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Supplementary Material

Ethyl-Iophenoxic acid as a serum marker for oral baiting of Tasmanian devils

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Methods

Negative electrospray ionisation

Electrospray ionisation was performed with a capillary voltage of 2.8 kV, and individually optimised cone voltages and collision energies for each MRM transition, as described below. The desolvation temperature was 450°C, nebulising gas was nitrogen at 950 L/h and cone gas was nitrogen at 50 L/h. SIM analysis for IPA utilised the deprotonated molecule [M-H]- (m/z) 570.70 with a cone voltage of 15 V. MRM transitions monitored for IPA were [M-H]- (m/z) 570.70 to 442.80 (cone voltage 15 V; collision energy 17 V) and (m/z) 570.70 to 126.80 (cone voltage 15 V; collision energy 13 V. Dwell time per channel was 161 ms.

Table S1. Identification, sex, age and location of devils whose serum samples were used for the external calibration step of the Et-IPA detection method

Devil ID	Sex	Age at sampling (years)	Location
Gwen	female	4	captive
Kwasi	male	2	Maria Island
Little Sitkin	Female	3	Maria Island
Lone Ranger	Male	2	Maria Island
Miss Marvel	Female	2	Maria Island
Volt	Male	2	Maria Island
Thor II	Male	2	Maria Island
Silverbeet	Male	2	Stony Head
Pan	Male	1	Stony Head

Table S2. Results of linear regression analysis of serum Et-IPA levels over time for devils TD1 to TD8 (Data shown in Figure 2 of manuscript). Statistical analysis performed by GraphPad Prism 8.0.

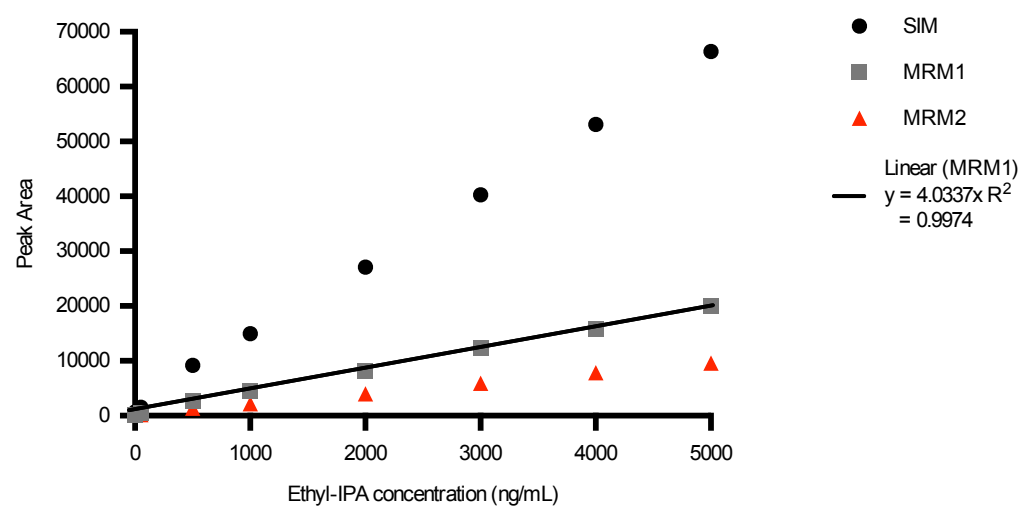
	TD1	TD2	TD3	TD4	TD5	TD6	TD7	TD8
R^2	0.944	0.927	0.992	0.997	0.990	0.995	0.992	0.994
Sy.x	0.230	0.385	0.123	0.107	0.108	0.104	0.128	0.098
F	67.71	50.51	260.6	590.7	311.2	651.8	357.4	508.1
DFn, DFd	1, 4	1, 4	1, 2	1,2	1,3	1,3	1,3	1,3
P value	0.001	0.002	0.004	0.002	<0.001	<0.001	<0.001	<0.001
Equation	Y = -0.01263 *X + 3.820	Y = -0.01829 *X + 3.399	Y = -0.01405 *X + 4.072	Y = -0.01837 *X + 4.110	Y = -0.01321 *X + 3.628	Y = -0.01832 *X + 3.301	Y = -0.01678 *X + 3.044	Y = -0.01525 *X + 2.777

Table S3. Results of linear regression analysis of serum Et-IPA levels over time for devils TD1 to TD8 (Data shown in Figure 3 of manuscript). Statistical analysis performed by GraphPad Prism 8.0.

	Day 1	Day 56	Day 180-206
R^2	0.364	0.056	0.337
Sy.x	0.456	0.527	0.628
F	3.428	0.359	3.049
DFn, DFd	1,6	1,6	1,6
P value	0.114	0.571	0.131
Equation	Y = 0.1040*X + 3.328	Y = 0.03894*X + 2.475	Y = 0.1351*X + 0.3398

Figure S1. Three modes of analysis for detecting Ethyl-IPA in Tasmanian devil serum samples (day 1). First, single ion monitoring (SIM) detection of the Et-IPA precursor deprotonated molecular ion $[M-H]^-$ (m/z) 570.7. Second and third are the multiple reaction

monitoring (MRM) molecular transitions 1 & 2, where the precursor ion is then fragmented into two specific product ions, (m/z) 442.8 and 126.8 respectively. The MRM1 data is that used to undertake the actual calibration, and this is the data that is displayed for the samples


70000
60000
50000
40000
30000
20000
10000
0
Peak Area
0
1000
2000
3000
4000
5000
Ethyl-IPA concentration (ng/mL)
SIM
MRM1
MRM2
Linear (MRM1)
y = 4.0337x R2
= 0.9974