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Membrane Filter and Emergency Water Supply Control

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Though a gala occasion created this sanitary emergency, the measures put together on the spot—and applied so successfully to assure a safe potable water supply under unusual and hazardous conditions—will recommend themselves to any health department suddenly confronted with a potential epidemic created by disaster, whether man-made or natural.

✣ Americans came to their Capital for the January 20, 1953, Presidential Inaugural celebration by every means of modern transportation. Particular interest from a public health engineer's viewpoint centered about some 8,000 of them who lived in the railroad yards. In attempting to meet the tremendous housing demand imposed on the District of Columbia during this period, the Baltimore and Ohio and Pennsylvania Railroads planned special "hotel" trains to accommodate organized groups. The groups (such as state delegations or veterans' clubs) lived aboard the same sleeping cars that transported them to and from the District. As it finally emerged, "Pullman City" consisted of 299 sleeping cars, parked in five "boroughs" located in as many railroad sidings scattered throughout the District. It was necessary to use freight sidings, despite their lack of sanitary facilities; the passenger terminal had to be free of parked cars in order to meet the tremendous transportation problem. Pullman City was in existence from January 17 through January 23.

During its brief existence, Pullman City posed serious problems for the District of Columbia Health Department. Many months in advance, the Bureau of Public Health Engineering began planning protective measures to insure the adequate and safe handling of water, ice, sewage, garbage, and rubbish. Because some unique approaches were made in the handling, protection, and control of the water supplied to the railroad cars, it is believed that this account will be of interest and value to persons in water supply work and other public health fields. The outstanding feature of the entire operation was the first reported, emergency field application of the membrane filter ever made in this country.

Remoteness of connections to the municipal water supply dictated the use of street-flusher tank trucks to carry water from fire hydrants to three of the yards. Water tanks of the railroad sleeping and dining cars were filled directly from the trucks.

At the other two yards, temporary canvas and rubber pipe lines were laid to carry city water from nearby hydrants. These lines paralleled the tracks supporting the cars to be serviced. Hose bibs were tapped in these lines at intervals, to permit the watering of each car by portable hose.

Both of these methods required "handling" of the municipal water, ex-

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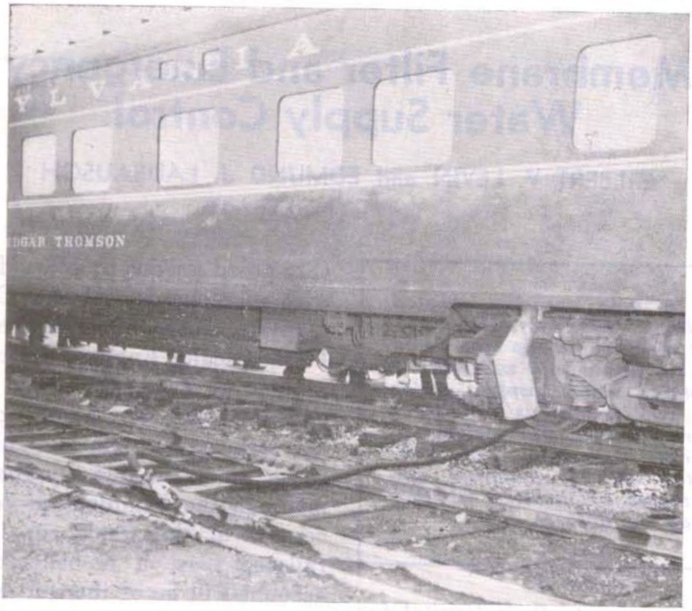


FIGURE 1—Temporary pipe line and hose watering railroad car. Note soil can directly above hose

posing it to serious potential contamination hazards. These hazards were very real, in that sewage was an ever-present factor. Watering operations had to be carried out, and the water loaded in the cars, within two or three feet of soil cans used to collect the sewage from the cars (Figure 1). Although collection and handling of the sewage were carefully planned, it would be folly to assume that no sewage would be spilled on the ground or cars, or even on the water hose and pipe lines. Together with contamination factors which are always present when handling water by temporary means, this condition presented all the ingredients of a serious epidemic. To assure maximum protection to the visiting public, the Bureau of Public Health Engineering carefully planned and supervised the handling procedures, and prepared the following instructions which were distributed to the responsible parties:

Procedure for Sterilizing Water Tank Trucks

Calcium hypochlorite (70 per cent available chlorine) shall be used as the sterilizing agent; chlorine strength of the sterilizing solution shall be 100 ppm.

