ORAL REHYDRATION SALTS (ORS)

WATER DISINFECTANT RESEARCH

Submitted to:

U.S.A.I.D.

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ORS WATER DISINFECTANT RESEARCH

BACKGROUND

Need for Disinfectants

Oral rehydration therapy saves the lives of many children who suffer from dehydration due to diarrhea (1). While its therapeutic value is unquestioned, there are potential hazards in the preparation and use of oral rehydration solutions in developing countries. One of these problems is the proliferation of pathogenic organisms in oral rehydration solutions (ORS) prepared with contaminated well water (1, 2). Microbial growth in ORS is promoted by two essential conditions: a microbial source (typically, contaminated well water or an unclean storage container) and a nutrient source (provided by glucose and salts of the ORS and organic matter present in well water). Microbial growth in ORS preparations can yield populations in excess of 10(5) bacteria per ml within 24 hours after initial preparation.

The contamination of ORS with undesirable microbes may result in reinfection or prolongation of the diarrheal symptoms. ORS preparations are commonly used during a period of 24 hours or longer to treat an ill child. It is generally recommended that ORS preparations be discarded after 24 hours. Mothers or care-givers, however, are likely to keep the solution for longer periods rather than discard it due to its cost and the inconvenience of making a fresh preparation.

The goal of this project was to identify an ORS disinfectant with the ability to kill microorganisms in typical village water sources. The proposed disinfectant would be packaged with the ORS components and would kill these contaminants within an hour of reconstitution with water.

Prior Research

Previous studies have examined methods to prevent the outgrowth of microbes in ORS preparations. Aluminum potassium sulfate (potash alum) has been shown to inhibit bacterial growth at levels of 0.05 to 0.1% in ORS (3). Exposure of prepared ORS to sunlight is another disinfection method which has been examined, using transparent containers to allow penetration of UV, thereby inhibiting bacterial growth (4). However, potential chemical disinfectants for ORS have not been thoroughly examined for their safety, effectiveness, and cost.

Disinfectants Evaluated

Potential disinfectants were selected following literature searches and discussions with Dr. Neil McCormick at the U.S. Army facility, Natick, Massachusetts. Laboratory tests were initially conducted in the U.S., using the following potential ORS disinfectants:

1. Aluminum hexacarbamide sulfate triiodide (AHST)

   (source: ICN, catalog # 203474)

AHST is used for decontamination of drinking water in emergencies, at levels sufficient to yield 8 ppm of active iodine. Typically it is sold in combination with talc as a tablet for water purification. At this concentration, the iodide component contributes an off-flavor to the water.

2. Lactoperoxidase + glucose oxidase + sodium thiocyanate

   (source: Fina Research, Belgium)

Lactoperoxidase is one of the protective proteins found in mammalian milks where it acts together with thiocyanate and hydrogen peroxide (provided by white blood cells) to inactivate a variety of bacteria. The commercial source of lactoperoxidase is bovine milk. This Fina Research product includes the required thiocyanate plus glucose oxidase, which

generates hydrogen peroxide in the presence of glucose. The commercially available lactoperoxidase is a proposed food additive used to control spoilage. For example, in a cottage cheese experiment, 30 ppm lactoperoxidase inhibited outgrowth of three types of bacteria. Effects on palatability were not recorded. Although lactoperoxidase toxicity is not a problem because it is a natural protein, thiocyanate is not presently permitted as a food additive.

3. Aluminum potassium sulfate dodecahydrate (alum)

(source: Aldrich Chemicals, catalog #23,708-6)

Alum is a GRAS (generally regarded as safe by the FDA) food additive used as a firming or neutralizing agent. It is odorless with a sweet taste. There are no side effects or contraindications to use.

4. Lauricidin (LC)

(source: Lauricidin, Inc., Galena, Illinois)

Lauricidin is a monoglyceride found naturally in mother's milk which is commercially available from coconut oil processing. It is currently used topically to treat animal skin diseases but is nontoxic for animals when ingested orally, even at 25% levels. There are no known side effects. Lauricidin inactivates some viruses and bacteria and has a bitter flavor.

