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Elimination of Botulinum Neurotoxin (BoNT) Type B from Drinking Water by Small-Scale (Personal-Use) Water Purification Devices and Detection of BoNT in Water Samples

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Seven small-scale drinking water purification devices were evaluated for their capacity to eliminate botulinum neurotoxin (BoNT) type B from drinking water. Influent water inoculated with toxic *Clostridium botulinum* cultures and effluent purified water samples were tested for the presence of BoNT by using a standard mouse bioassay and two commercial rapid enzyme immunoassays (EIAs). The water purification devices based on filtration through ceramic or membrane filters with a pore size of 0.2 to 0.4 μm or irradiation from a low-pressure UV-lamp (254 nm) failed to remove BoNT from raw water (reduction of <0.1 log_{10} units). A single device based on reverse osmosis was capable of removing the BoNT to a level below the detection limit of the mouse bioassay (reduction of >2.3 log_{10} units). The rapid EIAs intended for the detection of BoNT from various types of samples failed to detect BoNT from aqueous samples containing an estimated concentration of BoNT of 396,000 ng/liter.

Data on the purification capacities of various water purification devices and techniques are essential for the assessment of drinking water safety. Water sources and drinking water supply systems can become fecally contaminated but may also be targets for bioterrorism or sabotage (8, 9, 20, 22). Botulinum neurotoxins (BoNT; types A to G) produced by *Clostridium botulinum* and by some other related clostridia are the most potent biotoxins known, and as tasteless and odorless lethal compounds they would generate great concern if weaponized (5, 13, 27).

Several small-scale devices from different manufacturers are commercially available for drinking water purification. These devices, mostly based on filtration through ceramic or membrane filters, are needed especially by soldiers, hikers, or workers of aid organizations operating in primitive wilderness or under disaster conditions (6). Similar filters are also marketed for point-of-use in single households. To ensure consumer safety, it is essential to compare the microbial and chemical purification capacities of different devices through independent evaluation tests (12, 23). Data are available on the purification capacity of some filters, but usually these data are based on the capacity of the filters to remove microbial organisms, e.g., *Escherichia coli*, coliforms, or *Cryptosporidium* oocysts (15, 26, 28). There are some reports in which water purification devices or techniques were tested for elimination of microbial toxins, mainly cyanobacterial toxins (1–3, 17, 18, 25, 29, 33).

Free chlorine at a concentration of 5 mg/liter of water (5 ppm) for 30 min (32) and heating water at 80°C for 30 min (19) were shown to be effective for inactivation of BoNT. Assumptions on the capacity of reverse osmosis to remove BoNT from drinking water have been made (32), but no research to test this capacity has been performed. Reverse osmosis is assumed to eliminate BoNT effectively based of the 150-kDa molecular size of the toxin.

Rapid and sensitive tests are needed for BoNT detection under field conditions as well as for rapid screening of suspect samples (7). At present, the few rapid tests available have not shown sufficient sensitivity or specificity to replace the standard mouse bioassay, which remains the only standard method available for BoNT detection (4, 34). Apart from being time-consuming, the mouse bioassay poses ethical, economic, and safety concerns. Some enzyme-linked immunosorbent assays and enzyme immunoassays (EIAs) are available that show sensitivity similar to sensitivity level of the mouse bioassay (10, 11, 16, 35).

The aim of the present study was to obtain data on the capacity of commercial water purification devices based on various methods to eliminate BoNT from intentionally contaminated drinking water. Furthermore, two commercially available rapid EIAs for BoNT detection were evaluated in comparison to the standard mouse bioassay.

MATERIALS AND METHODS

Inoculated raw water. A total of 70 liters of tap water from the municipal drinking water supply system of Helsinki was stored in an open plastic container for 24 h to reduce the free chlorine concentration. Seven proteolytic *C. botulinum* strains producing BoNT type B (Table 1) were cultured separately in 100 ml of tryptone-peptone-glucose-yeast extract liquid broth medium (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) anaerobically at 37°C for 72 ± 2 h, followed by subculture at 37°C for 16 h. The broth cultures (seven cultures, 100 ml each) were added to the tap water (influent water).