1. Flush truck with fresh city water (discharging through all outlets).
2. Drain truck and close all outlets.
3. Sterilize all removable fittings and filling hose for one hour in 100-ppm chlorine solution.
4. Replace fittings and add required amount of calcium hypochlorite as powder or in concentrated solution (three pounds for 2,600-gallon flusher) and fill flusher with city water.
5. Circulate this water through flusher by sterilized hose from right front truck outlet back to tank for one hour.
6. Stop circulation and allow to stand for four hours.
7. Again circulate water in same manner for one hour.
8. Drain flusher completely through all outlets.
9. Flush truck two complete times through all outlets.
10. Close all outlets and fill truck with fresh city water, and flusher is ready for use.

This operation should be done as nearly as possible to the time of use of the truck. Steps 8, 9, and 10 should not be taken more than one day before such time. Use only right front outlet of flusher for all railroad car filling operations. Keep all other outlets closed tightly at all times after sterilization. The nozzle on the right outlet shall be covered with a tight-fitting (rubberband or tape), clean, clear, plastic bag at all times when it is not in use. The fitting on the truck-filling hose also shall be so protected from the time that it is sterilized. Similar protection shall be afforded the fittings of the hose used to fill the railroad cars.

All portable water hoses used to water railroad cars from hydrants or temporary manifolds shall be protected at both ends by plastic bags tightly fitted, and shall be stored off the ground when not in use. In the yards where temporary hose is connected and used as a pipe system, care shall be taken so that the location of the hose couplings shall be staggered in relation to the car soilhoppers, in order to prevent sewage from splashing on the couplings. Hose connections for temporary pipelines shall be elevated on special wooden blocks which will keep the connection six inches off the ground. Every connection shall be protected by clear, plastic wrapping extending six inches each side of the fitting. When hose is connected, it shall be sterilized by flushing entire system with 100-ppm chlorine solution from filled 2,600-gallon flusher. System shall then be flushed with city water from truck, and all exposed fittings covered with clear, plastic bags to remain in place except when in actual use.

To insure some residual during all handling operations, it was also required that 1 ppm of chlorine dosage be added to the water in the tank trucks, despite the 0.2 to 0.4 ppm normally present in the municipal water supply.

To confirm and maintain the effectiveness of the aforementioned precautions, an extensive program was planned. Chlorine residuals were to be checked throughout the handling operations and at the taps in the cars. Frequent bacteriological tests were planned as the mainstay of the sampling program. Here, the great shortcoming of the Standard Methods for the Examination of Water and Sewage tube test for coliform organisms became evident. From 48 to 96 hours would be required for the completion of the test, during which time the water would constantly be consumed. Some of the trains would have departed before the bacteriological results were known! An ideal and challenging situation was thus created for the application of recently developed membrane-filter technics, which could reduce to less than one-third the time elapsed between sampling and reporting of results. It was believed that valuable field experience could be gained by using this new tool under emergency water supply conditions comparable to those which might exist in any large city during disaster or attack.

In view of the twofold objective of protecting the Inaugural residents of Pullman City and of testing the membrane filter under emergency field conditions, the District of Columbia Health Department requested the Public Health Service to participate in the investigations. The Environmental Health Center and the Division of Sanitation cooperated by supplying the necessary equipment and assigning a sanitary engineer who had participated in recent MF research and developmental work at the center, and other professional personnel.

It was agreed that, in order best to accomplish these objectives, each sample would be tested by both the conventional, fermentation-tube MPN method and the newer MF method. In this way, bacteriological control would be established, and it would be possible both to obtain the benefits of the quick results afforded by the membrane filter and to determine its suitability as a single control test under unusual or simulated emergency conditions.

Sample Collection and Methods of Handling

Samples were collected in sterile, wide-mouthed, glass-stoppered bottles, of approximately 250-ml. capacity, that had been seeded with a few crystals of sodium thiosulfate in the usual manner. Pending the collection of samples, all bottles were protected with tight-fitting paper covers and were properly stored.

