Blue whale earplug reveals lifetime contaminant exposure and hormone profiles

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Lifetime contaminant and hormonal profiles have been reconstructed for an individual male blue whale (*Balaenoptera musculus*, Linnaeus 1758) using the earplug as a natural aging matrix that is also capable of archiving and preserving lipophilic compounds. These unprecedented lifetime profiles (i.e., birth to death) were reconstructed with a 6-mo resolution for a wide range of analytes including cortisol (stress hormone), testosterone (developmental hormone), organic contaminants (e.g., pesticides and flame retardants), and mercury. Cortisol lifetime profiles revealed a doubling of cortisol levels over baseline. Testosterone profiles suggest this male blue whale reached sexual maturity at approximately 10 y of age, which corresponds well with and improves on previous estimates. Early periods of the reconstructed contaminant profiles for pesticides (such as dichlorodiphenyltrichloroethanes and chlordanes), polychlorinated biphenyls, and polybrominated diphenyl ethers demonstrate significant maternal transfer occurred at 0–12 mo. The total lifetime organic contaminant burden measured between the earplug (sum of contaminants in laminae layers) and blubber samples from the same organism were similar. Total mercury profiles revealed reduced maternal transfer and two distinct pulse events compared with organic contaminants. The use of a whale earplug to reconstruct lifetime chemical profiles will allow for a more comprehensive examination of stress, development, and contaminant exposure, as well as improve the assessment of contaminant use/emission, environmental noise, ship traffic, and climate change on these important marine sentinels.

cetaceans | cerumen | persistent organic pollutants

The blue whale (*Balaenoptera musculus*) is the largest animal on Earth and is listed as "endangered" (International Union for Conservation of Nature Red List of Threatened Species) primarily due to drastic reduction in population during the whaling era and a slow postwhaling recovery (1, 2). Besides whaling activities, well-known anthropogenic activities including fishery entanglement, chemical use/emission, environmental noise, ship strikes, and climate change are impacting whales, with evidence found through morphological or epidemiological analysis as well as chemical profiles from tissue, fecal, and exhalation samples (3–6). However, we are currently unable to monitor and therefore assess the lifetime impacts of such anthropogenic pollution/activities on whales.

Many anthropogenic contaminants and biogenic physiological markers (i.e., hormones) are routinely measured in lipid-rich matrices such as blubber. Fatty tissues act as natural sinks for lipophilic compounds such as historic-use pesticides, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), methyl mercury, and hormones (7–9). Whereas biogenic compounds are produced by life processes, many anthropogenic compounds arrive in these tissues through long-range transport and bioaccumulation through the food web (10–12).

Many large baleen whale (family *Balaenopteridae*) species are known to accumulate layers of cerumen (i.e., ear wax) from birth in the ear canal, yielding an earplug (13–16). This waxy plug is composed of lipids, waxes, and keratin, accumulating in a continuous fashion, producing alternating dark- and light-colored laminae, or layers, which have been shown to be associated with periods of feeding or migration (17). Historically, the analysis of earplugs has enabled the accurate estimation of age in a manner that is similar to counting growth rings in trees (13–18).

Increasing global contaminant emissions, environmental noise, and shipping, combined with changing climatic conditions, make it imperative to improve our ability to reconstruct, evaluate, and interpret chemical profiles in sentinel species and their ecosystems. The objective of this study was to use a male blue whale earplug to age and reconstruct lifetime profiles (i.e., birth to death) of a wide range of hormones, persistent organic pollutants (POPs), and mercury. Profiles were reconstructed using known aging techniques (lamina counts) (13–18) and a wide range of analytical methods. Our investigations demonstrate that contaminants and hormones that routinely accumulate in whale blubber also accumulate in whale earplugs at quantifiable concentrations. In addition, our investigation reconstructed previously unknown lifetime contaminant and hormone profiles. For a majority of the species on the planet, lifetime profiles such as these are simply unattainable.