5. Dichloroisocyanuric acid (DCIC)

(source: Aldrich Chemicals, catalog #21,892-8)

DCIC has been examined in rats and dogs, who were unaffected by the intake of up to 333 ppm daily in the diet for six months. Rats were also unaffected by its inclusion in their drinking water for 30 days at 33 ppm. The manufacturer states that 3570 mg/kg is the lowest recorded dose for a human fatality by oral ingestion, a dose which is >800 fold higher than that tested in this study. Side effects include irritation of mucous membranes

if inhaled or swallowed in large quantity. Palatability problems in ORS were noted at levels >15 ppm in sensory testing at Land O'Lakes.

BENCH SCALE TESTS CONDUCTED IN THE U.S.

Objective

To evaluate the five potential ORS disinfectants for their bactericidal activity in ORS preparations.

Methods

Three microorganisms were selected for growth in ORS: *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhimurium*. These three are Gram negative organisms with the potential to induce gastrointestinal disease. Although they may not be identical to the pathogens in Pakistan village water sources, they were suitable as models for bench tests.

Each strain was cultured overnight in tryptic soy broth at 37° C. Cells were harvested by centrifugation and washed three times with sterile phosphate buffered saline to remove growth media. After resuspension to original volume in phosphate buffered saline, each cell preparation was diluted into prepared ORS at a final concentration of 500-15,000 per ml.

Various biological factors may have been responsible for the ranges of cell concentrations in these experiments. These potential factors which are difficult to control include the physiologic condition of the bacterial inoculum, slight differences in centrifugation conditions, culture medium or temperature, and to a lesser extent, dilution precision. Unfortunately, it is not possible to determine the bacterial concentrations prior to the start of the experiment, because chemical exposure must occur on a freshly grown culture and evaluation of cell concentrations requires 18 hours. Because the bactericidal

effect was examined after 24 hours of disinfectant exposure, the variations in initial bacterial concentrations were not significant.

The ORS containing the microorganisms was divided into two equal portions, one of which was supplemented with the chosen ORS disinfectant. The other unsupplemented portion served as a control to monitor bacterial growth in ORS. Bacteria were counted by standard dilution and plating techniques after 45-60 minutes of exposure to disinfectant and again after 4 and/or 24 hours of incubation at ambient temperature (approximately 23° C).

Results

Three of the disinfectants examined appeared effective after 24 hour exposure to ORS containing the three microorganisms. These were: LPO (25 ppm) + glucose oxidase + thiocyanate, AHST at 100 ppm, and DCIC at 20 ppm. The lauricidin, potash alum and AHST at 10 ppm were less effective. See Table I for complete results.

Additional tests were then conducted with two of the above ORS disinfectants to examine bactericidal activity following a one, four, and 24 hour exposure to the three microorganisms. The LPO system was not effective after one hour, partially effective at four hours and killed all three strains by 24 hours of incubation. The DCIC was tested against a pool of the three microorganisms and was completely effective at one hour, four hours and 24 hours of incubation (Table II). The DCIC was chosen to be the best disinfectant for testing in Pakistan.

An additional experiment examined the effect of flavoring compounds on the effectiveness of DCIC (Table III). Using the same three bacterial strains, ORS was inoculated with each organism and incubated with and without DCIC in the presence of each flavoring compound: orange, mango, lemon-lime, and peppermint. DCIC bactericidal activity for E. coli and S. typhimurium was inhibited by mango and peppermint flavors.

Orange and lemon-lime did not interfere with the bactericidal activity of DCIC for E.coli nor S. typhimurium. The survival or growth of the Klebsiella organism was apparent independent of flavor addition to the ORS (Table III). Therefore, this experiment demonstrated a reduced bactericidal effect for DCIC against Klebsiella pneumoniae even in the absence of flavors. This result differed from that observed in the previous experiment when the three organisms were tested in combination. No immediate explanation is available for this difference in the two experiments.