Water purification devices and testing. The water purification devices were selected from among commercially available products based partly on the suitability for field operation. Six devices, representing various types of filters and purification methods, were selected from four manufacturers (Table 2). In addition, an experimental sand filter was developed (Table 2). All devices were portable (weight, <10 kg) and functional without electricity or chemical supple-

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TABLE 1. Proteolytic *C. botulinum* type B strains used in the study

Strain name (alternative name)	Origin	Source[a]
ATCC 7949 (Hegarty 213 B)	Canned onions	ATCC
ATCC 17841 (McClung 1347 B)	NK[b]	ATCC
93/24 (FT243)	NK	IFR
93/36 (4 B)	NK	IFR
M15/18	Soil	DFEH
M18/2	Soil	DFEH
M46/15	Soil	DFEH

[a] ATCC, American Type Culture Collection; IFR, Institute of Food Research, Norwich, United Kingdom; DFEH, Department of Food and Environmental Hygiene, University of Helsinki, Finland.
[b] NK, not known.

mentation. The devices were used manually according to the instructions of the manufacturer. Prior to use, each device was rinsed with 1 to 2 liters of sterile water. A total of 3 liters of purified effluent water was produced with each device from inoculated influent water. The purified effluent water samples were collected in sterile glass bottles for further investigation.

Sampling. From the inoculated influent water the following samples were taken prior to using the purification devices: a 100-ml sample for total aerobic bacterial count; a 200-ml sample for total and free chlorine, conductivity, and pH analyses; and two 200-ml samples for BoNT analyses. From the purified effluent water produced by each device, a 100-ml sample for total aerobic bacterial count, a 200-ml sample for conductivity and pH, and two 200-ml samples for BoNT analyses were taken.

Bacteriological and physicochemical analysis. As a process indicator, the total aerobic count was analyzed by using 3 M Petrifilm aerobic count plates (3M Corp., St. Paul, Minn.) of 1-ml influent and effluent water samples. The plates were incubated at 35°C for 24 ± 2 h. Free and total chlorine were analyzed by using the Spectroquant Colorimeter Picco Cl_2 spectrophotometer (Merck KGaA, Darmstadt, Germany). Temperature (Delta Ohm HD8601P; Padua, Italy), pH (Eutech Cybernetics pHScanWP2; Singapore, Republic of Singapore), and conductivity (HACH Model C0150 conductivity meter; Loveland, Colo.) were measured with portable devices.

BoNT analysis. BoNT was analyzed by using a mouse bioassay (24, 31) with the permission of the State Provincial Office of Southern Finland. Samples from influent water inoculated with toxic *C. botulinum* cultures and purified effluent waters were sterile filtered through 0.45-μm-pore-size bacteriological membrane filters and diluted 10-fold (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}). One undiluted sample from inoculated influent water was heated at 100°C for 10 min to destroy the toxin prior to testing for possible nonspecific reactions in the mouse bioassay. A 0.8-ml volume of each undiluted and diluted sample and of heated sample was injected intraperitoneally into two 20-g laboratory mice. The mice were observed for typical symptoms of botulism for 4 days. The test results were used to estimate the BoNT concentration in the water samples and a 50% lethal dose of 1.2 ng of BoNT type B per kg of body weight was used in this estimation (13).

Two commercial rapid EIAs (Bot Tox *BioThreat Alert* Test Strip, Tetracore, Inc., Gaithersburg, Md., and BADD BoNT Rapid Detection Kit, Osborn Scientific Group, Lakeside, Ariz.) were used to further analyze the undiluted and diluted samples of inoculated influent water and undiluted samples of purified effluent water. The tests were qualitative and based on the use of dye-labeled anti-botulinum toxin antibodies, which in the presence of BoNT were intended to appear as visible colored lines. Both tests were performed according to the manufacturers' instructions. A 0.5-ml volume of sample was diluted with 0.5 ml of the buffer solution provided with the test kits, and a 0.15-ml volume of this dilution was dispensed onto the sample port of the test strips. The tests were interpreted as positive if two colored lines appeared in the test strip within 15 min: one in the test or sample location and one in the control location. The tests were interpreted as negative if only the control line appeared and as invalid if no line appeared at the control location.

