Pyrrolizidine Alkaloids in Teas, Herbal Infusions, Plant-Based Food Supplements and Honey

FSA Executive Summary

Introduction

Pyrrolizidine alkaloids (PAs) are toxins found naturally in a wide variety of plant species. Over 6,000 plant species are known to contain PAs, and they are produced by plants as a defence mechanism against herbivores. They are widely distributed and affect wildlife, livestock and humans. Cases of human toxicity have been shown to occur following contamination of staple foods, generally grain crops which are accidentally contaminated with PA containing weeds, and after consumption of some herbal remedies. Other possible food sources of exposure include milk, honey, offal and eggs, although cases of human poisoning resulting from exposure through these sources have not been reported. The main toxic effects of PAs are on the liver and lungs. A sub-group of PAs such as the 1,2-unsaturated PAs are genotoxic and those that have been tested cause liver cancer in experimental animals. The European Food Safety Authority (EFSA) has concluded that 1,2-unsaturated PAs may act as carcinogens in humans (EFSA, 2011).

The European Commission recommended the monitoring of relevant food and feed commodities for the presence of PAs in order to inform future EFSA evaluations. The Food Standards Agency (FSA) therefore commissioned a survey to measure the levels of PAs in teas, herbal infusions, plant-based food supplements and honey. The data collected as part of this survey were submitted to EFSA for their evaluation.

Results and Discussion

55 samples of tea from *Camelia sinensis* (common black and green teas), 70 samples of herbal infusions, 45 samples of plant-based supplements and 54 samples of honey were analysed in this study. Details of the samples tested, analytical methods and results are given in Appendices 1 - 3.

The samples were purchased from a range of national supermarkets, smaller retailers, health/natural/organic food stores and UK internet/mail order retailers between February and March 2014. The sample plan was intended to reflect the main products and brands within the major categories, taking into account available market share data. The levels observed are a snapshot of what was present at the time of sampling. The absence of any particular brand from this survey means only that the brand was not included in the survey. No further meaning should be read into the absence of any brand.

The analytical results are summarised in the table below:

Type of Tea	Total no. of samples	No. of samples in which PAs were detected	0 - 100 µg/kg	100 - 500 µg/kg	500 - 1000 µg/kg	1000 - 3000 µg/kg	>3000 µg/kg	Range (µg/kg)
Teas (black, green and Earl Grey)	55	11	6	4	-	1	-	< LOQ - 1,170
Herbal infusions	70	35	9	12	8	4	2	< LOQ - 52,508 *
Plant-based supplements	48[#]	5	2	3	-	-	-	< LOQ - 344
Honeys	54	35	29	6	-	-	-	< LOQ - 251

* The highest levels were from borage and comfrey infusions which are known to contain high levels of PAs.
[#] Three of the 48 samples could not be tested.

The results of this survey are similar to the levels reported by Bundesinstitut fur Risikobewertung (BfR, 2013), EFSA (EFSA, 2011 and 2015) and in scientific literature.

Teas and herbal infusions:
PAs were present at low levels in the samples in which they were detected; most of them below 500 µg/kg. Just two samples of herbal infusions containing borage and comfrey had very high levels (> 50,000 µg/kg). Borage and comfrey are known to contain high levels of PAs. None of the other plants used in teas and herbal infusions contain PA producing plants; therefore, the low levels of PAs detected in these samples are most likely to be from adventitious contamination from weeds which may coexist with the crop.

Plant-based food supplements:
PAs were not detected in most of the samples; only five samples of the 45 tested had detectable levels.

Honeys:
The levels detected in most of the samples were very low (29 samples contained measurable levels of PAs at concentrations of < 50 µg/kg). Samples of borage honey (110 - 163 µg/kg), one sample of blended honey (185 µg/kg) and one sample of heather honey (251 µg/kg) had slightly higher levels.

Discussion on exposure and risk

Doses associated with acute and short-term toxicity in humans are in the region of 1 mg PA/kg body weight (b.w.) per day or more. The lowest known dose associated with longer-term toxicity is reported to be 15 µg PA/kg b.w. per day. Based on the

available genotoxicity and carcinogenicity data, it is concluded that exposure to 1,2-unsaturated PAs should be reduced to as low as reasonably practicable (ALARP). A reference point on the dose range in animal studies for 10% increased tumour incidence (BMD_{10}) and its lower confidence limit ($BMDL_{10}$) of 237 µg/kg b.w. per day has been identified for the PA riddelliine. Margin of Exposure (MOE) = $BMDL_{10}$ divided by estimated dietary exposure. MOE > 10,000 based on $BMDL_{10}$ from animal data is "*of low concern from a public health point of view ……. low priority for risk management action*" (EFSA, 2005).

Exposure to PAs has been estimated from the results of the retail survey of PAs in the following products together with consumption data from the National Diet and Nutrition Survey (NDNS) (Bates *et al.*, 2014; Bates *et al.,* 2016; Roberts *et al.,* 2018) or portion sizes: (i) teas and herbal infusions, (ii) plant-based food supplements and (iii) honeys. Details of the assessments are given in Appendix 3.

The estimated exposures from herbal infusions indicate a concern for increased risk of cancer, particularly in the case of borage and comfrey infusions. The 97.5th percentile exposure estimates for PAs from consumption of borage and comfrey infusions for toddlers were at levels where human toxicity has occurred indicating that toddlers who drink large amounts of borage and comfrey infusions could also be at risk of liver damage. Black and green teas were found to have lower levels of PAs indicating a low level of concern.

Of the 45 samples of food supplements that were analysed, exposure at the consumption levels of marshmallow capsules, barley grass powder and maca powder recommended by the manufacturers indicated a concern for increased cancer risk. PAs were also detected in the mixture of organic herbs, but at a level that would only be a concern if the product is taken in amounts exceeding the level recommended by the manufacturer.

The levels of PAs in a small number of honey samples could indicate a risk. However, due to the very low sample number affected and following further results from honey producers, this does not indicate a concern at present. This will be reassessed in future, if necessary.

Conclusions and follow up

The levels of PAs found in this survey of 227 samples comprised of teas, herbal infusions, plant-based supplements and honeys are comparable to previously published literature reports (EFSA, 2017).

Although the levels of PAs in most of the samples tested in this study were low (except those with borage and comfrey), efforts should be taken to reduce exposure to PAs as they could increase the risk of cancer.

Specifically, herbal infusions with comfrey may contain significant levels of PAs and consumption of these should be avoided. This reiterates previous advice provided by

the Committee of Toxicity in 2008[1], and 1992[2]. Since borage infusions have also been found to contain high levels of PAs, like comfrey, consumption of herbal infusions made with borage is not recommended. PAs in borage oil is currently not an issue (EFSA, 2011).

As it is evident that PAs are present in teas and herbal infusions as a result of contamination from PA containing weeds, more rigorous quality control and good agricultural practices including better weed control, harvesting and processing are being put in place to minimize PA levels[3].

The FSA has been working with the producers of teas, herbal infusions, plant-based supplements and honey in identifying measures that will reduce levels of PAs in these foods. The Food Business Operators (FBOs) have identified and implemented good agricultural practices in the growing and harvesting of the plant material used in the production of these products. FBOs have shown that subsequent testing, since 2014 when this work was carried out, of these foods has indicated that the mitigatory measures have been successful in reducing the levels of PAs. The FSA will continue to monitor the levels of PAs in food.

The FSA has been working to develop risk management measures with the European Commission and other Member States to ensure that levels of PAs in teas, herbal infusions, food supplements and honey to ensure that levels of PAs are as low as reasonably achievable.

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List of Appendices:

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Appendix 1
Sample details

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010725	PG Tips	Regular Pyramid	Tesco	160 bags	Unknown	Jul-15	L40342P132 09:00
S14-010727	PG Tips	The Fresh One	Tesco	80 bags	Unknown	Apr-15	L33161B132 16:09
S14-010556	PG Tips	The Rich One	Asda	80 bags	Unknown	May-15	L33393B132 06:09
S14-010561	PG Tips	Zesty Lemon	Asda	20 bags	Unknown	Dec-15	L33363L620
S14-010584	PG Tips	The Strong One	Sainsbury's	80 bags	Unknown	Apr-15	L33311C132 06:43
S14-010597	Simpli-Special	English Breakfast Luxury loose leaf tea	Amazon	100 grams	Unknown	Aug-15	not declared
S14-010726	PG Tips	Loose Tea	Tesco	250 grams	Unknown	Jun-15	L40091 A620
S14-010724	PG Tips	Decaf Pyramid tea bags	Tesco	160 bags	Unknown	May-15	L33471F132 08:17
S14-010560	PG Tips	Pure Green Tea	Asda	20 bags	Unknown	Dec-15	L33403L620
S14-010732	Tetley	Extra Strong (black tea)	Tesco	80 bags	Unknown	Mar-15	L 3 252 14:22
S14-010731	Tetley	Easy Squeeze	Tesco	80 bags	Unknown	Jul-15	L4024
S14-010733	Tetley	Green Tea Pure	Tesco	50 bags	Unknown	Jan-16	L4028
S14-010547	Tetley	Green Tea Decaf	Morrison	50 bags	Asia	Nov-15	L3316 09:10
S14-010734	Tetley	Green Tea Lemon	Tesco	50 bags	Unknown	Jan-16	L4027
S14-010589	Tetley	Blend of Both Tea Bags	Asda	80 bags	Africa/Asia/Assam	May-15	L3326 17:51
S14-010548	Tetley	Estate Selection	Asda	80 bags	Kenya	Dec-14	3176C
S14-010779	Tea makers of London	Luxury supreme Earl Grey black loose tea	Amazon	125 grams	Unknown	Jul-17	not declared
S14-010736	Twinings	Earl Grey Loose Tea	Tesco	125 grams	Unknown	Oct-15	4081884-296
S14-010735	Twinings	English Breakfast Loose Tea	Tesco	125 grams	Unknown	Dec-15	4369220-344
S14-010591	Twinings	Everyday	Asda	160 bags	China	Jan-16	A0000010619
S14-010730	Tesco	Original Loose Tea	Tesco	250 grams	Unknown	May-15	L4037 P1
S14-010728	Tesco	Everyday Value Tea bags	Tesco	80 bags	Unknown	May-15	L4035 2B