CHEMICAL COMPOSITION OF DICHLOROISOCYANURIC ACID

Based on results of bench scale tests conducted in the United States, the ORS disinfectant chosen for further work in Pakistan was dichloroisocyanuric acid (DCIC). The 1990 edition of the United States Pharmacopeia, The National Formulary lists this compound on page 280 as dichloro-s-triazine-2,4,6-trione. According to this reference, DCIC is a white, slightly hygroscopic crystalline powder. Its active ingredient is approximately 70% available chlorine and it is commonly used in bleaches, dishwashing compounds, and scouring powders. Telephone communication with Dr. Ed Powers at the U.S. Army facility in Natick, Massachusetts, confirmed that the Army has approved its use as a water disinfectant at 20 mg/liter (20 ppm) in emergency or survival situations. FDA approval for DCIC as a water disinfectant for human consumption has not been sought by its manufacturer, Monsanto, as confirmed by telephone communication with Dr. Arthur Rakestraw, Monsanto, St. Louis.

PAKISTAN TESTING

Objective

To test DCIC as a chemical disinfectant for ORS prepared with village water.

Methods

Village water sources were tested for this work because it was speculated that they would offer the most challenging environment for the chemical disinfectant, due to their high organic content which may interfere with bactericidal activity. After visiting Pakistan, it was apparent that urban water supplies were also often contaminated with coliforms. Nevertheless, at project initiation, it was decided to study rural water sources to represent the worst-case situation.

Well or tap water from four village sources was collected into sterile bottles and refrigerated until use. ORS was prepared with each of these water sources. Each ORS preparation was divided into two portions: one was unsupplemented (control) and the second was supplemented with DCIC at 25 or 40 mg/liter (25 or 40 ppm). After 45-60 minutes, aliquots from DCIC-supplemented and control samples were serially diluted 10-fold and plated onto Petrifilm (3M Company, St. Paul, Minnesota) to enumerate surviving coliform bacteria. ORS (with and without DCIC) was incubated at ambient temperature (28-30° C) and the coliform enumeration was repeated at 24 hours.

Results

1. The four village water sources used to prepare ORS differed in their initial coliform levels as measured 45 minutes after preparation without supplementation with DCIC (Table IV). The range of coliforms was <10 (Mandala tap water) to 670 per ml (Malpur well).

2. DCIC addition was less effective at 25 ppm than at 40 ppm in reducing coliform populations (Table IV). Mandala and Bhandar water-prepared ORS solutions were effectively decontaminated with the chemical at 25 ppm, but ORS solution using Jhang well water required 40 ppm to eliminate coliforms at 24 hours. Malpur well water used to prepare ORS remained contaminated after 24 hour exposure to 25 ppm DCIC. Testing at 40 ppm was not done.

SENSORY EVALUATION OF FLAVORED ORS CONTAINING DCIC

Objective

To determine if any unpleasant taste associated with DCIC could be masked by the addition of flavors to the ORS.

Methods

Permission was obtained from Colonel Dr. Mohammad Akram Khan at the National Institute of Health, Islamabad, to conduct the sensory evaluations with adult Pakistani women. Because the U.S. Army had approved a similar level of DCIC for emergency use, this level of chemical was not expected to be harmful, especially since volunteers tasted only teaspoon quantities. In addition, each sample was also tasted by the researcher prior to the village evaluations to ascertain degree of chemical unpleasantness.

The sensory tests were conducted with mothers rather than children because, typically, mothers taste foods and drinks that their children consume and they are also decision-makers in purchasing.

Three liters each of unflavored, orange, mango, and lemon-lime flavored ORS were prepared using bottled mineral water. Each preparation was divided in half. One portion was supplemented with DCIC at 40 mg/liter (40 ppm); the remaining portion of each

flavored or unflavored ORS was not supplemented. All ORS preparations were then coded by number so that DCIC supplementation was not obvious to the test population nor to those administering the taste test. Field visits were conducted in three villages (Rawat, Mandra, Morra Konian) within 45 km of Islamabad. Thirty Pakistani adults (25 mothers of children less than five years old and five men) tasted each of the paired (with and without DCIC) ORS samples and indicated their preference within each pair.