Results

Following a ship strike resulting in mortality, a 25.4-cm earplug was harvested from a 21.2-m male blue whale in 2007 near Santa Barbara, CA (Fig. 1). The sectioned earplug yielded 24 discrete laminae, providing an estimated age of death at 12 y ± 6 mo (Fig. 1*D*). This was validated with length-to-age estimates commonly used to estimate the age of whales (19). The length–sexual maturity relationship of the blue whale sampled during this study (21.2 m) agrees with previous findings of the larger subspecies

Significance

Currently, obtaining lifetime chemical profiles (i.e., from birth to death) is extremely rare and difficult for most of Earth's animals. We have developed a unique approach to quantify hormone and contaminant lifetime profiles for an individual blue whale with a 6-mo resolution using the wax earplug as a natural matrix capable of archiving and preserving these temporal profiles. Using a male blue whale earplug, chemical analysis reveals lifetime patterns of mercury and organic pollutant exposure as well as fluctuating hormone levels. Specifically, we quantified contaminant maternal transfer, time to sexual maturity, and the doubling of stress over the animal's lifespan. We anticipate that this technique will fundamentally transform our ability to assess human impact on these environmental sentinels and their ecosystems.

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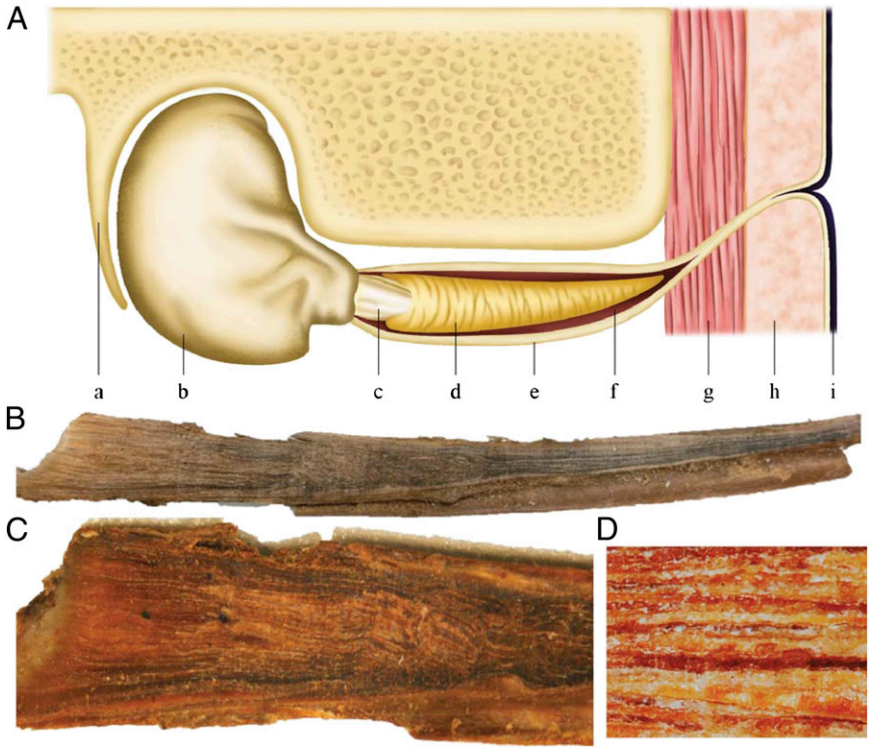

Fig. 1. Illustration of a blue whale earplug. (*A*) Schematic diagram showing the location of the earplug within the ear canal: (*a*) whale skull, (*b*) tympanic bulla, (*c*) pars flaccida/tympanic membrane ("glove finger"), (*d*) cerumen (earplug), (*e*) external auditory meatus, (*f*) auditory canal, (*g*) muscle tissue, (*h*) blubber tissue, and (*i*) epidermis. (*B*) Extracted blue whale earplug; total length 25.4 cm. (*C*) Earplug longitudinal cross-section. (*D*) View (20×) of earplug cross-section showing discrete laminae.

Antarctic blue whale (*Balaenoptera musculus intermedia*). In a seminal paper by Mackintosh and Wheeler (1929) (20), Antarctic blue whale sexual maturity was estimated by plotting lengths of hundreds of whales, estimating growth rates, and correlating with reproductive maturity. Key findings from this research concluded the mean length of sexual maturity of female Antarctic blue whales to be 23.7 m, whereas the males were estimated to reach sexual maturity at 22.6 m. Cortisol (stress related), testosterone (development), POPs, and mercury lifetime profiles were reconstructed from the 24 discrete laminae (lamina 1 being the oldest).

