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POTASSIUM DICHROMATE AND ETHYL ALCOHOL AS BLOOD PRESERVATIVES FOR ANALYSIS OF CHLORINATED ORGANICS

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Introduction

This is the first known study comparing potassium dichromate, ethanol, and freezing, as methods of preserving blood for the analysis of chlorinated organics. Historically, freezing blood specimens has been a standard method of blood preservation for chlorinated organics analyses. During research in Southeast Asia and elsewhere investigating contamination of the environment, food, and humans, we found substantial difficulties keeping blood samples frozen in tropical climates, often in rural areas of countries with little or no electricity or without dry ice [1-5]. Shipment on samples of dry ice has proved to be expensive and often uncertain even in the USA or Europe using established express shipping companies. On occasion, samples were held in customs, and at times we came close to having valuable specimens obtained in Vietnam, Laos, or Cambodia in danger of spoilage. We have designed this pilot study to test some other methods of blood preservation that might provide us with alternatives to freezing for shipment and storage of biological specimens.

In this study we used two different approaches to preserve human blood samples for chlorinated organics analysis:

1. Use of different concentrations of absolute alcohol, 20% and 40% per volume of sample: Alcohol has sometimes been used for the preservation of biological specimens but its use in blood preservation for analysis of persistent organic pollutants such as PCBs and dibenzodioxins has not been thoroughly tested.

2. Use of potassium dichromate: Potassium dichromate has been used in preservation of cow's milk [6-8] and is included in an AOAC Official Method as possibility for this purpose[9-10]. Dichromate tablets for preservation of milk samples are commercially available allowing an easy and elegant way of preservation. Therefore, for the Third Round of WHO-coordinated exposure studies on levels of PCBs, PCDDs and PCDFs in human milk, the use of dichromate for preservation of human milk samples was recommended for sending samples for analysis [11]. The reference laboratory of this round of WHO-coordinated exposure studies developed an easy procedure to destroy excess dichromate before analysis to avoid problems with dichromate during analysis or at waste disposal [12]: Addition of ascorbic acid reduces chromate to non-toxic Cr(III)-ions. With this experience, we wanted to test the hypothesis that it could be useful in preservation of human blood, as well.

Methods

We collected 1000 ml of left-over whole blood from anonymous donors at the Southwestern Medical Center clinics in Dallas, Texas, in 2002. There were 165 female and 84 male donors providing blood for this pooled sample. The average age of donors was 53 years, with a range from 18 to 85 years. This pooled sample was then divided into twelve smaller samples of 65 ml each. Three individual samples were frozen, three had total of 20% of absolute alcohol per volume of sample added, three had total of 40% of absolute alcohol, and two tablets (2 mg each)

of potassium dichromate were added to three blood samples. All samples were then shipped either on dry ice or not frozen in the case of the dichromate and alcohol blood samples to ERGO laboratory in Hamburg, Germany for dioxin analysis. Frozen samples were stored frozen until analyzed, while alcohol and dichromate samples were kept at room temperature for 6, 25, and 34 days when dioxin analysis was then performed on one of each preservation methods samples.

Seven dibenzodioxin, ten dibenzofuran and four coplanar polychlorinated biphenyl (PCB) congeners were analyzed by high resolution gas chromatography-mass spectrometry by ERGO laboratory in Hamburg, Germany[13]. This laboratory has been certified by the World Health Organization for congener specific analysis of dioxins, dibenzofurans, and PCBs in human tissues [14,15]. Levels of dioxin compounds are reported in pg/g or parts per trillion (ppt), lipid, and in World Health Organization (WHO) dioxin toxic equivalents. In addition, lipid content in each sample was gravimetrically measured to evaluate the stability and amount of lipid in the samples over time. German pooled blood samples were also analyzed as part of a quality control procedure. Two German samples were frozen, three had 20% and three had 40% alcohol per volume of sample added within an hour of analyses. No additional room temperature storage time occurred in these quality control samples after thawing.

Results and Discussion

Table 1 presents dioxin, dibenzofuran and non-ortho or coplanar PCB congeners specific data for twelve blood samples using four preservation methods. Overall, dioxin TEQ levels as well as amount of lipid per sample varied considerably depending on the method of preservation. We used the results of analyses of frozen blood samples as the standard to which we compared the results obtained by other methods. Preliminary analyses suggest that dichromate provides the best alternative to freezing which is currently the gold standard for preservation - from the point of lipid content and congener levels. The time of storage, with a maximum 36 days, did not seem to have any effect on the results of the analyses in this small pilot study.

Quality control pooled blood samples in the ERGO laboratory were also used to compare freezing to the use of 20 and 40% alcohol as preservatives. The congener specific results suggest that alcohol does not seem to be a good preservative for the whole blood even when there is no storage time (data not shown).

Figure 1 shows comparison of lipid content in US pooled blood samples for different preservation methods used. The amount of lipid measured in potassium dichromate preserved samples is very similar to the amount of lipid in blood samples preserved by freezing. In contrast, alcohol as a preservative at both 20% and 40% substantially increased the amount of lipid in comparison to freezing or potassium dichronate, possibly by cell breakdown.

Results of our study suggest that potassium dichromate may be a valuable alternative to freezing to preserve blood samples for dioxin and other chlorinated organics research. Further analyses on larger numbers of samples and for longer periods of storage time are required to confirm the results of this study. This method may prove to be valuable in facilitating further dioxin research in tropical countries, less developed countries including Southeast Asia, and also considerably reduce cost of shipping of biological specimens to laboratories for analysis. In the near future we also hope to evaluate this method for other groups of persistent organic pollutants including organochlorine pesticides, PCBs, DDT/DDE, HCH, brominated flame retardants and PAHs. The use of potassium dichromate in this study seemed to be simple, economical, and the specimens were easy to handle.

