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IR-MALDESI Mass Spectrometry Imaging of Rat Placenta Tissue After Exposure to Flame Retardants

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Introduction

Chemical exposures can adversely impact fetal development. For many compounds, such as flame retardants, the mechanisms by which this occurs remain unclear, but emerging evidence suggests that disruption at the level of the placenta may play a role. The placenta is a vital but ephemeral organ developed during pregnancy to deliver nutrients and facilitate gas exchange between mother and fetus. Disruption of placental function could result in poor fetal development and/or disease development later in life. It has been shown, for example, that flame retardants such as Firemaster® 550 (FM550) can disrupt placental production of neurotransmitters such as serotonin, which are critical to support fetal brain development.[1-2] Therefore, it is of high interest to develop tools for investigating how anthropogenic chemicals affect biological processes such as neurotransmitter production in the placenta.

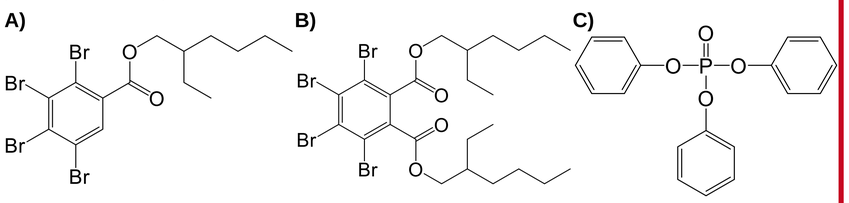

Figure 1. Primary components in flame retardant mixtures, FM550 and BZ-54. **A)** 2-ethylhexyl-2,3,4,5-tetrabromobenzoate, **B)** bis(2-ethylhexyl) tetrabromophthalate, **C)** triphenyl phosphate. BZ-54 contains only A and B components.

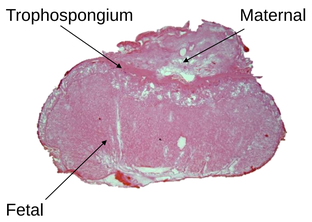

Figure 2. Optical image of the rat placenta depicting the maternal (mesometrium), trophospongium, and fetal (labyrinth zone) regions.

The primary objective of this study was to develop a method for detecting placental neurotransmitters and related metabolites without chemical derivatization so changes in the abundance and spatial distribution of neurotransmitters in rat placenta following chemical exposure could be determined using infrared matrix-assisted laser desorption electrospray ionization (IR-MALDESI) mass spectrometry imaging.[3]

Neurotransmitter Analysis by Mass Spectrometry Imaging

Table 1. Summary of neurotransmitter and related-metabolite detection in a single experiment by common mass spectrometry imaging (MSI) ionization techniques. Neurotransmitter analysis by MSI methods has historically required the use of chemical derivatization due to interfering organic matrix clusters. Inherent disadvantages to chemical derivatization include increased sample preparation time, additional reagents and resources, unknown reaction extent and functional group specificity.

Metabolites Detected	Sample Type	MSI Platform	Chemical Derivatization	Reference
5	Standards	MALDI TOF/TOF[1]	No	Segiura et al., *Anal Bioanal Chem*, **2012**, 403, 1851-1861
5	Rat Brain	MALDI LTQ Orbitrap XL	No	Ye et al., *ACS Chem Neurosci*, **2013**, 4, 1049-1056
9	Rat Brain	MALDI FT-ICR	Yes – Primary amines	Shariatgorji et al., *Neuron*, **2014**, 84, 697-707
12	Crab Brain	MALDI LTQ Orbitrap XL	No	Ye et al., *ACS Chem Neurosci*, **2013**, 4, 1049-1056
16	Rat Brain	IR-MALDESI Q Exactive Plus	No	Bagley et al., *Anal Bioanal Chem*, **2018**, 410, 7979-7986
35	Rat Brain	MALDI FT-ICR[1]	Yes - Phenol and primary amines	Shariatgorji et al., *Nat Methods*, **2019**, 84, 697-707

[1]MS/MS was performed on select molecules in these studies.

Experimental Design and Workflow

Dosing Group	Total Consumption	Animals per Group
BZ-54	1000 mg BZ54	1
High FM550	110 mg FM550	1
Low FM550	100 mg FM550	2
Control	Sesame Oil	2

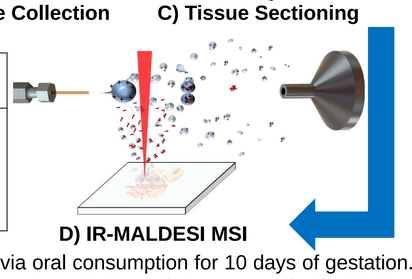

Figure 3. A) Pregnant rats were dosed daily via oral consumption for 10 days of gestation. **B)** Tissues were collected at GD18 and stored in -80°C until time of analysis. **C)** One placenta per sex was sectioned at 20 µm and thaw mounted onto glass slides. **D)** Tissue sections were kept at -10°C in a humidity-controlled enclosure for the entire experiment. A thin ice layer was formed by controlled exposure to ambient humidity.[4] Each tissue section was then imaged for neurotransmitters with IR-MALDESI coupled to a Q Exactive Plus Mass Spectrometer.[3] Ion images were generated and analyzed using MSiReader.[5]

Neurotransmitters and Metabolites Detected in Rat Placenta

Table 2. List of neurotransmitters and related-metabolites detected in control placenta tissue by IR-MALDESI. Each metabolite was detected with high mass and spectral accuracy.