Mean cortisol concentrations doubled over the male blue whale 12-y lifespan ($P < 0.05$; Fig. 2*A*). Overall mean cortisol concentration measured in the laminae was ∼150 pg·g^{-1} and ranged from 45 to 420 pg·g^{-1}. The lowest cortisol concentrations (i.e., baseline) found in the second lamina (age 6–12 mo) was measured below the method detection limit of 65 pg·g^{-1}) and the highest was found in lamina 22 (age 126–132 mo; Fig. 2*A*). Cortisol levels peaked at 420 pg·g^{-1}, which corresponds to a ∼800% increase over baseline at 126–132 mo of age (lamina 22).

Testosterone concentrations suggest sexual maturity was reached at 114–126 mo (Fig. 2*A*). Baseline testosterone levels (230 pg·g^{-1}) were measured in the terminal (24th) lamina. Testosterone levels increased from birth to approximately 3 y of age (2,500 pg·g^{-1}) where levels declined (530 pg·g^{-1}) until age 114–126 mo, whereupon levels increased over baseline by approximately two orders of magnitude, to a maximum of 93,000 pg·g^{-1} (Fig. 2*A*).

Cerumen samples were analyzed for 42 POPs, including 20 historic-use pesticides and metabolites, 15 PCBs, and seven PBDEs. Sixteen of the 42 POPs were measured at trace levels in blue whale cerumen. The sum of pesticides [*cis*- and transnonachlor, *o,p′*-dichlorodiphenyldichloroethylene (DDE), *p,p′*-DDE, and *p,p′*-dichlorodiphenyltrichloroethane (DDT)], measured in earplug laminae, ranged in concentrations of 120–830 ng·g^{-1}. *p,p′*-DDE, a metabolite of *p,p′*-DDT, had the highest concentration at 660 ng·g^{-1}. Eight of the 15 PCBs (∼53%) were also measured in whale cerumen, including PCB 105, 118, 138, 153, 156, 157, 167, and 187. ∑PCB concentrations ranged from 5.9 to 30 ng·g^{-1}. In addition, three PBDEs (47, 99, and 100) were also measured in blue whale laminae. ∑PBDE concentrations ranged from 0.19 to 5.9 ng·g^{-1}. The total organic chemical burden in the laminae ranged from 160 to 860 ng·g^{-1}. Ninety-six percent of the total organic burden was composed of four historic-use pesticides and their metabolites and 1 PCB: *p,p′*-DDE (80% burden) > *o,p′*-DDE (9.5%), > *p,p′*-DDT (3.9%) > transnonachlor (1.5%) > PCB 153 (1.4%). The mean ratio of *p,p′*-DDE to *p,p′*-DDT was 24 ± 10 and increased over the animal's lifetime to reach a maximum ratio of 54 in the last lamina (slope of 0.93 and a R^2 of 0.44).

All POP earplug concentrations spiked at 0–6 mo of age (Fig. 2 *B–D*). The mean mass of POPs measured in the first 6 mo represented ∼20% of the total POPs burden measured throughout the earplug. This percentage decreased as a function of age. The continual accumulation of POPs recorded in the cerumen throughout the animal's lifetime was relatively linear and reached a maximum of 5,200 ng·g^{-1} (Fig. 2*E*). This maximum represents an estimate of the total reconstructed lifetime POP burden (sum of POP concentrations measured in all laminae).

The lifetime mercury profile reconstructed from the 24 lamina showed different periods of peak exposure compared with the organic contaminants. The mean mercury concentrations were 14.1 ± 2.6 ng·g^{-1} and ranged from 10.6 to 20.8 ng·g^{-1} (Fig. 2*F*). Two distinct peaks were identified in the mercury profile, which corresponded to 60–72 mo (20.8 ng·g^{-1}) and 120–126 mo (18.7 ng·g^{-1}). Similar to the POPs, the continual accumulation of mercury recorded in the cerumen throughout the animal's lifetime was