Each respondent provided their name, age, sex, education, monthly income, and age of youngest child. Respondents were chosen to continue the interview if they had a child under five years of age. Each was also asked whether they use ORS to treat diarrhea symptoms, their preference for flavored or unflavored ORS, and where they purchase ORS.

Following the introductory questions, the respondents tasted a small quantity of a flavored ORS, with and without the DCIC and were asked if any difference was detected. If the two ORS samples were different, the respondent stated her preference and described what was liked or disliked about the samples. After all four pairs of ORS samples were tasted, a final question asked which flavor was preferred: mango, orange, lemon-lime, or unflavored.

Results

In all cases, the majority of the individuals preferred the ORS which did not contain the DCIC disinfectant. However, mango flavoring did appear to mask the chemical flavor well, because 53% of the population preferred the unsupplemented ORS versus 47% who chose the DCIC-containing ORS (Table V).

The additional information gathered from respondents regarding preferences for ORS flavors, treatments used for diarrhea, income, and education is compiled in Tables VI to XII.

CLINICAL TESTING PROTOCOL AND BUDGET

Objective

To evaluate by discussion with Pakistani physicians the safety of DCIC when ingested orally by children as an ORS supplement. If DCIC is considered safe by the physicians, plan a clinical evaluation of ORS containing DCIC in Pakistani children suffering from dehydration in a hospital setting.

Methods

Interviews were conducted with three Pakistani physicians:

Dr. K.A. Abbas, Consultant Pediatrician
National Coordinator W.H.O.; Mother and Child Health Programme
The Children's Hospital
Pakistan Institute of Medical Sciences
Islamabad

Dr. Shaukat H. Kiani
Medical Superintendent
Clinical Research Division
National Institute of Health
Islamabad

Dr. Arjumand Faisel
U.S.A.I.D.
Office of Health, Population and Nutrition
P.O. Box 1028
18-6th Avenue, Ramna-5
Islamabad

Results

Dr. Faisel was concerned with the safety of supplementing ORS dry packet preparations with DCIC at a level of 40 ppm. He indicated that the range for chlorine supplementation of Pakistan water was 1-5 ppm, similar to U.S. tap water. Results of this project indicate that >25 ppm (likely 40 ppm) of DCIC is needed to disinfect ORS prepared

with village water. Dr. Faisel said that if 40 ppm of DCIC were used as an ORS disinfectant, it could induce potential side effects such as chlorosis.

There may be less concern for the side effects of chlorine ingestion with adults. As mentioned previously, the U.S. Army has approved 20 ppm DCIC as a water disinfectant in emergency situations, but consumers in this case are healthy adults rather than children with gastrointestinal disease.

Conversations with Dr. Abbas identified parameters to measure if clinical trials using DCIC as a supplement to ORS are conducted. He recommended measuring kidney and liver function to evaluate potential side effects of DCIC ingestion. He also stated that clinical trials are rather difficult to accomplish in his hospital. Laboratories are already working at capacity and may not have time to monitor these blood chemistry parameters which would be essential for this type of study. Even though the equipment and expertise is available, analysis of any additional samples may be quite difficult.

Dr. Kiani reported that his hospital usually does clinical trials with children involving new drug treatments. His hospital would be able to monitor subjective indices of health (stool volume, duration of diarrhea, obvious signs of side effects) but may not be able to provide laboratory tests of blood chemistry analyses.

CONCLUSIONS AND RECOMMENDATIONS

This discussion mentioned several problems with pursuing a clinical trial of DCIC as a disinfectant for ORS. These were: toxicity potential, a requirement for biochemical testing to evaluate side effects in patients, and a potential lack of adequate personnel to complete the required blood chemistry analyses. For these reasons, a clinical trial cannot be recommended at this time.