	Molecule	$[M+H^+]^+$		Molecule	$[M+H^+]^+$
1	Imidazole-4-acetaldehyde	111.0553	26	Tryptamine	161.1073
2	Histamine	112.0869	27	Phenylalanine	166.0863
3	Phenethylamine	122.0964	28	3-methoxytyramine/synephrine	168.1019
4	Methylimidazole acetaldehyde	125.0709	29	Norepinephrine	170.0812
5	N-Methylhistamine	126.1026	30	1-Methylhistidine	170.0924
6	Imidazole-4-acetate	127.0502	31	Arginine	175.1190
7	4-Acetamidobutanal	130.0863	32	N-methyltryptamine	175.1230
8	N-Acetylputrescine	131.1179	33	5-hydroxyindole-3-acetaldehyde	176.0706
9	Adenine	136.0618	34	Serotonin	177.1022
10	N-methylphenethylamine	136.1121	35	Tyrosine	182.0812
11	Hypoxanthine	137.0458	36	Epinephrine	184.0968
12	Tyramine	138.0913	37	N-methylserotonin	191.1179
13	Urocanate	139.0502	38	L-Dopa	198.0761
14	Methylimidazoleacetic acid	141.0659	39	Spermine	203.2230
15	4-Acetamidobutanoate	146.0812	40	Tryptophan	205.0972
16	4-Guanidinobutanoate	146.0924	41	Formyl-5-hydroxykynurenamine	209.0921
17	Acetylcholine	146.1176	42	N-acetylserotonin	219.1128
18	Spermidine	146.1652	43	5-hydroxytryptophan	221.0921
19	L-Glutamine	147.0764	44	Melatonin	233.1285
20	Glutamate	148.0604	45	Homocarnosine/Anserine	241.1295
21	Guanine	152.0567	46	6-Hydroxymelatonin	249.1234
22	Dopamine	154.0863	47	5'-Deoxyadenosine	252.1091
23	L-Histidine	156.0768	48	Adenosine	268.1040
24	4-Imidazolone-5-propanoate	157.0608	49	Anandamide	348.2897
25	Indole-3-acetaldehyde	160.0757			

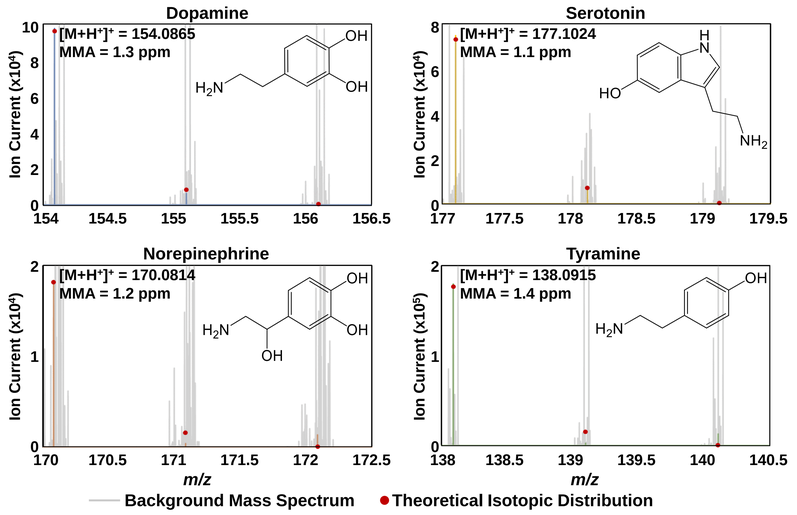

Figure 4. Four examples of neurotransmitters putatively identified with accurate mass (<2.5 ppm MMA) and spectral accuracy in placenta tissue from the female control group.[6] Red circles represent theoretical isotopic distribution, grey lines represent the background mass spectrum, and colored lines represent experimental isotopic distribution for each respective molecule.