Determination of purification capacities. The purification capacities of the devices were calculated as logarithmic ($\log_{10}$) reductions in concentrations of analyzed parameters (total aerobic count, conductivity, and estimated concentration of BoNT) between undiluted inoculated influent water and undiluted purified effluent water samples. The reduction in $\log_{10}$ units was calculated by using the following formula: $\log_{10}$ reduction = $\log_{10}$ (N_i/N_e), where N_i is the concentration in influent water before purification and N_e is the concentration in purified effluent water after purification.

If no aerobic bacteria were detected in the undiluted purified effluent water sample, the $\log_{10}$ reduction was estimated by using an aerobic bacteria count of 1 CFU/ml of sample. If the mouse bioassay was negative for BoNT in the undiluted purified effluent water sample, the maximum concentration of BoNT in the sample was estimated by using the detection limit of the mouse bioassay and the $\log_{10}$ reduction calculated as above.

RESULTS

Two of the water purification devices tested were able to eliminate some or all of the BoNT type B from the inoculated influent water (Tables 3 and 4). The device based on reverse osmosis removed >2.3 $\log_{10}$ units of BoNT to the level below the detection limit of the mouse bioassay, and the experimental sand filter reduced the level of toxin by 0.3 to 1.3 $\log_{10}$ units. The devices based merely on physical filtration through ceramic or membrane filters through 0.2- to 0.4-μm pores were not able to remove BoNT from the inoculated influent water (reduction, <0.1 $\log_{10}$ units), nor was UV irradiation from the low-pressure lamp able to destroy the toxin. All purification devices except for the sand filter reduced the level of total

TABLE 2. Water purification devices tested for elimination of botulinum toxin type B from drinking water

Device (manufacturer)	Purification technique (filter characteristics)[a]	Production capacity[a]	
		During operational hours (liters/h)	During lifetime of device (liters)
Katadyn Combi (Katadyn Products Inc., Wallisellen, Switzerland)	Ceramic filter (pores, 0.2 μm); activated carbon	72	50,000
Katadyn Pocket (Katadyn Products Inc.)	Ceramic filter (pores, 0.2 μm)	72	50,000
Katadyn Survivor MROD-35 (Katadyn Products Inc.)	Reverse osmosis	4.5	NA[b]
WaterWorksII (Mountain Safety Research, Seattle, Wash.)	Ceramic filter (pores, 0.3 μm); activated carbon; membrane (pores, 0.2 μm)	40	NA
Nerox 02 Filter (Nerox Filter Oy, Tampere, Finland/Plastec AS, Gjovik, Norway)	Membrane (pores, 0.4 μm)	5	2,500
Wedeco Aquada 4 (Wedeco AG Water Technology; Düsseldorf, Germany)	Low-pressure ultraviolet lamp (254-nm radiation)	3,870	NA
Sand filter (experimental device)	Sand filtration (0.6–1.2 mm silica sand granules; column height, 20 cm; column diameter, 20 cm)	30	NA

[a] Data according to the manufacturer.
[b] NA, data not available.

TABLE 3. Detection of botulinum toxin type B in *C. botulinum* culture broth, inoculated influent water, and purified effluent waters