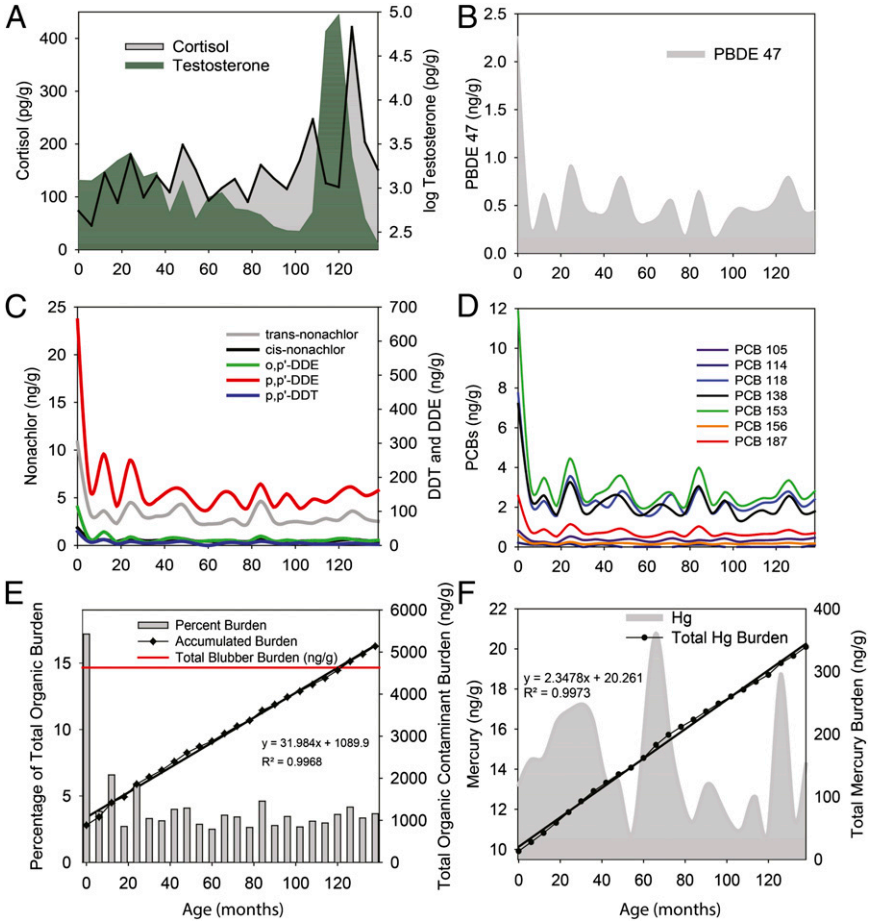

Fig. 2. Reconstructed chemical profiles measured in a blue whale earplug. Each measurement reflects the mean concentration during lamina production. (*A*) Cortisol levels increased with age, whereas testosterone peaked at 120 mo (~100× increase). (*B*) PBDE 47 profiles demonstrate considerable depuration from mother during periods of early development. (*C*) Historic-use pesticide profiles of DDT and metabolites and nonachlor compounds. (*D*) PCB profiles showed trends similar to the other pesticides with the highest levels measured in the first lamina. (*E*) Total mercury and organic contaminant deposition accumulation rate. (*F*) Life span mercury profile shows two unique peaks at 60–72 and 120–126 mo.

linear with a slope of 2.3, with a total reconstructed mercury burden of 340 ng·g^{-1} (Fig. 2*F*).

Discussion

Using the earplug from a single blue whale, we have been able to demonstrate that: (*i*) lipophilic compounds accumulate in cerumen; (*ii*) contaminants and hormones that accumulate in the cerumen lamina are chronologically archived; (*iii*) trace analysis techniques can be used to measure contaminants and hormones in individual lamina; and (*iv*) by combining chemical concentrations from individual lamina, we can reconstruct lifetime profiles for an individual whale (i.e., birth to death).

This study reports previously uncharacterized lifetime cortisol profiles in a baleen whale (Fig. 2*A*). Cortisol is a biomarker of stress in mammals with concentrations directly associated with responses to a combination of environmental, physical, chemical, and social factors (21–24). During this response, glucocorticoid steroid hormones are released into the blood stream with the amount reflecting the severity of the stressor (25). This cortisol profile highlights several prominent peaks as well as episodic variability over the animal's lifetime. Baseline cortisol concentrations were 45 pg·g^{-1} (6–12 mo), whereas cortisol peaked at 420 pg·g^{-1} (126–132 mo). This change in cortisol corresponds to an ~800% increase over the initial baseline. This peak in cortisol concentration immediately follows the largest peak in testosterone (114–126 mo) (Fig. 2*A*). This suggests that the cortisol maximum was due to breeding competition or social bonds formed during sexual maturity (26). Interestingly, the mean cortisol concentrations doubled over the life of this blue whale (Fig. 2*A*). The general increase in cortisol over the animal's lifetime could be associated with a multitude of factors including weaning, development, sexual maturity, migration, food availability, environmental conditions, changes in social status, accumulated contaminant exposure, and/or environmental noise.