Sanitary inspectors, when collecting the samples, usually accumulated about 20 before returning to the laboratory. No special preservation measures were employed as, in most cases, analyses were started within two to three hours after collection. Where a backlog of routine laboratory work prevented this, samples were stored temporarily in a refrigerator.

During a three-day period commencing January 18, 207 samples were collected at representative points in each of the distribution systems, flusher trucks, and temporary pipe lines. Just after removal of the 51-ml. sample required for MPN and agar pour-plate tests, 100 ml. were withdrawn for the membrane-filter test.

Laboratory Procedures

Total Count

Standard agar plate counts were observed for each sample, incubated for 24 ± 2 hr. at 37° C.

Tube Tests

Limitations in availability of media, laboratory equipment, incubator space, and labor restricted the number of fermentation tubes to not more than five per sample. The majority of water samples to be examined were expected to contain no, or only a moderate amount of, pollution; therefore, presumptive coliform testing was chosen, to consist of inoculation of five 10-ml. portions of each sample in lactose broth.

All presumptive gas-positives (those tubes showing any amount of gas in 48 hours upon incubation at 35° C.) were confirmed in 2 per cent brilliant green bile. The EMB streak-plate technic was used to supplement the liquid-confirmatory procedure. The examinations were not carried out to the completed stage.

Membrane-Filter (MF) Tests

The wide use of membranes for bacteriological analyses by the Germans during World War II was largely necessitated by the critical shortages of laboratory equipment and agar. The results of their work stimulated interest in this country in postwar years, leading to the successful application, simplification, and improvement of this technic.[1-5] Basically, the method entails the deposition of organisms on the surface of a thin, highly-porous disc (membrane), and their subsequent surface growth by the utilization of liquid nutrients diffusing upward through the membrane pores, the axes of which are normal to the plane of the disc. The domestic membranes previously described[4] were used for these investigations.

Prior to use, the membranes had to be sterilized. Ethylene oxide has been used for this purpose in the past, but more recent studies by the Public Health Service have indicated that, when properly executed, heat sterilization can be employed, apparently without seriously affecting the unique characteristics of the filter membrane.[4] For this project, about 12–15 membranes, individually separated by paper blotters, were placed in standard Petri dishes and autoclaved for 10 minutes at 10 pounds per square inch (psi) (115.5° C.). Steam was then exhausted from the autoclave to prevent damage of the membrane.

The nutrient pads were prepared prior to the actual sampling by placing them in a large beaker of distilled water and autoclaving for 15 minutes at 15 psi. They were subsequently drained, dried in an oven at 80 to 90° C., and aseptically stored in sterile containers until needed. This simplified procedure replaced the one previously described,[1] which was used when only inferior quality pads were available.

The filter apparatus used for this investigation was of a commercially available type, and was prepared and employed essentially in the manner described by other investigators.[1, 5] All filtrations were performed in the District of Columbia laboratory, where vacuum lines were available to facilitate sample filtration. Two filtration assemblies were used simultaneously. At the start of each day's operations, each filter-holder apparatus was individually wrapped in Kraft paper and autoclaved for 15 min. at 15 psi. Between individual sample filtrations, the vital parts were rinsed (with the membrane still in place) with sterile phosphate buffer dilution water (about 100 ± ml.) to wash away and deposit on the membrane any specimen droplets retained on the inner walls of the receptacle. When properly executed, the carry-over of contaminants in this procedure is insignificant for ordinary examinations. The inoculated membrane was then aseptically removed from the support.

Sample portions of 100 ml. were filtered in all cases for the following reasons: (1) it was anticipated that the high disinfectant dosage rates would yield samples with low bacterial counts; (2) sufficient graduates to handle the number of samples expected were lacking and clean, sterile, standard dilution-blank bottles, marked at 99 ± ml., were available in sufficient number to assure for each sample an accurate measurement, aseptically accomplished; (3) it was believed that this quantity would be sufficient to detect the relatively small contamination anticipated, while, on the other hand, the portion would not be large enough to produce overgrowth on the membranes.