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010729	Tesco	Finest Fair Trade Black Leaf Tea	Tesco	80 bags	Unknown	Dec-14	L3255 2
S14-010585	Typhoo (Apeejay)	Lift Instant Lemon Tea	Sainsbury's	150 bags	Unknown	Jul-15	4013 19:50
S14-010558	Typhoo (Apeejay)	Lift Instant Apple Tea	Sainsbury's	300 grams	Unknown	Jul-15	4015 14:56
S14-010647	Morrison	Extra Strong tea bags	Morrison	80 bags	Unknown	Apr-15	L3329 2
S14-010555	Typhoo (Apeejay)	Lift Instant Peach Tea	Morrison	300 grams	Unknown	Jul-15	4016 01:06
S14-010587	Yorkshire Tea	Everyday Loose Tea	Asda	250 grams	Unknown	Apr-15	L3309 0 36 10:39
S14-010588	Yorkshire Tea	Gold Loose Tea	Morrison	250 grams	Unknown	Mar-15	L3301 E 16 09:09
S14-010544	PG Tips	Loose tea	Morrison	250 grams	Unknown	Mar-15	L32771A620 12:52
S14-010582	Morrison	Red Label Loose Tea	Morrison	250 grams	Unknown	Jun-15	L4030 P1
S14-010551	Typhoo (Apeejay)	Leaf Tea	Morrison	250 grams	Unknown	Jul-16	L3204 B3 11:14
S14-010760	Whittard of Chelsea	English Breakfast Loose Tea	Whittard	100 grams	Unknown	Feb-15	112015 302174
S14-010545	Twinings	Assam Loose Tea	Morrison	125 grams	North East Assam	Feb-15	3661623 2213 13:08
S14-010740	Make Mine A Builders	Strong Cuppa	Morrison	80 bags	Unknown	May-15	3332C
S14-010549	Yorkshire Tea	Gold Tea Bags	Asda	160 bags	Assam/East Africa	Mar-15	3287WDE 12:56
S14-010683	Yorkshire Tea	Tea Bags	Morrison	160 bags	Unknown	May-15	3346WB0 12:43
S14-010553	Yorkshire Tea	Tea Bags for Hard Water	Asda	160 bags	Unknown	Oct-15	3164WBH 09:08
S14-010766	PG Tips	The Mellow One	Morrison	80 bags	Unknown	Jan-15	L32243B132 10:03
S14-010552	Clipper	EverydayTea	Morrison	80 bags	East Africa/ India/Sri Lanka	Oct-15	E9647
S14-010593	PG Tips	Decaffeinated Tea Bags	Asda	80 bags	Unknown	Jun-15	L40173J132 06:12
S14-010758	Whittard of Chelsea	Breakfast Earl Grey Loose Tea	Whittard	100 grams	Unknown	Feb-15	062015 302257
S14-010590	Tetley	Tea Bags	Asda	160 bags	Africa/Assam	Feb-15	L3352 08:40
S14-010554	Tetley	Decaffeinated Tea Bags	Morrison	160 bags	Unknown	May-15	L3308 17:37
S14-010592	PG Tips	Tea bags	Asda	160 bags	Unknown	Jun-15	L40313P132 13:40

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010550	Asda	Chosen by you	Asda	160 bags	Unknown	Jun-15	4002A 17:33
S14-010546	Asda	Assam tea	Asda	80 bags	Unknown	Jul-15	4021C 16:31
S14-010557	The Co-operative	Earl Grey Tea	The Co-operative	100 bags	Unknown	Feb-15	3240C 17:29
S14-010776	Sainsbury's	Basics Everyday tea	Sainsbury's	80 bags	Unknown	Aug-15	4059 N 07:52
S14-010583	Sainsbury's	Taste the difference English Breakfast tea	Sainsbury's	80 bags	Unknown	Jan-15	4007
S14-010559	PG Tips	Refreshing Peppermint (Fresh Peppermint)	Asda	20 bags	Unknown	Dec-15	L33473L620 (x8), L33433L620(x7)
S14-010765	PG Tips	Delicate Camomile	Asda	20 bags	Unknown	Dec-15	L33441L620 (x14), L33443L620 (x1)
S14-010640	Tesco	Lemon & Ginger Infusion	Tesco	40 bags	Unknown	Jul 15	L4042 9C & L4027 9C
S14-010763	PG Tips	Aromatic Spices & Mint	Asda	20 bags	Unknown	Dec-15	L33473L620
S14-010764	Twinings	Comforting Liquorice Tea	Sainsbury's	20 bags	United Kingdom	Jan-16	0000011423 UK014 17:54
S14-010737	Twinings	Settling Ginger Tea bags	Tesco	80 bags	Unknown	Jan-16	120000011559 UK 020
S14-010738	Asda	Camomile	Asda	80 bags	Unknown	Dec-14; Jun-15	3171; 4015
S14-010775	Neuners	Baby stomach tea	Amazon	20 bags	Austria	Dec-16	521303329
S14-010687	Hipp	Instant Fennel tea for babies	Amazon	200 grams	Unknown	Jun-15	L307860
S14-010711	Hipp	Hipp Instant Calming Tea for Babies	Amazon	200 grams	Unknown	Aug-15	L307525
S14-010634	Whittard of Chelsea	Apple & Elderflower Infusion	Whittard	125 grams	Unknown	Mar-15	CH 0587944
S14-010609	Tesco	Tesco Finest Forest Fruits Pyramid Tea	Tesco	20 bags	Unknown	Mar-15; Feb-15	02290150248; 03264149082
S14-010817	Pukka	Detox organic aniseed fennel & cardamom	Sainsbury's	20 bags	United Kingdom	Jan-17	15:16 L4014

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010607	Pukka	Cleanse organic nettle fennel & peppermint	Whole Foods Market	20 bags	United Kingdom	Dec-16	L3339 22:44
S14-010755	Whittard of Chelsea	Nettle, Fennel & Peppermint Tag & Envelope Teabags	Whittard	40 grams	Unknown	Aug-15; Oct-15	15/08/2013; 01/10/2013
S14-010653	Morrison	Citrus Ginseng Sling	Morrison	20 bags	Unknown	Mar-15; May-15	L3355 9B; L3282 9B
S14-010614	Hampstead tea	Organic Rosehip Hibiscus	Whole Foods Market	20 bags	Unknown	31/1/17: 12/8/16: 21/10/16	L4031; L3294; L3224
S14-010649	Morrison	Aromatic Green tea	Morrison	40 bags	Unknown	May-15	L3339 9C
S14-010652	Morrison	Hint of Mint Green tea	Morrison	20 bags	Unknown	Feb-15; Mar-15	L3269 9B; L4020 9B; L3303 9B
S14-010815	Apotheke	Tea for kids Easy digestion	Amazon	20 bags	Czech Republic	Nov-15	L0026 A
S14-010656	Tetley	Green Tea Decaf	Morrison	50 bags	Unknown	Dec-15	L3351
S14-010822	Sainsbury's	Peppermint Infusion	Sainsbury's	20 bags	Unknown	Jul-15	4037 00:57
S14-010823	Sainsbury's	Liquorice infusion	Sainsbury's	20 bags	Unknown	Jun-15	4007
S14-010690	Sainsbury's	Strawberry Raspberry & Cranberry infusion	Sainsbury's	20 bags	Unknown	Jul-15	4027 14:25
S14-010794	Apotheke	Natural Cherry Tea for babies and children	Amazon	20 bags	Czech Republic	Mar-15	L0015 A
S14-010811	Luaka	Ceylon tea Pure loose tea from Luaka tea	Amazon	125 grams	United Kingdom	Jul-16	B/N 6191
S14-010596	Clipper	Snore & Peace	Sainsbury's	20 bags	EU/non-EU	Sep-15	S1156 12:08
S14-010768	Tesco	Camomile	Tesco	40 bags	Unknown	Jun-15	L4022 9A
S14-010600	Clipper	Clipper Infusion Dandelion & Burdock Tea, Organic	Amazon	20 bags	EU/non-EU	May-15	K0327
S14-010812	Clipper	Clipper organic Nettle Tea	Amazon	20 bags	EU/non-EU	Feb-16	L0788 08:55

