Characterization of POPs in California Condors using Comprehensive Two-Dimensional Gas Chromatography (GCxGC) with High Resolution Time of Flight Mass Spectrometry and Novel Spectral Analysis Tools

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Introduction

The California condor (*Gymnogyps californianus*) was driven near the point of extinction by the end of the 20th century. In 1987, their breeding population was reduced to a total of 22 birds. Fortunately their numbers have rebounded to over 400 individuals at the present time, with half of them as part of wild populations in California, Arizona, and Baja California thanks to numerous individuals participating in conservation efforts. There is evidence that inadvertent lead and pesticide consumption may have played a significant role in their decline[1,2] However, it should be noted that lead and DDE exposure may not be the only factor impeding the survival and recovery of this critically endangered species. More recent studies[3] have targeted a number of well-known and recognized halogenated organic compounds (HOCs) such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), but a more comprehensive analytical approach may be warranted for the rigorous characterization of other potentially harmful compounds in condor plasma. Some of these substances may include additional classes of endocrine disrupting compounds (EDCs) that could contribute to reproductive complications of condors. The goals of this study included the development and implementation of an effective workflow for comparison of coastal and inland condors, and to increase the number of confidently identified HOCs and other persistent organic pollutants (POPs) in plasma samples. The goals were achieved through improved non-targeted screening of samples using a combination of GCxGC, high resolution time-of-flight mass spectrometry (HRT) and novel spectral analysis tools. This methodology facilitated identification of physiologically harmful xenobiotics present in California condor plasma.

Materials and methods

Inland and coastal condor plasma was extracted with formic acid, measured for total lipid content, purified using gel permeation chromatography and analyzed using GCxGC-TOFMS to detect commonly monitored HOCs as well as screen for new and emerging POPs. GCxGC-HRT was implemented for identity confirmation and characterization of additional compounds in plasma. Extracts were injected into an Agilent 7890B gas chromatograph equipped with a first dimension R*xi*-5ms column (30m x 0.25mm x 0.25µm) with 5m Integra guard column and 2nd dimension R*tx*-

17ms (0.79m x 0.10mm x 0.10µm) column. The data was collected at 150 spectra per second with a mass range of 50-1000 m/z. The analysis workflow included a combination of ionization methods (EI & CI) and GC-HRT technology. Data were processed using comprehensive, untargeted Peak Find for discovery and retrospectively using target analyte finding for rapid data processing once compounds of interest were identified. Compounds were characterized using a combination of spectral similarity searches of deconvoluted data against large, well-established databases and formula determinations for high resolution accurate mass fragment, molecular and adduct ions. The implementation of novel spectral analysis tools aided in the characterization of unknowns.

Results and discussion

Preliminary GCxGC-TOFMS analyses[4] demonstrated that coastal condor plasma samples contained a greater number of HOCs as compared to inland birds. These HOCs included PCBs, PBDEs and pesticides (e.g., DDE, etc.). Coastal plasma contained an average of 66 halogenated organic compounds, including 34 PCBs, compared to an average of 9 HOCs and no PCBs in inland condor plasma. Twelve unexpected compounds were identified including three DDT-related compounds, three halogenated natural products and twelve compounds of unknown structure or origin.

The workflow applied in this study resulted in confident characterization of numerous compounds in California condor plasma. Comprehensive analysis of condor plasma samples using GCxGC-HRT confirmed the presence of HOCs, but also facilitated the identification of a variety of compounds. The compounds included aromatic compounds, bisphenols, phthalates, polyaromatic hydrocarbons (PAHs), halogenated PAHs, heterocyclic and persistent organic pollutants (Table 1). The representative set of compounds listed below exhibited average spectral similarity and absolute mass accuracy values of 879/1000 and 0.54 PPM, respectively.

Table 1. Representative compounds in condor plasma

Name	Mass Accuracy (ppm)	Similarity
Naphthalene, 1-methyl-	-0.42	940
Naphthalene, 2,6-dimethyl-	-0.31	825
Naphthalene, 1-bromo-	-0.08	743
Acenaphthene	0.60	902
Dibenzofuran	-0.28	804
Naphthalene, 1,6,7-trimethyl-	-0.73	849
Diethyl Phthalate	-0.53	950
2,6-Diisopropylnaphthalene	-0.21	829
1,1'-Biphenyl, 2,2',5,5'-tetramethyl-	-0.01	774
Tri(2-chloroethyl) phosphate	N/A	891
α-Lindane	N/A	900
Phenanthrene	-0.25	766
Anthrone	-0.06	807
Phenanthrene, 4-methyl-	0.04	831
1,1'-Biphenyl, 2,2',5,6'-Tetrachloro-	0.32	866
9,10-Anthracenedione	0.67	932

Name	Mass Accuracy (ppm)	Similarity
Bisphenol AF	-0.87	874
1,1'-Biphenyl, 2,3',4',5-tetrachloro-	0.39	919
Pyrene	0.02	912
DDMU	0.50	923
1,1'-Biphenyl, 2,2',3',4,6-Pentachloro-	-0.31	934
Fluoranthene	0.55	932
Nonachlor	3.11	812
p,p'-DDE	0.05	898
o,p'-DDE	0.46	869
1,1'-Biphenyl, 2,2',3,4,4',6-Hexachloro-	-0.52	951
1,1'-Biphenyl, 2,2',3,3',4,4',5-heptachloro-	-0.25	942
Dibromophenyl ether	-0.28	941
1,1'-Biphenyl, 2,2',3,3',4,4',5,6'-octachloro-	0.31	884
3,3',4,4'-Tetrabromodiphenyl ether (IS)	-0.56	915
2,2',4,4',6-Pentabromodiphenyl ether	-0.81	934
2,2',4,4',5,-Pentabromodiphenyl ether	-0.97	917

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