The technic employed for coliform analyses was that developed at the Environmental Health Center. This procedure minimizes the shock effect of the inhibitory reagent, and permits superior coliform recovery. Essentially,

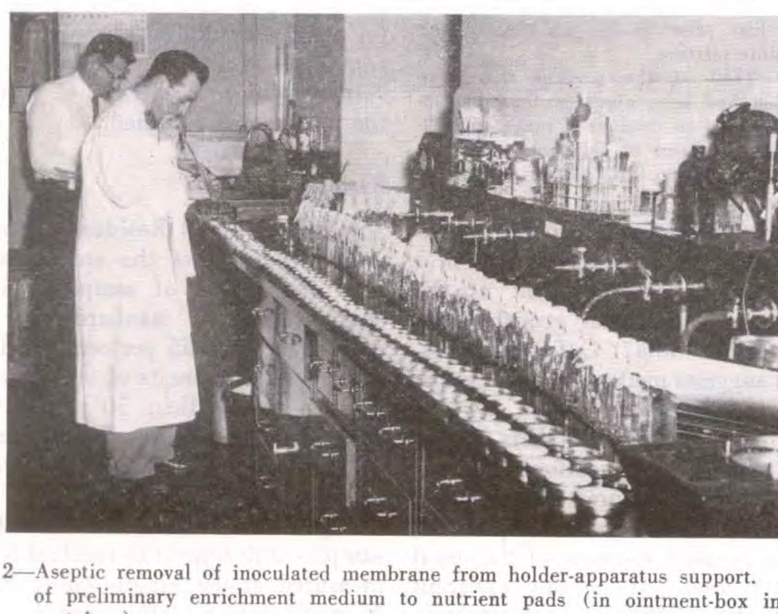


FIGURE 2—Aseptic removal of inoculated membrane from holder-apparatus support. Addition of preliminary enrichment medium to nutrient pads (in ointment-box incubation container)

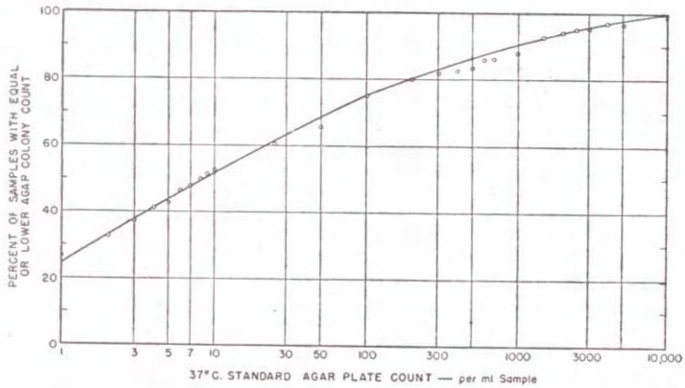


FIGURE 3—Distribution of samples in relationship to total colony counts

it consists of a 2-hour incubation on Difco's dehydrated M-enrichment medium, and 16 ± hours of additional incubation on modified EHC Endo broth prepared as described by Kabler,[3] Clark, et al.,[1, 2] with the following modifications later developed and adopted at EHC:

1. The lactose solution was prepared separately, autoclaved for 10 min. at 10 psi, and added to the basal medium just prior to use. It was added in the same proportion (3 ml. of 20 per cent lactose to 30 ml. of basal medium) just prior to the addition of the fuchsin-sulfite mixture.

2. Only 7/10 of the average dye-sulfite ratio, which had been observed through biological titration [4] to produce a range of optimum results in terms of coliform production and sheen characteristics, was used.