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010821	Simpli-Special	Yummy Berry Fruit tea for all the family	Amazon	100 grams	Unknown	Sep-15	not declared
S14-010704	Leros	Detsky caj s klidne sny-Children restful sleep Herbal Tea	Amazon	30 grams	Unknown	Mar-14	05/12/2012
S14-010657	Tetley	Camomile	Morrison	40 bags	Unknown	Aug-15	L3228
S14-010659	Tetley	Peppermint	Morrison	150 bags	Unknown	Dec-15	L3364
S14-010650	Morrison	Pure Peppermint	Morrison	40 bags	Unknown	Jun-15	L4002 9B; L4042 9C
S14-010645	Twinings	Invigorating Peppermint	Morrison	80 bags	Unknown	Aug-15	120000004654 UK 241
S14-010628	Simpli-Special	Simpli-Special Sencha Kyoto Cherry Rose Luxury loose tea Leaf	Amazon	100 grams	Unknown	Sep-15	not declared
S14-010773	Pukka	Three Fennel	Whole Foods Market	20 bags	United Kingdom	Dec-16	L3354 11:24
S14-010655	Twinings	Calming Camomile	Morrison	80 bags	Unknown	Sep-15	120000006195 UK 269
S14-010632	Number One Brand	Milk Green Tea	Amazon	200 grams	Thailand	Feb-16	MFD 21/2/14
S14-010762	Clipper	Organic Sleep Infusion	Whole Foods Market	20 bags	EU/non-EU	Feb-16	H0806 11:07
S14-010813	Clipper	Clipper Green Tea with Ginseng	Amazon	20 bags	EU/non-EU	Mar-15	V0990
S14-010710	Special tea company	Special tea Avena Dream herbal Loose leaf tea	Amazon	85 grams	Unknown	Dec-15	MB1957
S14-010630	Simpli-Special	Simpli-Special Hibiscus-herbal tea Loose leaf	Amazon	100 grams	Unknown	Aug-15	not declared
S14-010705	Special tea company	White hibiscus herbal loose leaf tea	Amazon	85 grams	Unknown	Jan-15	FB029
S14-010638	Fresh & Wild Organic	Organic Nettle Tea	Whole Foods Market	25 bags	Poland	Feb-17	L4057
S14-010723	Tick Tock	Tick Tock Rooibos	Tesco	80 bags	Unknown	Nov-16	3345

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010722	Dragonfly	Dragonfly Rooibos Vanilla Tea	Tesco	40 bags	South Africa	Oct-16	3294 17:33
S14-010646	Waitrose	Waitrose Rooibos caffeine free	Waitrose	80 bags	Republic of South Africa	Jan-15	05-D2
S14-010648	Tetley	Redbush & Vanilla (rooibos)	Morrison	40 bags	Unknown	Aug-15; Oct-15	L3291; L3228
S14-010804	Heath & Heather	Heath & Heather Rosehip & Hibiscus Herbal Infusions	Holland & Barrett	50 bags	European Union	Jan-17	L4015 9C
S14-010761	Sussex Wholefoods	Comfrey Leaves (Loose Tea)	Healthy supplies	50 grams	Hungary	Mar-15	6797
S14-010707	Hipp	Hipp Instant Digestion Facilitating Tea for Babies	Amazon	200 grams	Unknown	Jun-15	L307816
S14-010611	North American Herb & Spice	WildPower Tea	Amazon	56 grams	Unknown	Jul-15	Lot E12206
S14-010712	Holle	Multipack Holle Organic Baby Teas	Amazon	120 bags	czech Republic	Nov-15	1023151113 CS
S14-010816	Apotheke	Calming Tea For Children Organic	Amazon	20 bags	Czech Republic	Dec-15	L0029
S14-010577	Whittard of Chelsea	Very Berry Burst	Whittard	100 grams	Unknown	Jun-15	1065505-1
S14-010777	Qi	Qi Ginseng Vitality Tea	Amazon	25 bags	Unknown	Apr-16	43826161
S14-010629	Simpli-Special	Simpli-Special Red Ginseng Green Sencha Loose Leaf Wellness Tea	Amazon	100 grams	Unknown	Sep-15	not declared
S14-010767	The Kent and Sussex Tea & Coffee Co	Lemon Balm Tea	Tea and coffee	500 grams	Unknown	Feb-15	not declared
S14-010778	Indigo Herbs	Lemon Balm (Melissa Officinalis) Loose Herbal Tea	Amazon	50 grams	Spain	Oct-15	IH3014516
S14-010623	Birt & Tang	Birt & Tang Ginseng herbal tea	Holland & Barrett	20 bags	China	22/04/2016, 21/2/15	23/04/2013, 22/2/12

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010809	Dragonfly	Dragonfly Tea, Naturally Caffeine Free Rooibos Earl Grey	Holland & Barrett	40 bags	South Africa	Oct-16	3294 19:38
S14-010808	Qi	QI Teas Organic Fairtrade Green Tea With Ginkgo	Holland & Barrett	25 bags	China	Apr-16	P171013E 64746262
S14-010793	Heath & Heather	Heath & Heather Fennel Herbal Infusions	Holland & Barrett	50 bags	European Union	Jan-17	L4014 9C
S14-010701	Suki tea	Suki tea Loose green tea Sencha (organic)	Amazon	100 grams	China	Jul-15	L1400904
S14-010806	Clearspring	Clearspring Organic Loose Sencha Tea	Planet Organic	125 grams	Japan	Jul-15	24/07/2013
S14-010756	Whittard of Chelsea	Cinnamon Chai Caffeine Free Herbal Infusion	Whittard	125 grams	Unknown	Jun-15	201290
S14-010757	Whittard of Chelsea	Acai and Goji Berry Caffeine Free Fruit Infusion	Whittard	125 grams	Unknown	Jul-15	224600
S14-010759	Whittard of Chelsea	Crème Caramel Flavour Rooibos Loose Tea	Whittard	100 grams	Unknown	Feb-15	022015 301432
S14-010774	Celestial	Celestial Seasoning herb tea Tension Tamer	Amazon	20 bags	United States	Jul-15	not declared
S14-010807	Clearspring	Clearspring Organic Genmaicha Tea	Planet Organic	20 bags	Japan	Jul-15	24/07/2013
S14-010631	Whittard of Chelsea	Blueberry Blaster Infusion	Whittard	125 grams	Unknown	Apr-14	CH:0550655
S14-010618	The Cozy Leaf	Organic Lactation Blend for Nursing Mommy Loose Tea	etsy.com	124 grams	United States	not declared	not declared
S14-010622	Urban Earth	Abundance Organic herbal blend for nursing moms (with borage)	etsy.com	113 grams	Unknown	not declared	not declared
S14-010782	Holland & Barrett	Cranberry tablets	Holland & Barrett	250 tablets	Unknown	Mar-16	72966103

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010810	Simply supplements	Gingko Biloba	Amazon	360 tablets	Unknown	Feb-16	11975
S14-010686	Nature's Answer	Elder Berry Herbal Supplement	Whole Foods Market	120 ml	Unknown	Feb-17	130147
S14-010820	Solgar	Olive Leaf Extract	Whole Foods Market	60 capsules	Unknown	Aug-16	747373-03
S14-010606	Nature's Garden	Fenugreek	Holland & Barrett	100 capsules	Unknown	Sep-16	73096601
S14-010620	Good'n natural	Raspberry leaf	Holland & Barrett	100 capsules	Unknown	Feb-15	332840-03
S14-010718	Now	Triple Strength Liquid Chlorophyll	Whole Foods Market	473 ml	United States	Aug-15	1566690 2117
S14-010792	Holland & Barrett	Saw palmetto	Holland & Barrett	100 capsules	Unknown	Jul-16	751937-01
S14-010697	Udo's Choice	Beyond Greens	Whole Foods Market	255 grams	United States	Oct-14	102313
S14-010598	Synergy Natural	Barley Grass	Whole Foods Market	200 grams	Unknown	Feb-16	S557
S14-010699	Synergy Natural	Wheat Grass	Whole Foods Market	200 grams	Unknown	Oct-16	S643
S14-010715	Organic Burst	Wheat Grass powder	Whole Foods Market	60 grams	United Kingdom	Nov-15	25017
S14-010796	Naturya	Maca Powder	GNC	300 grams	Peru	Nov-15	408011
S14-101577	GNC	Sage leaf	GNC	90 capsules	Unknown	Sep-16	73058102
S14-010635	Pukka	Organic Gotu Kola & Turmeric	Whole Foods Market	90 capsules	Unknown	Aug-14	lot 12/26/9
S14-010642	Nature's Garden	Ginger root	Holland & Barrett	100 capsules	Unknown	Sep-16	73076601
S14-010633	Nature's Garden	Fennel Seed capsules	Holland & Barrett	100 capsules	Unknown	Jul-16	73055701
S14-010803	GNC	Siberian Ginseng	GNC	90 capsules	Unknown	Jun-15	73012503
S14-010802	GNC	Triple Ginseng	GNC	30 capsules	Unknown	Oct-14	72881601
S14-010569	Pukka	Organic Andrographis & Holy Basil	Whole Foods Market	90 capsules	Unknown	Mar-15	B:110636
S14-010579	Pukka	Organic Andrographis	Whole Foods Market	90 capsules	Unknown	Jan-16	lot 13/42/12