The increase of androgens during postnatal development is a key factor defining sexual maturity in male mammals (27). The significant peak (400-fold over baseline) in testosterone observed during this study at ~114–126 mo provides a strong indication of sexual maturity (Fig. 2*A*). This unique approach provides chemical verification of sexual maturity (via lifetime testosterone profiles) and offers improved resolution over historical methods such as age–length estimates (20, 28), ear plug lamina counts (14), and ovarian corpora counts–length data (29), which cumulatively have previously estimated sexual maturity of male blue whales to be between 60 and 180 mo (30). Testosterone concentrations sequestered in the cerumen from this male blue whale ranged from ~230 to 93,000 pg·g^{-1}. The lifetime testosterone profile reported here from a single animal was in agreement with a study by Kjeld et al. (3) who measured serum testosterone concentration in 278 male fin whales at varying ages, where testosterone concentration also ranged over two orders of magnitude (35–14,000 pg·g^{-1}). Kjeld et al. (3) used earplugs for aging and histological and

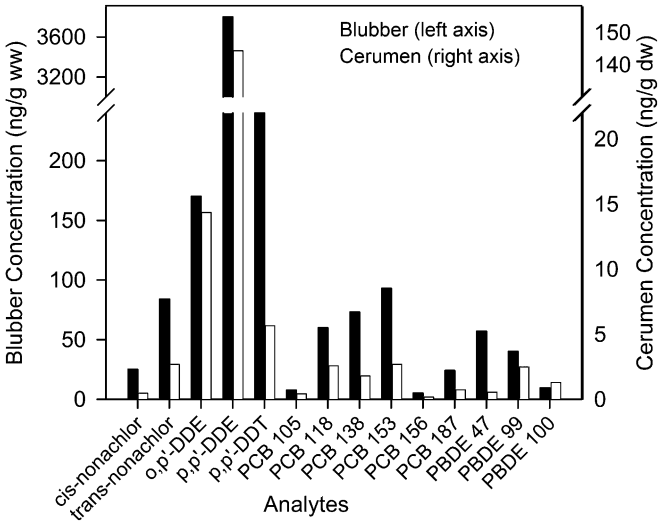

Fig. 3. Male blue whale blubber (left axis) and earplug (right axis) contaminant profiles. Blubber was sampled near muscle blubber interface and compared with the lamina corresponding to 72–78 mo in the earplug, which represents a midpoint in the organism lifespan.

anatomical indices to determine the medium age of sexual maturity in male fin whales to be 120 mo (10 y).

The reconstructed POP profiles of this male blue whale demonstrate that a substantial maternal transfer occurred during gestation and/or lactation. A review by Wagemann and Muir (31) highlighted similar maternal transfer of contaminants in a large number of marine mammals throughout the north hemisphere. The maternal transfer of POPs for this individual blue whale was equal to ∼20% of its total lifetime burden. This substantiates previous hypotheses regarding the ability of organic contaminants to undergo maternal transfer during gestation and lactation in large marine mammals (32, 33). Further, our lifetime POP burden measured in this blue whale is in agreement with the lifetime physiologically based pharmacokinetic fugacity model developed by Hickie et al. (1999) (Fig. 2*E*) (32). In our study, the lifetime accumulative lipophilic contaminants burden describes the uptake, metabolism, and excretion of POPs by the organism over its entire lifetime. The lifetime accumulative POP burden was recorded in the lamina of the earplug and totaled 5,200 $ng{\cdot}g^{-1}$. Blubber tissue collected from the same male blue whale provided a total POP burden of 4,700 $ng{\cdot}g^{-1}$ or 90% of the total accumulative burden recorded in the earplug (Fig. 2*E*, red vertical line). This 10% decrease may be due to differences in uptake, metabolism, and excretion process specific to blubber compared with cerumen. Regardless, under the assumption that both the whale blubber and earplug can provide estimates of accumulative contaminant burden, we see striking similarities between these two matrices. Specifically, *o,p′*-DDE, *p,p′*-DDE, *p,p′*-DDT, *cis*- and transnonachlor, PCB 105, 118, 138, 153, 156, and 187, as well as PBDE 47, 99, and 100 were identified in both blue whale earplug cerumen and blubber (Fig. 3). The major difference between the two matrices is that s.c. blubber cannot estimate when the exposure transpired, whereas earplugs provide lifetime accumulative contaminant burden with, in this case, a 6-mo resolution.