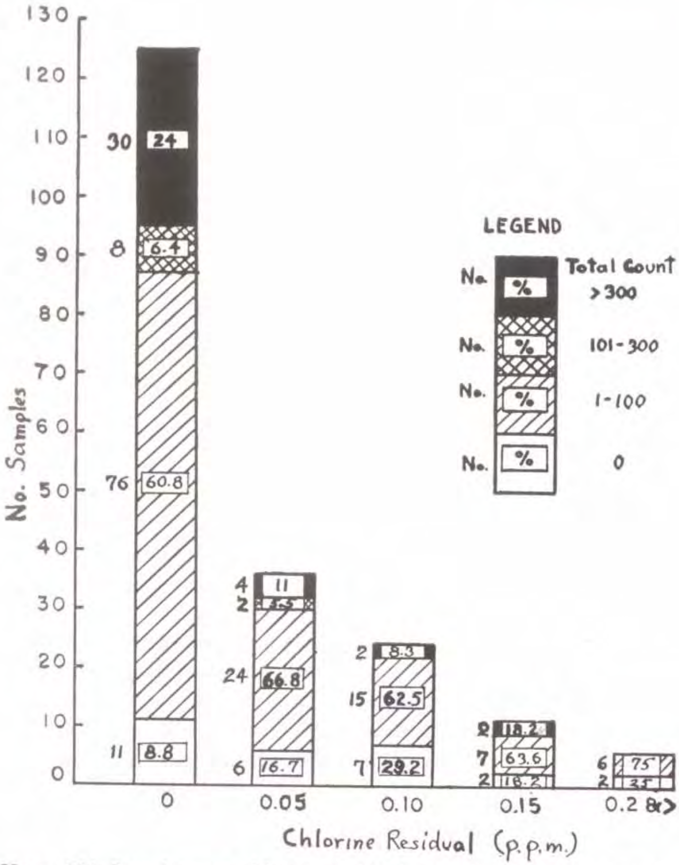
The literature [5] indicates the desirability of incubating the membranes in a highly-humidified environment at the temperature of optimum growth — approximately 35° to 37° C. for coliforms, and also suggests methods by which this can be accomplished. Work by the Public Health Service and others in connection with field applications of membrane filtration (in press) has shown that a tin box (ointment tin or pill box, e.g.) can be used in place of the usual glass Petri dish without any apparent toxic effect, reduction in quantitative coliform recovery or degradation in sheen characteristics. Two-ounce tin boxes were used, and yielded satisfactory results—Figure 2. These containers have other advantages—they are inexpensive and readily available, and can be autoclaved. Moreover, they can be used in the conventional-type, hot-air, 35° C. incubator available in the laboratory, since they are sufficiently tight to prevent evaporation of the nutrient broth and drying of the membrane, and to retain sufficient moisture for optimum culture environment. These containers were simply stacked in an inverted position in the 35° C. incubator for the periods specified.

Results

Total Counts and Residual Chlorine

Figure 3 shows the cumulative per cent distribution of samples, with respect to 37° C. standard agar plate counts. Almost 25 per cent of all samples tested had counts of one or less per ml., and more than 50 per cent had counts of 10 or less. The upper limit of total count was recorded for two samples at 12,000 colonies per ml.

Figure 4 shows the distribution of samples with respect to residual chlorine measured at the sample collection site. More than half of the samples had no



Note: 204 Samples—no chlorine residual recorded for three samples

FIGURE 4—Chlorine residual—total count.

residual chlorine, and less than 10 per cent had a residual in excess of 0.1 ppm. Practically all samples with a colony count in excess of 300 per ml. had no, or merely a trace of, chlorine when collected.

MPN and MF Tests

Table 1 shows the results obtained by each of the various laboratory technics employed. Only 11, out of 207 samples tested, produced any gas in lactose within 48 hours. Of these, only one (sample 110) was positive within 24 hours, with all of the five 10-ml. portions showing a rapid rate and large amounts of gas production. Within 48 hours, three of the 11 samples were confirmed in brilliant-green lactose bile and on eosin methylene-blue agar.

By the filter method, two of the 207 samples developed colonies having a metallic sheen characteristic of coliforms and intermediates on the differential medium; these were also positive for coliforms by the MPN method. Approximately 120 well defined surface colonies with excellent sheen characteristics were counted on the membrane upon which the 100-ml. portion of sample 110 had been concentrated. Since the corresponding MPN was indeterminate, quantitative comparison could not be made. Those colonies with

Table 1—Postive Laboratory Results Obtained by Various Analytical Procedures Employed

Sample Number	D.C.* Water	Cl_2 Res. ppm	37° C. Standard Agar-plate Count	MPN † 48 hr. Presump.	MPN 48 hr. Conf.	MF ‡ Count	EMB ** Colony Type
			per ml.		per 100 ml.		
102	No	0.05	250	16	..	0	
110	No	0.0	2,100	16+	16+	120	Typical aerogenes
161	No	0.05	9,000	2.2	..	0	
163	No	0.05	46	9.2	2.2	1	Typical coli
166	No	0.05	250	2.2	..	0	
167	No	0.05	3,600	2.2	..	0	
176	No	0.15	51	9.2	..	0	
217	Yes	0.05	8	2.2	..	0	
263	Yes	0.05	0	2.2	2.2	0	Atypical aerogenes (?)
277	Yes	0.05	3,000	5.1	..	0	
305	Yes	0.0	100	2.2	..	0	

* D.C. Water = Water loaded on railroad cars in the District of Columbia as opposed to water already in cars upon arrival.