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010797	GNC	Acai	GNC	120 capsules	United States	Sep-14	72710604
S14-010580	Pukka	Organic Ginger	Whole Foods Market	90 capsules	Unknown	Jan-16	lot:13/42/10
S14-010615	Terra Nova	Artichoke Leaf	Whole Foods Market	50 capsules	Unknown	Nov-16	1300328
S14-010819	Terra Nova	Garlic	Whole Foods Market	50 capsules	Unknown	Nov-16	13507
S14-010570	A.Vogel	Echinaforce	Whole Foods Market	120 ml	Unknown	Aug-14	01072B
S14-010789	Holland & Barrett	Liquorice root	Holland & Barrett	100 capsules	Unknown	Apr-16	501451-01
S14-010706	A.Vogel	Avena Sativa	Whole Foods Market	50 ml	Unknown	Jun-17	25083
S14-010576	GNC	Extra Strength Peppermint Oil	GNC	60 capsules	Unknown	Feb-15: Dec-14	72817401: 72653101
S14-010698	A.Vogel	Dandelion	Whole Foods Market	50 ml	Unknown	Jun-16	16113
S14-010578	A.Vogel	Ginkgo Bilboa	Whole Foods Market	100 ml	Unknown	Dec-15	10092
S14-010571	A.Vogel	Milk Thistle	Whole Foods Market	100 ml	Unknown	Feb-16	3083
S14-010713	A.Vogel	Plantago	Whole Foods Market	50 ml	Unknown	Sep-18	12014
S14-010714	A.Vogel	Neem Oil	Whole Foods Market	100 ml	Unknown	Nov 15; Jan 17	12K2208; 14A1606
S14-010791	Holland & Barrett	Beetroot extract	Holland & Barrett	90 tablets	Unknown	Sep-16	73052001
S14-010790	Good n natural	Hawthorn berries	Holland & Barrett	100 capsules	Unknown	Jan-15	333958-01
S14-010781	Holland & Barrett	Odour controlled Garlic	Holland & Barrett	100 tablets	Unknown	Oct-16	73067303
S14-010787	Holland & Barrett	Manchurian Ginseng	Holland & Barrett	50 tablets	Unknown	Apr-16	72978902
S14-010689	Nature's Plus	Papaya Enzyme	Whole Foods Market	180 tablets	United States	May-15	1243621
S14-010641	Nature's Garden	Slippery Elm	Holland & Barrett	100 capsules	Unknown	Jul-16	73053303
S14-010644	Forever Young	Whole Leaf Aloe Vera	Whole Foods Market	500 ml	Central America	May-15	10412E
S14-010581	Lily of the Desert	Aloe vera Gel Whole Leaf	Whole Foods Market	473 ml	United States	Oct-15	29433

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010786	Holland & Barrett	Guarana	Holland & Barrett	90 tablets	Unknown	Jan-16	72923002
S14-010619	Good'n natural	Cayenne capsules	Holland & Barrett	100 capsules	Unknown	Jul-17	752228
S14-010716	Aloe Pura	Aloe vera Juice with Manuka Honey	Whole Foods Market	500 ml	United States	Jan-15	3009-2
S14-010688	Good n natural	Marshmallow	Holland & Barrett	100 capsules	Unknown	Apr-14	73023501
S14-010700	Holland & Barrett	Agnus Castus	Holland & Barrett	60 tablets	Unknown	Sep-14	3D340323S
S14-010626	Holland & Barrett	Evening Primrose Oil	Holland & Barrett	60 capsules	Unknown	Jul-16	73085201
S14-010684	Wilkin & Sons Ltd	Manuka Active 10+ honey	Morrison	340 grams	New Zealand	Apr-15	H2943
S14-010742	Rowse	Manuka Honey 10+	Asda	250 grams	New Zealand	Jul-15	4021B 21.09
S14-010627	Littleover Apiary	Pure Organic Forest honey	Amazon	340 grams	New Zealand	Oct-18	not declared
S14-010780	Odysea	Wild Thyme and fragrant herb honey	Amazon	450 grams	Greece	May-15	L31013T
S14-010595	Holland & Barrett	Australian honey	Holland & Barrett	340 grams	Australia	Jan-15	3198C 08:25
S14-010621	Hilltop Honey	Chilli honey	Holland & Barrett	340 grams	United Kingdom	Apr-16	not declared
S14-010685	Gale's	Set Honey	Morrison	454 grams	Unknown	Jun-15	3352H 13:52
S14-010784	Rowse	Rowse Organic Set Honey	Amazon	340 grams	Unknown	Apr-15	3283B
S14-010664	Gale's	Blossom Honey pure & natural (Squeezy Honey)	Morrison	340 grams	Unknown	May-15	3312H 08:16
S14-010695	Pure Gold	Pure Gold Active 18+ Manuka Honey	Holland & Barrett	250 grams	New Zealand	Sep-17	13/A084
S14-010604	Holland & Barrett	Clear Acacia Honey	Holland & Barrett	340 grams	Unknown	Jul-15	4028B 12:55
S14-010771	Hilltop Honey	Raw British wildflower honey	Amazon	340 grams	United Kingdom	Apr-16	not declared
S14-010639	Nelson Honey	Active Manuka honey 15+	Amazon	500 grams	New Zealand	Jul-18	140713 MH
S14-010663	Rowse	Squeezy Acacia Honey Light & mild	Morrison	340 grams	Unknown	May-15	33290 13:33

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010601	Medibee	Medibee Active 5+ Manuka Honey	Holland & Barrett	250 grams	New Zealand	Aug-16	18260557
S14-010602	Tropical Forest	Organic Forest Honey	Holland & Barrett	340 grams	Zambia	Jan-16	not declared
S14-010594	Holland & Barrett	Holland & Barrett Clear Blended Honey	Holland & Barrett	907 grams	Unknown	Jul-15	4020A 08:18
S14-010660	Morrison	Squeezy Australian Honey	Morrison	340 grams	Australia	Jun-15	3343D 04:17
S14-010661	Morrison	Squeezy Acacia Honey	Morrison	340 grams	Unknown	Jul-15	4020D 12:59
S14-010717	Queen Bee	Manuka honey 12+ Active	Amazon	340 grams	New Zealand	Mar-15	LOT:089318/A
S14-010693	Manuka Doctor	Active Manuka Honey 20+	Holland & Barrett	250 grams	New Zealand	Aug-16	130448
S14-010662	Morrison	Morrisons savers Clear Honey	Morrison	340 grams	Non European Union	Jul-15	4008A 16:17
S14-010741	Asda	Extra Special New Zealand Thyme Honey	Asda	340 grams	New Zealand	Jul-15	4014B 07.13
S14-010744	Sainsbury's	Rich and Floral Manuka with runny honey	Sainsbury's	340 grams	Unknown	May-15	3323B 9:47
S14-010745	Sainsbury's	Rich and Intense Manuka Honey active 10+	Sainsbury's	340 grams	New Zealand	Aug-15	4034B 13:41
S14-010739	Rowse	Australian honey	Asda	340 grams	Australia	Jul-15	4021D 22:11
S14-010669	Steens	Raw 5+ Manuka Honey	Whole Foods Market	250 grams	New Zealand	Aug-15	217
S14-010670	Steens	Raw 10+ Manuka Honey	Whole Foods Market	250 grams	New Zealand	Jul-15	211
S14-010671	Epicure	Wild Blossom Clear Honey	Whole Foods Market	454 grams	Non European Union	Oct-17	L141013
S14-010672	Epicure	Wild Blossom Set Honey	Whole Foods Market	454 grams	Non European Union	Dec-17	L301213
S14-010673	Epicure	Acacia Honey	Whole Foods Market	340 grams	Hungary	Nov-17	L192123
S14-010680	Gran Luchito	Honey with rare smoked mexican chillies and mexican honey	Whole Foods Market	250 grams	Mexico	Dec-14	L13178
S14-010676	J Friend & Co	Beechwood Honeydew Honey	Whole Foods Market	160 grams	New Zealand	Apr-18	Vintage April 2011
S14-010677	J Friend & Co	Kamahi Honey	Whole Foods Market	16 grams	New Zealand	Jan-14	Vintage January 2009

Sample number	Brand name	Product Description	Retail Outlet Name	Pack Size	Country of Origin	Expiry Date	Batch/Lot Number
S14-010681	GFM	Organic Honey with Royal Jelly	Whole Foods Market	230 grams	Unknown	Jul-17	not declared
S14-010667	Ogilvys	Zambezi Plains Organic Honey	Whole Foods Market	280 grams	Zambia	Jun-16	L13162/13
S14-010668	Ogilvys	Balkan Black Locust	Whole Foods Market	280 grams	Serbia	Nov-14	L11330/5
S14-010674	Seggiano	Sunflower Raw Honey	Whole Foods Market	500 grams	Italy	Dec-15	306
S14-010675	Seggiano	Woodland Honeydew Raw honey	Whole Foods Market	500 grams	Italy	Dec-15	305
S14-010665	Heather Hills Farm	Scottish Heather Honey	Whole Foods Market	340 grams	United Kingdom	Feb-18	not declared
S14-010666	Heather Hills Farm	Scottish Blossom Honey	Whole Foods Market	340 grams	United Kingdom	Nov-17	not declared
S14-010679	Alberfedy Oatmeal	Pure Scottish Flower Blossom Honey	Whole Foods Market	227 grams	United Kingdom	Jan-15	208
S14-010678	Wye Valley Apiarie	Wye Valley Honey	Whole Foods Market	227 grams	United Kingdom	Dec-15	not declared
S14-010785	Raw Health	Acacia Blossom honey	Amazon	350 grams	Romania	Dec-15	L3823046-A
S14-010605	Comvita	Comvita Active 5+ Manuka Honey	Holland & Barrett	250 grams	New Zealand	Dec-15	17042412
S14-010772	Raw Health	Organic Tropical Forest honey	Amazon	350 grams	Brazil	Nov-15	L3822880-F
S14-010692	Tesco	Squeezy Clear Honey	Tesco	340 grams	Unknown	Aug-15	4049U
S14-010694	New Zealand Honey Co	New Zealand Honey Co Manuka Honey 15+	Holland & Barrett	250 grams	New Zealand	Apr-14	1096
S14-010603	Manuka Pharm	Manuka Pharm Active Manuka Honey 12+	Holland & Barrett	250 grams	New Zealand	Feb-16	130376
S14-010743	The Co-operative	Clear honey all natural	The Co-operative	340 grams	Non European Union	Nov-14	3142A 20.36
S14-022697	Wilkin & Sons Ltd	Tiptree English Borage Honey	Amazon	340 grams	England	Jan-15	L1833 / H 0873 G
S14-022752	The Hive Honey Shop	Borage Blossom Honey	TheHiveHoneyShop.co.uk	340 grams	England	Sep-19	39
S14-022753	The Hive Honey Shop	Fresh Honeycomb in English Honey	TheHiveHoneyShop.co.uk	340 grams	England	not declared	not declared
S14-022754	The Hive Honey Shop	The Hive's Own English Borage Honey	TheHiveHoneyShop.co.uk	not given	England	Nov-19	618