Source of sampling	Dilution level	Detection of botulinum toxin type B[a]			Concentration of botulinum toxin in undiluted sample as estimated by mouse bioassay[b] (ng/liter)
		Mouse bioassay (no. of mice surviving/ no. of mice used)	Bot Tox *BioThreat Alert* Test Strip	BADD Botulinum Toxin Rapid Detection Kit	
C. botulinum cultures in TPGY broth[c]	Undiluted	Positive (0/2)	Negative	Negative	396,000–<598,500
Influent water inoculated with *C. botulinum* cultures, heated (100°C, 10 min)	Undiluted	Negative (2/2)	Negative	Negative	<30
Influent water inoculated with *C. botulinum* cultures	Undiluted	Positive (0/2)	Negative	Negative	3,960–<5,985
	10^{-1}	Positive (0/2)	Negative	Negative	
	10^{-2}	Positive (0/2)	Negative	Negative	
	$10^{-2.12}$	Positive (0/2)	ND	ND	
	$10^{-2.30}$	Negative (2/2)	ND	ND	
	$10^{-2.60}$	Negative (2/2)	ND	ND	
	10^{-3}	Negative (2/2)	Negative	Negative	
Effluent from Katadyn Combi (Katadyn Products Inc. Wallisellen, Switzerland)	Undiluted	Positive (0/4)	Negative	Negative	3,000–<30,000
	10^{-1}	Positive (0/4)	ND	ND	
	10^{-2}	Positive (0/4)	ND	ND	
	10^{-3}	Negative (4/4)	ND	ND	
Effluent from Katadyn Pocket (Katadyn Products Inc.)	Undiluted	Positive (0/4)	Negative	Negative	3,000–<30,000
	10^{-1}	Positive (0/4)	ND	ND	
	10^{-2}	Positive (2/4)	ND	ND	
	10^{-3}	Negative (4/4)	ND	ND	
Effluent from Katadyn Survivor MROD-35 (Katadyn Products Inc.)	Undiluted	Negative (4/4)	Negative	Negative	<30
	10^{-1}	Negative (4/4)	ND	ND	
	10^{-2}	Negative (4/4)	ND	ND	
	10^{-3}	Negative (4/4)	ND	ND	
Effluent from WaterWorksII (Mountain Safety Research, Seattle, Wash.)	Undiluted	Positive (0/4)	Negative	Negative	3,000–<30,000
	10^{-1}	Positive (0/4)	ND	ND	
	10^{-2}	Positive (0/4)	ND	ND	
	10^{-3}	Negative (4/4)	ND	ND	
Effluent from Nerox 02 filter (Nerox Filter Oy, Tampere, Finland/Plastec AS, Gjovik Norway)	Undiluted	Positive (0/4)	Negative	Negative	3,000–<30,000
	10^{-1}	Positive (0/4)	ND	ND	
	10^{-2}	Positive (0/4)	ND	ND	
	10^{-3}	Negative (4/4)	ND	ND	
Effluent from Wedeco Aquada4 (Wedeco AG Water Technology, Düsseldorf, Germany)	Undiluted	Positive (0/4)	Negative	Negative	3,000–<30,000
	10^{-1}	Positive (0/4)	ND	ND	
	10^{-2}	Positive (0/4)	ND	ND	
	10^{-3}	Negative (4/4)	ND	ND	
Effluent from sand filter (experimental device)	Undiluted	Positive (0/4)	Negative	Negative	300–<3,000
	10^{-1}	Positive (0/4)	ND	ND	
	10^{-2}	Negative (4/4)	ND	ND	
	10^{-3}	Negative (4/4)	ND	ND	

[a] Bot Tox *BioThreat Alert* Test Strip (Tetracore, Inc., Gaithersburg, Md.); BADD Botulinum Toxin Rapid Detection Kit (Osborn Scientific Group, Lakeside, Ariz.); ND, not done.

[b] Concentration estimated by using the result of the mouse bioassay at various dilutions when 0.8-ml volume of sample was injected intraperitoneally into 20-g mice and by using an estimate of 1.2 ng/kg of body weight as the 50% lethal dose of botulinum toxin for mice.

[c] A mixture of seven toxic *C. botulinum* type B strains (Table 2) enriched in tryptone-peptone-glucose-yeast extract (TPGY) broth anaerobically at 35°C for 72 ± 16 h.

aerobic count from the inoculated water by >3.3 $\log_{10}$ units (sand filter, reduction by 0.8 $\log_{10}$ units). The device based on reverse osmosis was the only device able to reduce conductivity during the purification process (reduction by 1.6 $\log_{10}$ units). The total aerobic count in the inoculated influent water was 2,000 CFU/ml of water, conductivity was 322.2 μS/cm, and the concentration of free and total chlorine was <0.01 mg/liter.