Spatial Distributions of Representative Neurotransmitters

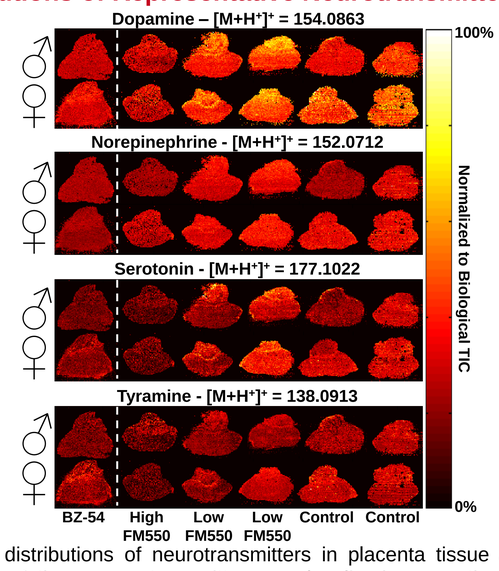

Figure 5. Spatial distributions of neurotransmitters in placenta tissue as a function of exposure. All four of these neurotransmitters are localized across the whole placenta. While dopamine and norepinephrine appear to be unaffected by exposure levels, serotonin and tyramine have lower normalized abundance in the BZ-54 and high FM550 exposure groups compared to the control group. Neurotransmitters are normalized to a biological TIC which consists of 10 homogenous ions specific to placenta tissue. Dotted white line is used to separate BZ-54 from FM550 exposed tissues.

Pathway Analysis of Neurotransmitters in Rat Placenta

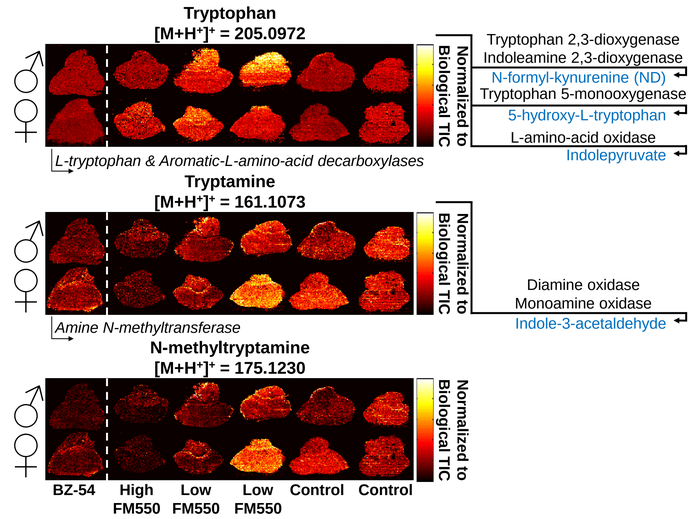

Figure 6. Ion image metabolic pathway of tryptamine. Tryptamine is produced from tryptophan which has homogenous distributions across exposure groups. In the metabolism of tryptophan, the control tissues produce more tryptamine compared to the higher dosed tissues. This indicates a possible disruption in the metabolism of tryptophan to tryptamine. Other metabolites (blue) and their respective enzymes (black) involved in this pathway are shown on the right. Dotted white line is used to separate BZ-54 from FM550 exposed tissues.

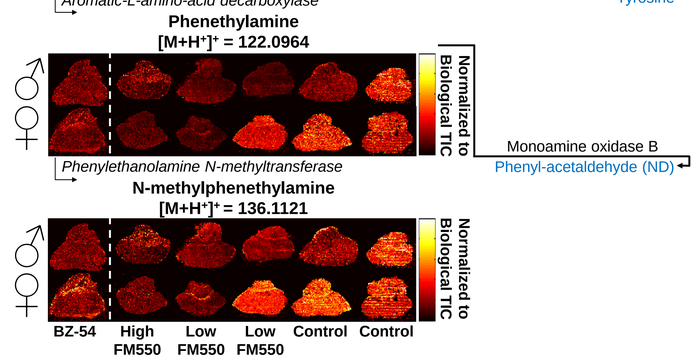

Figure 7. Phenylalanine metabolic pathway depicting the production and metabolism of phenethylamine. Phenylalanine is more abundant in the maternal region of the placenta but does not have a dosing group effect. Phenethylamine decreases in abundance with increasing exposure to flame retardants. This implies that the reaction to form phenethylamine could be impaired when exposed to flame retardants. Other metabolites (blue) and their respective enzymes (black) involved in this pathway are shown on the right. Dotted white line is used to separate BZ-54 from FM550 exposed tissues.

Conclusions

- This method presents the capability of deep neurotransmitter coverage not previously achieved in a single imaging experiment without chemical derivatization.
- IR-MALDESI detected 49 neurotransmitters and metabolites in their native form in untreated rat placental sections after gestational exposure to flame retardants.
- A few neurotransmitters appeared to have been impacted by exposure to one or both flame retardant mixtures.
- Initial biological investigations indicate that the enzyme activity could be disrupted in the production of neurotransmitters.

Literature Cited

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Acknowledgments

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