicated the presence of mutagenic chemicals as well as giving a positive test in TA1538 and TA1537 without the addition of S-9 mix. It should be noted, that examination of the background lawn of the plates in the 80 mL sample equivalent indicated that we were observing a toxic as well as a mutagenic effect.

Based on previous studies of Hamilton Harbour waters we expected to find significant mutagenic activity in the muds of Hamilton Bay. However, Samples 8, 9 and 10 proved to be negative, perhaps due to inefficient or inappropriate extracting procedures or sample volume or they truly may have been negative.

TABLE 3a. SUMMARY OF WATER SAMPLES GIVING A POSITIVE RESULT (INCREASE IN REVERSION RATE) IN THE SALMONELLA TYPHIMURIUM MICROSOME TEST. DATA ARE REPORTED WITH (+S9) AND WITHOUT (-S9) THE ADDITION OF MICROSOMES

Sample	Concentration Procedure	Equivalent Original Volume	Tester Strains with Mean of Triplicate Counts					
			TA98		TA1538		TA1537	
			-S9	+S9	-S9	+S9	-S9	+S9
11	Base	80	43	103	133[t]	73	180[t]	58
	Extraction	60		124		58		56
		40		123	20	60	6	37
		20		93		35		44
		8	43	68	17	34	13	39
		4	35	56	16	30	13	32
Control	Spontaneous		36	52	20	28	12	18
Control	DMSO	0.2	35	49	19	30	14	24
12	Base	80	55	120	27	67	10	51
	Extraction	8	46	73	20	39	9	25
		4	53	61	21	36	11	32
Control	Spontaneous		36	52	20	28	12	18
Control	DMSO	0.2	35	49	19	30	24	24
29	Base	80	37	115	16	100		
	Extraction	8	29	52	19	42		
		4	42	48	23	37		
Control	Spontaneous		42	57	22	28		
Control	DMSO	0.2	45	48	21	30		
20a	Acid	80			22	100		
	Extraction	40				36		
		20				31		
		8			20	37		
		4			17	36		
Control	Spontaneous				22	28		
Control	DMSO	0.2			21	30		

t = sample toxic to cells.

TABLE 3b. SUMMARY OF WATER SAMPLES GIVING A POSITIVE RESULT (INCREASE IN REVERSION RATE) IN THE SALMONELLA TYPHIMURIUM MICROSOME TEST. DATA ARE REPORTED WITH (+S9) AND WITHOUT (-S9) THE ADDITION OF MICROSOMES

Sample	Concentration Procedure	Equivalent Original Volume	Tester Strains with Mean of Triplicate Counts			
			TA98		TA1538	
			-S9	+S9	-S9	+S9
20a	Base	80	28	119	21	101
	Extraction	40		102		56
		20		84		48
		8	24	51	20	42
		4	28	38	19	40
Control	Spontaneous		42	57	22	28
Control	DMSO	0.2	45	52	21	30
20b	Base	80	39	324	22	380
	Extraction	8	30	85	24	96
		4	39	73	25	29
Control	Spontaneous		38	58	20	28
Control	DMSO	0.2	39	50	17	31
20b	Acid	80			29	77
	Extraction	8			19	40
		4			20	40
Control	Spontaneous				20	28
Control	DMSO	0.2			17	31
20c	Base	80	55	291	33	337
	Extraction	8	36	94	17	72
		4	43	60	16	59
Control	Spontaneous		38	58	20	28
Control	DMSO	0.2	39	50	17	31
20c	Acid	80			46	131
	Extraction	8			21	40
		4			30	33
Control	Spontaneous				20	28
Control	DMSO	0.2			17	31

Toxicity Tests

In testing unconcentrated and even 10X concentrated water samples, it has been found that the majority of the waters in southern Ontario are stimulatory to the organisms used in the Microtox test and enhanced light production occurs. Thus, instead of observing light inhibition which is related to toxicity levels, we observed light stimulation, an event also reported by Chang et al. (1981). Certain compounds at low concentrations such as ethanol are known to stimulate light production. Thus, when testing a sample, if such substrates are present they could be the cause of the light stimulation or they could change the availability of other components, i.e., blocking a potential toxic effect (Nealson, 1970).

Since it is very difficult to obtain an EC_{50} concentration (that concentration causing a 50% reduction in light) under these conditions, we have arbitrarily chosen any sample that inhibits at least 25% of the light as being positive by the Microtox technique. A summary of the positive (toxicity present) Microtox and Spirillum volutans test are shown in Table 4. Table 4 also contains examples of three negative samples; lake, creek and effluent.

When the 25% light inhibition end point is used for the Microtox test there is an excellent correlation with Spirillum volutans acute toxicity test. All samples giving a positive indication of toxicity by the Microtox test (greater than 25% light inhibition) also gave a positive Spirillum volutans test. Similarly, all samples with the exception of Sample No. 29, Red Hill Creek, found to be non toxic by the Spirillum volutans test were also negative for toxic activity by the Microtox test.

Although Sample No. 11, when tested for mutagenic acitivty, showed signes of toxic activity when 200 mL equivalents of sample were tested, no comparable toxic effects were found by either toxicity testing procedure. The answer to this is probably related to the mL equivalents which were tested, 200 by the Salmonella typhimurium procedure, 8 by Spirillum volutans and 4.5 by the Microtox test.

TABLE 4. SUMMARY OF WATER SAMPLES HAVING POSITIVE MICROTOX AND SPIRILLUM VOLUTANS TOXICITY TESTS. THREE NEGATIVE SAMPLES ARE SHOWN FOR COMPARISON

	Microtox Test Percent Light Inhibition After 10 Minutes Incubation			Spirillum volutans 120 Minute Test	
	Sample			Concentration	
Sample	4.5X	2.5X	1X	10X	1X
7	+*21.0	+17.0	+10.0	Negative	Negative
14	10.4	8.9	3.9	Negative	Negative
27c	5.4	3.0	+26.8	Negative	Negative
17a	+9.9	+5.4	44.3	Positive	Negative
20a	36.0	23.0	1.2	Positive	Positive
20b	37.0	39.0	48.0	Positive	Negative
20c	76.0	60.0	25.0	Positive	Negative
27a	74.0	56.0	23.0	Positive	Negative
29	+16.0	+15.0	+17.0	Positive	Negative

+* Light stimulation.

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