† MPN 48 hr. Presump. = Most probable number of coliform organisms per 100 ml. of sample as determined by Standard Methods for the Examination of Water and Sewage.

‡ MF Count = Number of coliform organisms per 100 ml. of sample indicated by the membrane filter method of analysis.

** EMB Colony Type = Probable type of bacterial colony as identified by culturing on eosin methylene-blue agar plates.

sheen were observed to be capable of fermenting lactose and BGB with gas production, and exhibited characteristics typical of coliforms when cultured on EMB agar plates. Two replicate 100-ml. portions of sample 163 with a MPN of 2.2 were found to contain a single coliform each by this procedure.

The two cases cited were the only ones for which coliform-like colonies developed on the membrane. Sample 263 (with a MPN of 2.2 per 100 ml.), the only local sample suspected of containing coliforms, failed to produce any coliform-like colony within 24 hours by the filter method.

Field Observations

Three Health Department inspectors were assigned to collect the water samples for bacteriological analysis from taps in the cars, the flusher trucks, the filling hoses, and the temporary pipe lines. On-the-spot chlorine residual determinations were made in each case.

Where the temporary pipe lines were laid, it was noted that metal discs welded to the hose couplings were used as substitutes for wooden blocks to elevate the couplings above the ground. These discs were too small and, for effective use, it is recommended that the discs be at least 8 in. in diameter.

The protection offered by the plastic bags on the couplings and fittings seemed well worth while. It is believed that this innovation not only provided a marked degree of physical protection to the pipe and hose lines, but (probably equally important) considerably impressed the laborers carrying out the operations with the care required for water-handling. Some of the covers, however, did not fit tightly enough.

Chlorine-residual tests made on the trains shortly after fresh water had been loaded rarely showed any residual, despite the 1-ppm. dose applied to the flusher tank-trucks, indicating that the car tanks contained materials exerting a high chlorine demand. The addition of chlorine to the city water was well warranted; it is believed that the dosage might even be increased to 1.5 or 2.0 ppm in the future.

The value of the membrane filter was

excellently illustrated in the case of sample 110. This sample was collected from a Pullman car on January 18 at 11:40 a.m. At 8.00 a.m. on the morning of the 19th, it was quantitatively reported as coliform-positive by the membrane-filter test. Actually, coliform colonies could have been detected several hours sooner, perhaps within 12–15 hours after the sample had been filtered. Railroad officials were immediately called and, within two hours, the car tank from which the sample had been drawn was emptied, disinfected with chlorine solution, flushed, and refilled. It was not until the morning of January 20 that the Standard Methods confirmed test verified the contamination.

Discussion

It is believed that the foregoing sanitary precautions, laboratory procedures, and discussions have greater general significance than that attached to the particular instance of use reported here. This wider field of application might well encompass natural disasters to populated areas, civilian defense, and military field use. In supplying water under such emergency conditions, it is necessary to insure the safety of the supply with a minimum of misdirected or unneeded precautionary or control measures. The precautionary sanitary measures used in the Pullman City water supplies were based on what was considered to be sound engineering practice and judgment. Further experimentation with modified sanitary precautions that can be readily evaluated by use of the membrane filter could be used to develop minimum sanitary practices consistent with satisfactory, emergency water-quality control.

Relatively few people were required to furnish and control a wholesome supply of drinking water to approximately 8,000 persons temporarily housed on trains. With the considerable amount of rolling stock in and near all major cities in the United States, a speedy method of providing emergency shelter to civilians made homeless by natural or military disasters is indicated. It seems entirely feasible that, with the use of the membrane filter, small compact laboratories that could be carried on a man's back would be capable of providing bacteriological control over the quality of drinking water provided by makeshift means. The highly satisfactory sanitary results afforded by the exercise of relatively simple precautions indicated that, given a safe source of drinking water, the dangers of contamination associated with the transportation, handling, and storage of that water to provide emergency supplies are readily surmountable.

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