The results for detection of BoNT with the standard mouse bioassay and commercial rapid tests are presented in Table 3. The BoNT concentrations were extrapolated from the mouse bioassay results to be in the range of 3,960 to 5,985 ng/liter in undiluted inoculated influent water and 100-fold higher in the tryptone-peptone-glucose-yeast extract broth used for inoculating the influent water. Both commercial rapid EIA kits determined that all the samples that were positive in the mouse bioassay were negative for BoNT. All samples negative in the mouse bioassay were also negative in the rapid EIAs.

DISCUSSION

The testing of the water purification devices produced information crucial to the assessment of drinking water safety and security. Based on the present study and some suggestive results from earlier studies (18), the only technique available for portable devices capable of eliminating BoNT from drinking water is reverse osmosis. To some extent sand filtration could be effective in reducing BoNT concentration, as suggested here as well as in studies on microcystin removal, but

TABLE 4. Purification capacities of seven drinking water purification devices

Device (manufacturer)	Reduction in log_{10} units during purification		
	Conductivity	Total aerobic count (35°C, 24 h)	Botulinum toxin type B
Katadyn Combi (Katadyn Products Inc., Wallisellen, Switzerland)	<0.1	>3.3[a]	<0.1
Katadyn Pocket (Katadyn Products Inc.)	<0.1	>3.3[a]	<0.1
Katadyn Survivor MROD-35 (Katadyn Products Inc.)	1.6	>3.3[a]	>2.3[b]
WaterWorksII (Mountain Safety Research, Seattle, Wash.)	<0.1	>3.3[a]	<0.1
Nerox 02 Filter (Nerox Filter Oy, Tampere, Finland/Plastec AS, Gjovik, Norway)	<0.1	>3.3[a]	<0.1
Wedeco Aquada 4 (Wedeco AG Water Technology, Düsseldorf, Germany)	<0.1	>3.3[a]	<0.1
Sand filter (experimental device)	0.0	0.8	0.3–1.3

[a] Not detected in purified effluent water, and the reduction in log_{10} units was estimated by using a count of less than one particle in a 1-ml sample.
[b] Not detected in purified effluent water. Reduction in log_{10} units was estimated by using the concentration of botulinum toxin in a sample less than the detection limit of the mouse bioassay used.

this most probably is strongly dependent on the thickness of the sand bed and properties of the sand used (21, 25).

In the present study, the 254-nm UV irradiation produced by the low-pressure lamp was not able to degrade BoNT in the water. Some studies indicated that direct sunlight can degrade BoNT by 90% in 1 h and 100% in 3 h (30). The degradation process probably required broad-spectrum UV irradiation coupled with oxidative spectrum produced by high-pressure UV lamps. Activated carbon combined with filtration through ceramic filters did not affect toxin removal in this study. A single previous study showed activated carbon to be effective against BoNT type A in water samples (14), but the removal was apparently due to the amount and type of activated carbon used as well as to the flow rate of the water and contact time with the carbon. In the present study, the activated carbon was either in the form of a thin layer or in a relatively small cartridge. Taking into account the purification techniques of individual filters, the results of aerobic bacteria removal and reduction in conductivity coincided with their theoretical purification capacities and with the results of earlier studies on some other drinking water purification filters (18, 28).

The rapid EIAs for detection of BoNT showed poor performance compared with the results of the standard mouse bioassay. Even the broth with toxic *C. botulinum* cultures at estimated BoNT concentrations of 396,000 to 598,500 ng/liter appeared to be negative for BoNT when evaluated with the rapid tests. It can be estimated from the toxicological data that only 1.2 to 1.8 ml of this broth constitutes the oral lethal dose for a 70-kg human being (4, 13, 27). The failure to detect BoNT is unexpected, since the estimated concentration of BoNT in this test was similar to or higher than the detection limits of the tests reported by the manufacturers. The intentional contamination of drinking water and water supply systems will apparently result in concentrations remarkably lower in distributed drinking water than in pure bacterial culture (8). However, the total intake of the toxin can still cause symptoms or death due to the total amount of water ingested. Therefore, the usefulness of these rapid tests is very limited due to their high detection limit and failure to detect lethal concentrations of toxin in drinking water. The negative test results will be misleading and may result in casualties if BoNT is intentionally released into drinking water supplies.

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