Appendix 2

FINAL REPORT

DETERMINATION OF PYRROLIZIDINE ALKALOIDS IN TEAS, HERBAL INFUSIONS, PLANT-BASED SUPPLEMENTS AND HONEY

Report Number:	FD 15/07
Author:	Colin Crews
Date:	March 2015
Sponsor:	Food Standards Agency Aviation House London
Sponsor's Project manager:	Gillian Bramley
Sponsor's Project number:	FS102056
Fera Contract Number:	A2JN 1000
Principal Workers:	Antony Lloyd, Dennis Speck, Jonathan Guest, Roy Macarthur and Colin Crews
Team leader:	Emma Bradley

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INTRODUCTION

Pyrrolizidine alkaloids (PAs) are found in many plant families, and those in the genera Senecio, Eupatorium, Symphytum, Cynoglossum, Heliotropium and Crotalaria contain the species most frequently associated with human poisoning. These genera are widely distributed throughout different climates. Each plant species has a characteristic distribution of PAs and a typical ratio of free base to *N*-oxide. Some may contain essentially only a single major pyrrolizidine alkaloid, but many contain between five and eight individual PAs.

The alkaloid content of PA containing plants varies between species, plant organ and season, but can be up to several per cent of the plant's dry weight.

Relatively high concentrations (up to 4 mg/kg) of pyrrolizidines have been measured in honey produced from PA-producing plants including *S. jacobaea* and *Echium plantagineum*, although in a recent study of almost 4000 honey samples the average PA concentration of positive samples was roughly 70 µg/kg with a median of 27 µg/kg with bulk honey containing about 2.5 times more PA than retail products (Dubecke *et al.*, 2011).

PA containing plants tend to flower late in the flowering season, when they are the predominant nectar source in some localities. There is therefore considerable potential for the localised contamination of honey. Pollen has been shown to be a major source of PAs in honey (Kempf *et al.*, 2011).

Herbal medicines provide another source of PAs to the human diet, particularly those containing *Symphytum* (comfrey), borage, or *Tussilago farfara* (coltsfoot) as these plants contain PAs naturally (HMSO, 1994).

Scientific Opinion on pyrrolizidine alkaloids in food and feed

The European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM) has identified a number of PAs as being of particular importance for food contamination based on the present knowledge of metabolism, activation, DNA adduct-formation, genotoxicity and carcinogenicity. The CONTAM Panel has concluded that 1,2-unsaturated PAs containing a hydroxymethyl ester group at position C1 or C7 of the ring system may act as genotoxic carcinogens in humans.

The PAs of concern in human poisoning (in animal poisoning through feed contamination) are the following bases and their corresponding *N*-oxides:

- Senecionine-type PAs: acetylerucifoline, erucifoline, integerrimine, jacobine, jacoline, jaconine, jacozine, retrorsine, senecionine, seneciphylline. These PAs occur particularly in the Senecioneae (which includes ragwort), but are also found in Crotalaria spp.
- Lycopsamine-type PAs: acetylechimidine and isomers, echimidine and isomers, echivulgarine, lycopsamine and isomers, vulgarine. These PAs occur mainly in the Boraginaceae family (borage and Echium) and in the Eupatorieae.
- Heliotrine-type PAs: europine, heliotrine, lasiocarpine. These PAs occur in Heliotropium spp.
- Monocrotaline-type PAs: fulvine, monocrotaline, retusamine, trichodesmine. These PAs occur in Crotalaria spp.

To date, legal limits for PAs in food have not been set within the EU. The generally accepted benchmark dose recommended by the BfR for an adult (60 kg b.w.) corresponds to about 0.42 µg of unsaturated PAs per day which corresponds to a margin of exposure (MOE) of 10,000 as recommended by the EFSA CONTAM Panel. This exposure level would be unlikely to be of concern for a cancer risk, but the Panel concluded that there is a possible health concern for those toddlers and children who are high consumers of honey.

The potential exposure to PAs from herbal infusions and related preparations was not considered in detail by the CONTAM Panel and no information on exposure from this source became available until the results of a survey carried out by the Federal Institute for Risk Assessment (BfR) in response to the EFSA call were published in 2013 (BfR, 2013).

In the BfR study, unexpectedly high PA contents were measured in some individual herbal infusion and tea samples. It concluded that acute health risk is regarded as improbable while frequent consumers are possibly at increased risk if they consume highly contaminated products over longer periods of time. But due to uncertainties and some lack of data further investigations are required.

The current survey was commissioned by the Food Standards Agency with sampling provided by another contractor. The survey was originally intended to comprise of 50 samples of tea from *Camelia sinensis* (common black and green teas), 75 samples of herbal infusions, 50 samples of plant-based supplement preparations and 50 samples of honey. After revision of the assignments of some samples to other categories on receipt of the samples the final composition was 55 *Camelia sinensis* teas (including three fruit flavoured instant infusions), 70 herbal infusions (including non-*Camelia* instant infusions), 48 plant-based supplement preparations and 54 samples of honey.

SAMPLES

The samples are listed in Table 1. They are described fully in the Excel file supplied by the sampling contractor and reported in Appendix 1 of this report.

Table 1a. Summary list of samples - *Camelia sinensis* teas

LIMS No.	Type	LIMS No.	Type
S14-010544	Black	S14-010597	Black
S14-010545	Black	S14-010647	Black
S14-010546	Black	S14-010649	Green
S14-010547	Green	S14-010656	Green
S14-010548	Black	S14-010683	Black
S14-010549	Black	S14-010701	Green
S14-010550	Black	S14-010724	Black
S14-010551	Black	S14-010725	Black
S14-010552	Black	S14-010726	Black
S14-010553	Black	S14-010727	Black
S14-010554	Black	S14-010728	Black
S14-010555	Instant	S14-010729	Black
S14-010556	Black	S14-010730	Black
S14-010557	Earl Grey	S14-010731	Black
S14-010558	Instant	S14-010732	Black
S14-010560	Green	S14-010733	Green
S14-010561	Green	S14-010734	Green
S14-010582	Black	S14-010735	Black
S14-010583	Black	S14-010736	Earl Grey
S14-010584	Black	S14-010740	Black
S14-010585	Instant	S14-010758	Earl Grey
S14-010587	Black	S14-010760	Black
S14-010588	Black	S14-010766	Black
S14-010589	Black	S14-010776	Black
S14-010590	Black	S14-010779	Earl Grey
S14-010591	Black	S14-010806	Green
S14-010592	Black	S14-010811	Black
S14-010593	Black		

Table 1b. Summary list of samples – Herbal infusions

LIMS No.	Type	LIMS No.	Type
S14-010559	Peppermint	S14-010712	Mixture of plants
S14-010577	Fruit	S14-010722	Rooibos & vanilla
S14-010596	Mixture of plants	S14-010723	Rooibos
S14-010600	Mixture of plants	S14-010737	Ginger
S14-010607	Nettle fennel & peppermint	S14-010738	Camomile
S14-010609	Fruit & hibiscus	S14-010755	Mixture of plants
S14-010611	Mixture of plants with borage	S14-010756	Mixture of plants
S14-010614	Mixture of plants with hibiscus	S14-010757	Mixture of plants with hibiscus
S14-010618	Mixture of plants	S14-010759	Rooibos
S14-010622	Mixture of plants with borage	S14-010761	Comfrey
S14-010623	Mixture of plants	S14-010762	Mixture of plants
S14-010628	Green tea-based mixture of plants	S14-010763	Mixture of plants
S14-010629	Green tea-based mixture of plants	S14-010764	Liquorice
S14-010630	Mixture of plants with hibiscus	S14-010765	Camomile
S14-010631	Fruit	S14-010767	Lemon Balm
S14-010632	Green tea-based mixture of plants	S14-010768	Camomile
S14-010634	Mixture of plants	S14-010773	Fennel
S14-010638	Nettle	S14-010774	Mixture of plants
S14-010640	Mixture of plants	S14-010775	Mixture of plants
S14-010645	Peppermint	S14-010777	Mixture of plants
S14-010646	Rooibos	S14-010778	Lemon Balm
S14-010648	Rooibos	S14-010793	Fennel
S14-010650	Peppermint	S14-010794	Mixture of plants
S14-010652	Green tea & mint	S14-010804	Mixture of plants with hibiscus
S14-010653	Ginseng	S14-010807	Green tea-based mixture of plants
S14-010655	Camomile	S14-010808	Green tea-based mixture of plants
S14-010657	Camomile	S14-010809	Rooibos
S14-010659	Peppermint	S14-010812	Nettle
S14-010687	Fennel (instant)	S14-010813	Green tea-based mixture of plants