Other consequences of disinfectant supplementation of ORS should also be further evaluated. These include the effect of DCIC on the glucose and other ORS components. As part of the U.S. testing in this project, a preliminary indication of interaction of DCIC with glucose was obtained. For this experiment, 5 ppm DCIC was added to water in the presence and absence of glucose at 2%, its concentration in ORS. After 10, 20 and 30 minutes, the free chlorine was measured. Regardless of the presence of glucose, >3.5 ppm of free chlorine was detectable, indicating the chlorine had not been bound by the glucose. At a molecular level the ratio of glucose to DCIC is greater than 100 to one. Even if the DCIC were binding and altering glucose, rather than binding and inactivating contaminating microorganisms, 99% of the original glucose would still remain. Further analytical work to confirm these observations would be very useful to determine whether or not a chlorine compound can be used to disinfect ORS preparations at safe levels.

The cost of any potential ORS disinfectant is also a factor in developing countries. Informal discussions with Dr. Rakestraw at Monsanto indicated that DCIC may be available for as little as one cent/dose (U.S. $ 0.01). If so, DCIC has potential as a cost-effective disinfectant.

In addition, these experiments demonstrate that DCIC adds an unpleasant taste to ORS, particularly in the unflavored formula. While the addition of flavor compounds can partially mask the off-flavor, these compounds may also inhibit the disinfecting activity of DCIC.

While DCIC is effective if used at adequate levels, and is low in cost, it is unlikely to be recommended as an ORS disinfectant due to the potential toxicity at the concentrations needed to disinfect village water sources. Further discussions with officials at the Minnesota Department of Public Health (Mr. Dick Clark), and the Environmental

Health Department, University of Minnesota (Dr. Rexford Singer) support the concerns of the Pakistani physicians. Additional research should focus on identifying a disinfectant which can be used at lower concentrations that maintain bactericidal activity.

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REFERENCES

1. Hirschhorn, N. and W.B. Greenough III. 1991. Progress in oral rehydration therapy. Scientific American 264:50-56.

2. Black, R.E., M.M. Levine, M.L. Clements, P. Angle and R. Robins-Browne. 1981. Proliferation of enteropathogens in oral rehydration solutions prepared with river water from Honduras and Surinam. J. Trop. Med. and Hyg. 84:195-197.

3. Mathur, R. and V. Reddy. 1983. Bacterial contamination of oral rehydration solution prepared from well water. Indian J. Med. Res. 78:814-818.

4. Ahmad, K., K. Jahan and I. Huq. 1984. Decontamination of drinking water by alum for the preparation of oral rehydration solution. Food Nutr. Bull. 6:54-57.

5. Acra, A. et al. 1980. Disinfection of oral rehydration solutions by sunlight. Lancet, ii:1257-1258.

TABLE I

DISINFECTANT EFFECT ON BACTERIAL GROWTH IN ORS

Bacterium	Disinfectant	Bacteria per ml 0 Hour	24 Hours
E. coli	None	570	72,000
	LPO		< 1
	Alum		13,000
	LC		16,000
	AHST		< 1
	DCIC*	14,000	2
K. pneumoniae	None	690	33,000
	LPO		< 1
	Alum		11,000
	LC		12,000
	AHST		< 1
	DCIC*	14,000	2
S. typhimurium	None	1,000	790
	LPO		< 1
	Alum		470
	LC		140
	AHST		< 1
	DCIC*	14,000	2

*Tested in a separate experiment at 20 ppm, with a pool of the three organisms.

TABLE II

EFFECT OF TIME ON DISINFECTANT ACTIVITY IN ORS

		Bacteria per ml			
Bacterium	Disinfectant	0 Hr	1 Hr	4 Hrs	24 hrs
E. coli	None	N.D.(a)	390	140	7600
	LPO	460	220	0	0
K. pneumoniae	None	710	650	170	9000
	LPO	430	10	0	0
S. typhimurium	None	700	500	10	370
	LPO	830	530	30	0
Pool	None	N.D.	14000	7200	14000
	DCIC	N.D.	<10	<1	2

(a) N.D. = not determined.