The POP burden measured in each lamina suggests that contaminants are recirculating throughout the body during both periods of feeding and fasting. The impact from the chronic and acute POP exposure on baleen whales is largely unknown, but may potentially be positively correlated with cortisol. This relationship has been difficult to identify using inconsistent sampling strategies (34–36). Contaminant and hormone profiles reconstructed from this earplug suggest that there may be a weak positive correlation between contaminant burden and cortisol concentrations as a function of time; however, with a sample size of $n = 1$, this should be considered a tentative assessment.

Anthropogenic mercury is ubiquitous in the environment and has received much attention among ecologists, environmental chemists, and toxicologists because of its ability to bioaccumulate and impair neurological development. Well-documented research involving humans reveals maternal transfer of mercury in utero and then to the neonate during lactation (37). Mercury profiles in this blue whale do not mirror maternal transfer to the same degree as the POPs (Fig. 2*F*). The mercury profile also highlights two pulse events ranging from 60 to 72 mo and from 120 to 126 mo. Because this blue whale appeared to routinely traverse the coast of California (ship strike near Santa Barbara, CA), we speculate that these pulse events may be associated with regional environmental and/or anthropogenic increases of mercury (38).

This article highlights significant advantages and research opportunities in the fields of biology and chemistry, specifically the reconstruction of lifetime chemical profiles (i.e., birth to death) in baleen whales. Lifetime profiles offer significant improvements over costly ship time and conventional intermittent sampling techniques that use blood (39), feces (40), blubber (41–43), morphometric measurements, and/or exhalations (4) as well as conservation advantages in the reduction in the samples (blood, blubber, etc.) required to address a specific research question (3, 41). Using earplugs to age and reconstruct lifetime chemical profiles allows for a more comprehensive examination of stress, development, and contaminants. In addition, earplugs allow for the simultaneous assessment of multiple research questions (e.g., concerning contaminants and hormones) thereby expanding opportunities to address more complex and integrated questions, such as the impact of POP burden on the lifetime stress of an animal. Finally, earplugs allow for examination of both persistent compounds (i.e., POPs) as well as compounds that are metabolized in the body (i.e., hormones). Earplugs may provide a unique opportunity to reconstruct exposure profiles for compounds such as polycyclic aromatic hydrocarbons, which typically undergo rapid biodegradation in tissues such as liver and blubber (44).

One of the most profound advantages offered by earplugs is the ability to retrospectively examine critical issues through the analysis of archived museum samples, some of which were harvested in the 1950s. A comprehensive database could be derived by combining the analysis of multiple earplugs harvest over multiple generations. Further, this innovative tool increases the feasibility of accurately assessing anthropogenic impact on everything from an individual organism to marine ecosystems. Without such data, there is no context with which to interpret the biological significance or anthropogenic impact of individuals or populations.

Materials and Methods

Briefly, the blue whale earplug was sectioned longitudinally to improve accessibility to internal lamina using an ultrafine-toothed band saw. Under 20× magnification, individual lamina were removed from each longitudinal section and stored in nitrogen at −30 °C. Hormone determination was performed using their respective Enzo Life enzyme immunoassay kits. Total mercury determination in cerumen was in accordance with US Environmental Protection Agency Method 1631, a dual preconcentration method using a Model 2600 Cold Vapor Atomic Fluorescence Spectroscopy Mercury Analysis system (Tekran Instruments). Organic contaminant determination in cerumen used a recently developed selective pressurized liquid extraction in-cell clean-up method with basic alumina, silica gel, and Florisil adsorbents followed by analysis with an Agilent gas chromatograph 7890 coupled to a Agilent mass spectrometer 5975C in electron capture negative ionization and electron impact modes.

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