Table 1b (continued). Summary list of samples – Herbal infusions

LIMS No.	Type	LIMS No.	Type
S14-010690	Fruit	S14-010815	Mixture of plants
S14-010704	Mixture of plants	S14-010816	Mixture of plants
S14-010705	Mixture of plants with hibiscus	S14-010817	Mixture of plants
S14-010707	Mixture of plants (instant)	S14-010821	Fruit
S14-010710	Mixture of plants	S14-010822	Peppermint
S14-010711	Mixture of plants (instant)	S14-010823	Liquorice

Table 1c. Summary list of samples – Plant-based supplements

LIMS No.	Type	LIMS No.	Type
S14-010569	Organic Andrographis & Holy Basil	S14-010699	Wheat Grass
S14-010570	Echinacia	S14-010700	Agnus Castus
S14-010571	Milk Thistle	S14-010706	Avena Sativa
S14-010576	Peppermint	S14-010713	Plantago
S14-010578	Ginkgo Bilboa	S14-010714	Neem Oil
S14-010579	Andrographis	S14-010715	Wheat Grass
S14-010580	Ginger	S14-010716	Aloe vera Juice with Manuka Honey
S14-010581	Aloe vera	S14-010718	Chlorophyll
S14-010598	Barley Grass	S14-010781	Garlic
S14-010606	Fenugreek	S14-010782	Cranberry
S14-010615	Artichoke Leaf	S14-010786	Guarana
S14-010619	Cayenne	S14-010787	Ginseng
S14-010620	Raspberry leaf	S14-010789	Liquorice root
S14-010626	Evening Primrose Oil	S14-010790	Hawthorn berries
S14-010633	Fennel Seed	S14-010791	Beetroot
S14-010635	Gotu Kola & Turmeric	S14-010792	Saw palmetto
S14-010641	Slippery Elm	S14-010796	Maca
S14-010642	Ginger root	S14-010797	Acai
S14-010644	Aloe Vera	S14-010802	Ginseng
S14-010686	Elderberry	S14-010803	Ginseng
S14-010688	Marshmallow	S14-010810	Gingko Biloba
S14-010689	Papaya Enzyme	S14-010819	Garlic
S14-010697	Mixture of plants	S14-010820	Olive Leaf
S14-010698	Dandelion	S14-101577	Sage leaf

Table 1d. Summary list of samples – Honeys

LIMS No.	Type	LIMS No.	Type
S14-010594	Blended	S14-010677	Kamahi
S14-010595	Blended	S14-010678	Blended
S14-010601	Manuka	S14-010679	Blended
S14-010602	Blended	S14-010680	Blended
S14-010603	Manuka	S14-010681	Blended
S14-010604	Acacia	S14-010684	Manuka
S14-010605	Manuka	S14-010685	Blended
S14-010621	Blended	S14-010692	Blended
S14-010627	Blended	S14-010693	Manuka
S14-010639	Manuka	S14-010694	Manuka
S14-010660	Blended	S14-010695	Manuka
S14-010661	Acacia	S14-010717	Manuka
S14-010662	Blended	S14-010739	Blended
S14-010663	Acacia	S14-010741	Thyme
S14-010664	Blended	S14-010742	Manuka
S14-010665	Heather	S14-010743	Blended
S14-010666	Blended	S14-010744	Manuka
S14-010667	Blended	S14-010745	Manuka
S14-010668	Black Locust	S14-010771	Blended
S14-010669	Manuka	S14-010772	Blended
S14-010670	Manuka	S14-010780	Blended
S14-010671	Blended	S14-010784	Blended
S14-010672	Blended	S14-010785	Acacia
S14-010673	Acacia	S14-022697	Borage
S14-010674	Sunflower	S14-022752	Borage
S14-010675	Honeydew	S14-022753	Borage
S14-010676	Honeydew	S14-022754	Borage

The *Camelia sinensis* teas category included 3 samples of instant tea which were sugar-based; 4 samples of Earl Grey tea and 9 samples of green tea (two with lemon).

The herbal infusion category also included 7 infusions based on green tea, one of which also contained mint, one which contained ginseng and one which contained gingko. Another (S14-010807 Genmaicha tea) is a blend of green tea with processed rice and S14-010632 was a blend of green tea with milk. Further details of the ingredients are given in Annex 1.

The plant-based supplements were mostly leaf or root powder contained in capsules or in tablet form. Others were alcohol extracts, water based, oils or aqueous gels.

METHODS

Analytical methods for PAs have evolved rapidly in recent years. The most sensitive and reliable methods are based on liquid chromatography with tandem mass spectrometry detection (LC-MS/MS). Sample preparation involves extraction into aqueous acid and clean up/concentration on solid phase extraction columns (SPE), usually based on strong cation exchange technology (SCX), although the trapping mechanism is unclear.

Application of these methods has been hindered by a lack of the analytical standards required for quantification, and by limited method validation by collaborative trial. Recently, separate methods for both honey and plant materials have been subject to validation trials organised by the EU Institute for Reference Materials and Measurements (IRMM), and the German Federal Institute for Risk Assessment (BfR). Fera participated in both of these trials and achieved good results. A report of the international collaborative study is available (BfR, 2015). However, the overall results for teas showed that there were problems with homogeneity, and that the sample
pre-treatment, had a critical influence on repeatability and reproducibility of the method. As a result of this the method for tea was revised. The survey reported here was carried out before this revision was available.

Fera applied the method used in its participation in the BfR trial with good results for all analytes (Bodi *et al*., 2014). A similar procedure was used successfully for Fera's participation in an IRMM test for the determination of PAs in plant material (hay) and honey (IRMM, 2012) although the variation in results submitted by all participants was too high for z-scores to be calculated. For this survey the methods provided by the BfR for these validation exercises were applied.

In addition analytical standards have become commercially available for some of the EFSA priority PAs (erucifoline, erucifoline *N*-oxide, jacobine, jacobine *N*-oxide, retrorsine, retrorsine *N*-oxide, senecionine, senecionine *N*-oxide, seneciphylline, seneciphylline *N*-oxide, echimidine, echimidine *N*-oxide, lycopsamine and its isomer intermedine, heliotrine, heliotrine *N*-oxide, lasiocarpine, lasiocarpine *N*-oxide, monocrotaline, monocrotaline *N*-oxide, retusamine, and trichodesmine).

To overcome the lack of individual reference standards, an alternative approach enabling determination of the total PA content by means of a sum parameter expressed as e.g. retronecine equivalent has been introduced (Cramer *et al*., 2013).

Important PA standards that were unavailable commercially at the time of this project included acetyllcopsamine, acetlyintermedine, acetylerucifoline, integerrimine, jacoline, jaconine, jacozine, acetylechimidine and isomers, echivulgarine and isomers, vulgarine, europine, fulvine, and their *N*-oxides, and also the *N*-oxides of heliotrine, lasiocarpine and trichodesmine. Therefore, these were not determined in this study.

Teas and herbal infusions

The principle of the method for teas is that PAs are extracted from the powdered tea with dilute aqueous sulphuric acid, neutralised, and trapped on to a C18 medium in a solid phase extraction (SPE) cartridge. The cartridge is washed with water to remove some of the co-extracted material, and PAs are then eluted with methanol. The methanol is evaporated and the PAs measured using LC-MS/MS with quantification against calibration standards prepared in a blank plant material matrix.

For the tea and herbal infusion samples the BfR method for plant materials was revised to replace the filtration step by a repeat of the centrifugation step as this provided cleaner extracts and was more rapid.

Teas and herbal infusion samples were prepared by taking 50-100 g of sample removed where necessary (most samples) from tea bags and grinding to a fine powder using an electric spice grinder. The powdered sample was passed through a 0.5 mm sieve to meet the BfR particle size requirement. The grinder was cleaned between samples with a fine brush followed by grinding a portion of rice grains to a powder and brushing clean again.

Plant-based supplements

No method specifically for plant-based supplements was available. However, most samples were either capsules containing fine plant material or tablets of compressed plant material with inorganic binder. Leaf-derived supplements were prepared by opening capsules or crushing compressed leaf tablets. These samples were

analysed using the BfR plant method. Other samples were alcoholic tinctures or aqueous extracts. Aliquots of these samples were warmed overnight at 40°C under a stream of nitrogen to evaporate most of the alcohol, mixed with water and analysed by the SCX honey procedure without heating during addition. Generally, a smaller quantity of supplement material, was available than for the teas. This varied considerably with the product type and brand.

The few oil-based samples were shaken with 0.05 M aqueous sulphuric acid, centrifuged and analysed using the SCX honey procedure without heating during addition.

The powdered materials were extracted with 0.05 M aqueous sulphuric acid. The extracts were neutralised with ammonia solution as specified in the BfR method and cleaned up by C18 SPE, filtered and analysed. The neutralisation step allowed the removal of a considerable quantity of co-extracted material by precipitation and centrifugation.