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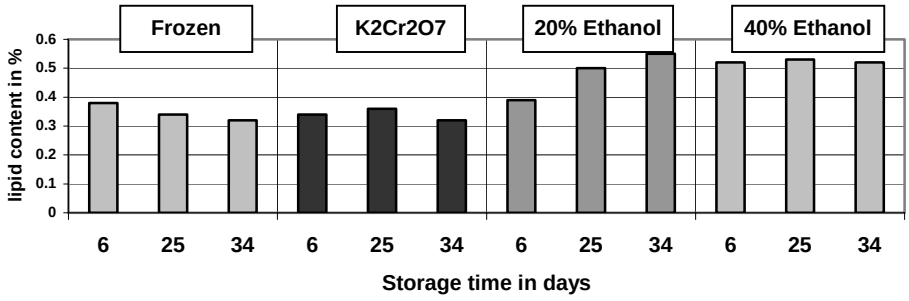
Table 1. Dioxin, dibenzofuran, and coplanar PCB congeners in US pooled blood sample using different preservation methods over period of time (pg/g or ppt, lipid).

	Frozen			**Potassium Dichromate**			**Alcohol 20%**			**Alcohol 40%**		
Days stored	6	24	32	6	24	32	6	24	32	6	24	32
Lipid content (%)	*0.38*	*0.34*	*0.32*	*0.34*	*0.36*	*0.32*	*0.39*	*0.50*	*0.55*	*0.52*	*0.53*	*0.52*
2.3.7.8-TCDD	3.2	3.6	3.6	4.1	3.8	4.1	3.5	2.6	2.1	2.3	2.1	2.6
1.2.3.7.8-PnCDD	5.4	6.7	7.7	7.5	5.9	7.0	7.4	5.7	4.5	5.1	4.5	4.3
1.2.3.4.7.8-HxCDD	9.7	6.6	7.3	7.3	7.6	9.6	9.3	5.1	6.1	5.7	5.2	5.4
1.2.3.6.7.8-HxCDD	29	35	38	42	32	36	41	29	25	26	28	25
1.2.3.7.8.9-HxCDD	4.5	6.2	7.5	6.1	5.6	5.9	5.7	4.9	4.0	4.0	4.0	3.5
1.2.3.4.6.7.8-HpCDD	53	55	64	60	50	55	58	41	36	40	36	36
OCDD	386	446	464	469	441	448	462	344	302	321	297	300
2.3.7.8-TCDF	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1	n.d.(1)	n.d.(1)	n.d.(1)
1.2.3.7.8-PnCDF	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	1.2	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)
2.3.4.7.8-PnCDF	5.1	5.4	6.4	5.3	5.6	5.6	5.4	4.2	3.3	4.0	3.6	3.2
1.2.3.4.7.8-HxCDF	5.9	7.5	7.7	8.7	7.0	6.8	6.9	5.2	4.4	5.5	5.3	4.2
1.2.3.6.7.8-HxCDF	3.5	3.7	5.2	3.9	4.0	4.3	3.7	2.9	2.4	2.7	2.9	2.6
1.2.3.7.8.9-HxCDF	n.d.(17)	n.d.(37)	n.d.(37)	n.d.(36)	n.d.(33)	n.d.(36)	n.d.(16)	n.d.(7)	n.d.(10)	n.d.(19)	n.d.(10)	n.d.(12)
2.3.4.6.7.8-HxCDF	1.3	1.3	1.4	1.3	1.5	n.d.(2)	1.8	n.d.(2)	1.0	1.0	n.d.(1)	n.d.(1)
1.2.3.4.6.7.8-HxCDF	n.d.(9)	5.9	6.5	4.3	n.d.(7)	6.5	6.2	n.d.(7)	3.7	n.d.(5)	n.d.(6)	4.2
1.2.3.4.7.8.9-HpCDF	n.d.(2)	n.d.(1)	n.d.(1)	n.d.(2)	n.d.(2)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)	n.d.(1)
OCDF	n.d.(8)	n.d.(4)	n.d.(4)	n.d.(13)	n.d.(9)	n.d.(4)	n.d.(12)	n.d.(6)	n.d.(3)	n.d.(9)	n.d.(6)	n.d.(3)
PCB 77	n.d.(69)	n.d.(110)	n.d.(114)	n.d.(68)	n.d.(74)	n.d.(11	n.d.(59)	n.d.(53)	n.d.(67)	n.d.(45)	n.d.(50)	n.d.(72)
TCB 81	3.3	7.1	3.7	3.4	3.0	6.5	3.6	2.4	n.d.(2)	2.5	2.9	4.5
PCB 126	36	51	51	46	44	58	39	32	29	25	32	34
PCB 169	16	27	27	20	18	28	21	14	17	13	18	17
Total PCDD/PCDF	507	584	619	619	564	590	610	445	395	418	389	390
Total coplanar PCB	56	84	82	70	65	93	63	49	46	41	53	56
Total TEQ	21	25	27	27	23	27	25	19	16	17	17	17

n.d. – Non detected, limit of detection in the brackets.

Figure 1. Lipid concentration in % in pooled blood samples over time using different blood preservation methods.



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