TABLE III

EFFECT OF FLAVORS ON DCIC ACTIVITY IN ORS

Bacterium	Flavor	Bacteria per ml 0 Hr	24 Hrs
E. coli			
	Unflavored	5600	40
	Orange	3000	< 10
	Lemon/lime	3500	< 10
	Peppermint	2900	80000
	Mango	5000	9900000
K. pneumoniae			
	Unflavored	10000	110000
	Orange	7000	120000
	Lemon/lime	14000	310000
	Peppermint	10000	8200000
	Mango	7000	940
S. typhimurium			
	Unflavored	10000	30
	Orange	17000	40
	Lemon/lime	7000	60
	Peppermint	13000	8800
	Mango	5500	90000

TABLE IV

DCIC BACTERICIDAL ACTIVITY IN ORS PREPARED WITH VILLAGE WATER IN PAKISTAN

Village	DCIC, ppm	Coliforms per ml 1 Hr	Coliforms per ml 24 Hrs
Malpur	0	670	2900000
	25	250	124000
Bhandar	0	40	550000
	25	12	9
Jhang	0	540	250000
	25	69	550
	40	<1	*
Mandala	0	<10	950
	25	<10	*
	40	<1	<1

*Non-coliform colonies present; organisms unidentified.

TABLE V

TASTE PREFERENCES OF ORS WITH AND WITHOUT DCIC

ORS Flavor	DCIC Present	Flavor Preferences
Unflavored	No	76.7%
	Yes	23.3%
Orange	No	73.3%
	Yes	26.7%
Mango	No	53.3%
	Yes	46.7%
Lemon-lime	No	63.3%
	Yes	36.7%

TABLE VI

AGE DISTRIBUTION OF TASTE PREFERENCE RESPONDENTS

Age	Number of Respondents
20	1
22	2
23	1
24	3
25	5
26	2
27	1
28	2
30	6
31	1
32	1
35	3
37	1
38	1
Total	30

TABLE VII

EDUCATION LEVELS OF TASTE PREFERENCE RESPONDENTS

Education, Years	Number of Respondents
0	3
5	7
8	8
10	9
12	2
14	1
Total	30

TABLE VIII

INCOME LEVELS OF TASTE PREFERENCE RESPONDENTS

Rupees, monthly	Number of Respondents
500 - 1000	6
>1000 - 2000	18
>2000 - 3000	4
5000	2

Approximately 24 rupees = $1.00

TABLE IX

TASTE PREFERENCE RESPONDENTS: NUMBER OF CHILDREN <5 YRS OF AGE

Number of children	Number of respondents
0	1
1	11
2	10
3	6
4	1
5	1
Total	30

TABLE X

ORS USE AMONG TASTE PREFERENCE RESPONDENTS

Number Which Used ORS	Type
29 / 30	Flavored = 16
	Unflavored = 16
	Both flavored and unflavored = 3

Sources of ORS	Number of Respondents
Dispensary	3
Doctor	4
Village market	6
Chemist	4
Hospital	7
Fauji Foundation	1
National Institute of Health	1
UNICEF	1
Survey team	1
General store	2
Medical store	6
Total	36

TABLE XI

TASTE PREFERENCE RESPONDENTS: TREATMENT FOR DIARRHEA

Treatment given	Number of respondents
ORS	25
Take to doctor	3
Give fruits (cold things)	1
Syrup + tablets	1
Rice, yogurt, soft foods	1
Orange juice + salt	1
Did not use ORS	2*
Total	34

*Children reportedly refused to take ORS

TABLE XII

FLAVOR PREFERENCES OF TASTE PANEL RESPONDENTS

Flavored Preferred Overall	Number of Respondents
Orange	10
Mango	9
Lemon-lime	5
Unflavored	2
Total	26

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W