Honeys

The principle of the method for honeys is that PAs are extracted from the honey with dilute aqueous sulphuric acid, and trapped on to a cation exchange (SCX) medium in a solid phase extraction (SPE) cartridge, washed with water to remove some of the co-extracted material, and eluted from the SPE cartridge with ammonia in methanol. The methanol is evaporated and the PAs measured using LC-MS/MS with quantification against calibration standards prepared in a blank honey material matrix. The use of SCX-SPE allows the sample to be more thoroughly isolated from the sugars present in the honey sample.

Honey samples were dissolved in 0.05 M aqueous sulphuric acid, filtered and cleaned up by SCX SPE, filtered and analysed. Slight variations were made to the honey method in that the application of the sample to the SPE cartridge was carried out inside an oven heated to 40°C. Warming of the sample is a common procedure in published methods for PAs in honey including that on which the BfR method was based.

It should be noted that for the survey carried out by the BfR a standards addition quantification procedure was applied that was not used in the collaborative trial or

provided as an option in the operating procedure document (Bodi *et al*., 2014). Depending on the method used the standards addition procedure can compensate for matrix effects, however quantification by standard addition requires analysis of (usually) a minimum of five spiked replicates of every sample, which was outside the scope of the project.

Quantitative results were obtained using an external standard calibration over the range 0 to 500 ng/ml for plant materials (equivalent to a maximum of 1000 µg/kg) and 0 to 100 ng/ml for honey (equivalent to a maximum of 10 µg/kg). The plant material calibration range exceeded that of the BfR method but was linear, it allowed quantification without dilution and its associated change in matrix effect. The standards were prepared according to BfR instructions in a blank matrix. As some difficulty was encountered in quickly finding a tea that contained no PAs to a very low level a matrix was prepared by drying privet leaves collected close to the laboratory at a time when no PA containing plants were in growth.

A calibration standard series prepared in a matrix (matrix matched standard) is important in many analyses as co-eluting compounds can affect the sensitivity of the instrument by enhancing or decreasing responses to the analyte.

The agreed quality assurance procedures were limited by the available time and budget to every tenth sample being analysed in duplicate. The method had not been validated in-house but had given good results in the BfR trial. In practice, replicate analyses of many tea samples were carried out and several were analysed more than twice in order to confirm relatively high levels of PAs. In addition, a plant-based reference material was prepared by adding small portions of comfrey, dried ragwort and dried echium to a green tea sample, and a honey reference material was prepared by adding an aqueous solution of the standards to a honey. The reference materials were mixed on a roller for 24 hours prior to use but could not be characterised or tested for homogeneity before the start of the project.

Three honey samples were analysed after spiking with a mixture of all of the target PAs.

METHOD PERFORMANCE

To assess the method uncertainty for the tea data measurement uncertainty was calculated for the 19 target PA analytes in 6 herbal infusion samples. Two replicate samples (green tea S14-010560 and herbal mixture S14-010623) were fortified with the PAs at 10 and 100 µg/kg. Six extracts were produced independently from each sample and measured in a single LC-MS/MS run which included two sets of matrix matched calibration standards. The measurement was repeated in a second run for some samples.

For each of the twenty four analytes in each sample the following was estimated to provide a summary of the measurement performance:

- The mean concentration of analyte, not corrected for recovery
- The standard deviation of concentration estimates across the six unfortified samples
- The average recovery of 10 µg/kg added to each sample
- The average recovery of 100 µg/kg added to each sample
- The standard errors of the recoveries
- The relative standard error associated with the calibration

The resulting estimated uncertainties are summarised in Table 2.

Fuller details of the statistical measurements and results for the calculation of the measurement uncertainty for teas are provided as a separate document.

Table 2. Estimates of expanded uncertainty

PA	Estimated concentration (µg/kg)	Expanded uncertainty (µg/kg)
Echimidine	109	23
Erucifoline	107	20
Erucifoline N-oxide	103	12
Heliotrine	4.6	3.1
Heliotrine	105	21
Heliotrine N-oxide	99	23
Intermedine N-oxide	99	18
Jacobine N oxide	101	12
Lasiocarpine	110	22
Lasiocarpine N-oxide	101	19
Lycopsamine	11.9	5.8
Lycopsamine	112	17
Lycopsamine N-oxide	101	15
Monocrotaline	112	22
Monocrotaline N-oxide	105	16
Retrorsine	109	15
Retrorsine N-oxide	101	14
Senecionine	110	24
Senecionine N-oxide	99	24
Seneciphylline	113	28
Senkirkine	102	17

The estimates of expanded uncertainty are valid if the variation associated with the extraction is larger than that associated with the instrumental analysis.

An estimate of measurement uncertainty was made for those analytes that demonstrated a relative standard deviation of ≤ 30%, a mean recovery of 50 to 200% on addition of 100 µg/kg and a calibration relative standard error ≤ 30%. This was based on the assumption that between-run analytical variation is small compared to between run extraction variation. The estimate of uncertainty was made by combining the observed variation with the uncertainty about recovery at 100 µg/kg addition and the uncertainty associated with the calibration.

For these 19 analytes, the method is probably capable of detecting and producing quantitative results for samples that contain analyte at concentrations at or above the estimated expanded uncertainty, i.e. around 20 µg/kg. This is based on the assumption that absolute uncertainties do not get larger as concentrations of analyte reduce.

Based on the calculations, for tea, herbal infusions and plant-based supplements the LOQ was set to 20 µg/kg.

For honey samples the limits of detection for PA was 1 ng/ml corresponding to 0.1 µg/kg with an LOQ of 0.3 µg/kg. These values are in good agreement with the BfR figures for in-house validation (LOQ 0.2 to 0.6 µg/kg).

RESULTS

For the tea, herbal infusions and plant-based supplement samples, quantitative results for repeated analysis of the same sample were consistent for low levels of PAs when the extract was compared with the matrix matched calibration. However, when samples with high PA levels were reanalysed after dilution the responses for some compounds were enhanced by the decrease in the matrix effect brought about by the dilution with solvent. The responses for some lower level PAs in the same sample were decreased by the dilution effect. Because of this variation the calibration range of the matrix matched standards was increased as described above and undiluted samples were measured against the wider range of standards.

Data are summarised in Tables 3 to 6. The tables show a summary of the PA concentrations in µg/kg subject to the limits of quantification of 20 µg/kg per alkaloid in teas, herbal infusions and supplements and 0.3 µg/kg per alkaloid in honey. The levels reported are mean values for all of the analyses conducted on each sample with the omission of early results made by comparison of diluted samples with solvent standards where mid-range values were much affected by the differences in matrix effects. Also discarded were results for which the confirmatory mass spectrometric transitions were unsatisfactory and a few results for certain PAs where a single PA was detected but not supported by repeated analysis and where no supporting response was present for another PA expected to be derived from the same plant.

Camelia sinensis teas

The results for the *Camelia sinensis* teas (black, green and Earl Grey) are shown in Table 3.

The results are presented on a basis of the received weight, i.e. the weight of the plant material provided without additional drying (all samples were either dry tea leaves or instant tea powders).

Table 3. Summary results for the tea category (µg/kg)

		Total	ECHI	INT	LYCO	LAS	LASNO	ER	ERNO	HEL	HELNO	JACOB	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SPYNO	SENK
S14-010544	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010545	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010546	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010547	Green	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010548	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010549	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010550	Black	1,170	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	201	718	56	195	nd	nd	nd
S14-010551	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010552	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010553	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010554	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010555	Instant	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010556	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010557	Earl Grey	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010558	Instant	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010560	Green	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010561	Green	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010582	Black	440	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	33	407	nd	nd	nd	nd	nd
S14-010583	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010584	Black	31	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	31	nd	nd	nd	nd	nd
S14-010585	Instant	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010587	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010588	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010589	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010590	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010591	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010592	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 3 (continued). Summary results for the tea category (µg/kg)

		Total	ECHI	INT	LYCO	LAS	LASNO	ER	ERNO	HEL	HELNO	JACOB	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SPYNO	SENK
S14-010593	Black	65	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	27	38	nd	nd	nd	nd	nd
S14-010597	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010647	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010649	Green	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010656	Green	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010683	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010701	Green	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010724	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010725	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010726	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010727	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010728	Black	33	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	33	nd	nd	nd	nd	nd
S14-010729	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010730	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010731	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010732	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010733	Green	227	nd	51	41	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	89	nd	46	nd	nd	nd
S14-010734	Green	485	nd	23	39	nd	nd	nd	nd	nd	nd	nd	nd	nd	47	376	nd	nd	nd	nd	nd
S14-010735	Black	43	nd	nd	43	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010736	Earl Grey	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010740	Black	269	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	61	180	nd	29	nd	nd	nd
S14-010758	Earl Grey	44	nd	nd	23	nd	nd	nd	nd	nd	nd	21	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010760	Black	43	nd	nd	23	nd	nd	nd	nd	nd	nd	20	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010766	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 3 (continued). Summary results for the tea category (µg/kg)

		Total	ECHI	INT	LYCO	LAS	LASNO	ER	ERNO	HEL	HELNO	JACOB	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SPYNO	SENK
S14-010776	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010779	Earl Grey	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010806	Green	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010811	Black	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = less than reporting limit of 20 µg/kg

LOQ = limit of quantification

Key

ECHI	Echimidine	MC	Monocrotaline
INT	Intermedine	MCNO	Monocrotaline *N*-oxide
LYCOP	Lycopsamine	RET	Retrorsine
LAS	Lasiocarpine	RETNO	Retrorsine *N*-oxide
LASNO	Lasiocarpine *N*-oxide	SEN	Senecionine
ERU	Erucifoline	SENNO	Senecionine *N*-oxide
ERNO	Erucifoline *N*-oxide	SPY	Seneciphylline
HEL	Heliotrine	SPYNO	Seneciphylline *N*-oxide
HELNO	Heliotrine *N*-oxide	SENK	Senkirkine
JACOB	Jacobine		

Of the 39 black tea samples eleven contained PAs, at total levels ranging from 31 to 1,170 µg/kg. Retrorsine and retrorsine *N*-oxide were the most common contaminants of the black teas, being present at concentrations of up to 201 and 718 µg/kg respectively in sample S14-010550 and moderately high in sample S14-010740 (61 and 180 µg/kg). Sample S14-010550 also contained lower levels of senecionine and its *N*-oxide, senecionine *N*-oxide was also detected in sample S14-010740. Lycopsamine was present in two samples of black tea (S14-010735 and S14-010760).

One of the four Earl Grey teas contained relatively low levels of lycopsamine and jacobine but its isomer compound intermedine was absent.

Of the nine green teas two contained relatively high levels of PAs. Samples S14-010733 and S14-010734 contained lycopsamine and its isomer intermedine. Sample S14-010733 contained retrorsine *N*-oxide and senecionine *N*-oxide; sample S14-010734 contained higher levels of retrorsine and retrorsine *N*-oxide.

None of the three black tea based instant teas contained detectable PAs.

Herbal infusions

The results of the analysis of the 70 herbal infusions are presented in Table 4.

The results are presented on a basis of the received weight, i.e. the weight of the plant material provided without additional drying (all samples were either dry tea leaves or instant tea powders).

Table 4. Summary results for the herbal infusion category (µg/kg)

Sample	Description	Total	ECH	INT	LYC	LAS	LASNO	ERU	ERNO	HEL	HELNO	JAC	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SEPHNO	SENK
S14-010559	Peppermint	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010577	Fruit	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010596	Mixture	132	55	77	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010600	Dandelion & Burdock	323	323	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010607	Nettle fennel, peppermint	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010609	Mixture with hibiscus	511	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	511	nd	nd	nd
S14-010611	Mixture	50245	49980	nd	59	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	206	nd	nd	nd
S14-010614	Mixture with hibiscus	408	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	408	nd	nd	nd
S14-010618	Mixture	93	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	20	nd	73	nd	nd	nd
S14-010622	Mixture with borage	2327	nd	1191	1136	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010623	Ginseng	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010628	Green based mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010629	Green based mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010630	Mixture with hibiscus	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010631	Fruit	232	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	232	nd	nd	nd
S14-010632	Green with milk	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010634	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010638	Nettle	408	370	nd	38	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010640	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010645	Peppermint	422	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	55	38	129	37	163	nd
S14-010646	Rooibos	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010648	Roobois	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010650	Peppermint	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010652	Green with mint	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010653	Ginseng	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010655	Camomile	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 4 (continued). Summary results for the herbal infusion category (µg/kg)

Sample	Description	Total	ECH	INT	LYC	LAS	LASNO	ERU	ERNO	HEL	HELNO	JAC	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SEPHNO	SENK
S14-010657	Camomile	690	186	224	62	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	55	111	nd	52	nd
S14-010659	Peppermint	76	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	42	nd	34	nd
S14-010687	Fennel (instant)	87	nd	nd	nd	87	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010690	Fruit	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010704	Mixture	57	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	27	nd	nd	30
S14-010705	Mixture with hibiscus	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010707	Mixture (instant)	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010710	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010711	Mixture (instant)	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010712	Mixture	57	nd	nd	nd	nd	57	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010722	Rooibos	937	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	59	228	127	490	nd	33	nd
S14-010723	Rooibos	641	33	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	58	149	113	288	nd	nd	nd
S14-010737	Ginger	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010738	Camomile	213	35	27	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	27	84	40	nd	nd
S14-010755	Nettle, fennel, peppermint	568	538	nd	30	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010756	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010757	Mixture with hibiscus	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010759	Rooibos	633	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	94	153	138	249	nd	nd	nd
S14-010761	Comfrey	52508	52	26359	26097	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010762	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010763	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010764	Liquorice	2881	nd	nd	33	70	579	nd	nd	208	1992	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010765	Camomile	1370	931	205	98	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	26	63	46	nd	nd
S14-010767	Lemon Balm	2516	20	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	71	552	86	935	88	764	nd

Table 4 (continued). Summary results for the herbal infusion category (µg/kg)

Sample	Description	Total	ECH	INT	LYC	LAS	LASNO	ERU	ERNO	HEL	HELNO	JAC	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SEPHNO	SENK
S14-010768	Camomile	133	22	87	24	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010773	Fennel	578	nd	nd	nd	44	534	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010774	Mixture	30	nd	nd	nd	30	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010775	Mixture	76	nd	nd	nd	21	30	nd	nd	nd	25	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010777	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010778	Lemon Balm	20	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	20
S14-010793	Fennel	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010794	Mixture	539	nd	nd	nd	nd	42	nd	nd	nd	106	nd	nd	nd	nd	nd	nd	391	nd	nd	nd
S14-010804	Mixture with hibiscus	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010807	Green based mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010808	Green based mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010809	Rooibos	369	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	52	191	97	nd	nd	29	nd
S14-010812	Nettle	165	165	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010813	Green based mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010815	Mixture	20	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	20	nd
S14-010816	Mixture	279	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	41	nd	136	39	63	nd
S14-010817	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010821	Fruit	145	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	145	nd	nd	nd
S14-010822	Peppermint	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010823	Liquorice	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = less than reporting limit of 20 µg/kg

LOQ = Limit of quantification

Key as Table 3.

Quantifiable PAs were found in 34 herbal infusion samples subject to a limit of quantification for the individual PAs of 20 µg/kg. The highest levels (> 50,000 µg/kg) were in teas based on borage and comfrey. Three samples that did not declare PA producing plants as ingredients contained over 1,000 µg/kg total PAs. These results agree well with those published by the BfR (BfR, 2013) and elsewhere (Bodi *et al*., 2014, Mathon *et al*., 2014, Schultz *et al*., 2015, Madge *et al*., 2015). In its research project on the "Determination of Pyrrolizidine Alkaloids in Food and Feed" the BfR analysed 221 different commercially available herbal infusions and tea samples and herbal drugs for 17 PAs. For a limited unrepresentative number of herbal infusion types (baby fennel, fennel, camomile, herbal, peppermint, nettle and melissa but not borage) the total PA contents ranged from < LOQ to 3,400 mg/kg of dry product.

Apart from infusions containing borage (which is known to contain PAs) the highest consistent contamination was found in camomile infusions. Of the five camomile infusion samples tested four contained echimidine and intermedine or lycopsamine or both. Three also contained senecionine and senecionine *N*-oxide and two of these contained seneciphylline. The predominant PAs were echimidine (22 to 931 µg/kg) and intermedine (up to 205 µg/kg).

One leafy fennel infusion (S14-010773) contained the heliotrope PAs lasiocarpine and lasiocarpine *N*-oxide. An instant infusion based on fennel (S14-010687) also contained lasiocarpine.

Two of three samples of nettle infusions (S14-010638, S14-010812) and a mixed infusion with nettle (S14-010755) contained PAs up to 568 µg/kg (principally echimidine).

Four out of six rooibos infusions contained high levels of retrorsine and senecionine and their *N*-oxides, with total PA content, where present, of between 369 and 937 µg/kg.

None of seven infusions based on green tea ingredients contained quantifiable PAs.

Relating ingredients to PA concentrations was hampered by the fact that the named packet ingredients on the packet face were frequently not the only or even the major components. A list of the ingredients of all of the herbal infusions is provided in Annex 1. Of the samples with a common named (front of packet) ingredient only one of five infusions named as simply camomile tea was free of PAs and the remainder

contained significant levels. Two of six infusions named as containing hibiscus contained high levels of senecionine *N*-oxide.

The *Senecio* alkaloids senecionine, seneciphylline, and retrorsine and their *N*-oxides were the most common contaminants of the herbal infusions. These compounds are found in a very wide variety of toxic plants around the world. The incidence of some other *Senecio* alkaloids (erucifoline and jacobine) was unexpectedly low.

Retrorsine is one of the main alkaloids present in *Senecio*. It is not a major component of the ragwort plant (*Jacobaea vulgaris*, previously *Senecio jacobaea*) found in northern Europe but is present in other *Senecio* species including *S. vulgaris* (common groundsel). These plants frequently contaminate hay and fodder in many countries.

The co-occurrence of lasiocarpine and its *N*-oxide, particularly in combination with heliotrine and its *N*-oxide is indicative of contamination with *Heliotropium lasiocarpum* or *Heliotropium europaeum*, annual weeds native to Western and middle Asia, the Caucasus, China, the Indian Subcontinent and to Europe, Asia, and North Africa but widely naturalized in Australia and North America (Prakash *et al.*, 1999, Parsons and Cuthbertson 2001). These plants have been reported to contain other PAs (europine and supinine) but these are not monitored by the current method.

The monoester PAs echimidine, intermedine, and its isomer lycopsamine are found principally in the family Boraginaceae which includes *Symphytum* (Comfrey), Borage and *Echium*. Echimidine is absent from most but not all plants of common comfrey (*S. officinale*) and is more usually associated with Russian comfrey (*S. x uplandicum*). The comfreys are also known to contain similar PAs not included in the analytical method including the acetyl esters of intermedine and lycopsamine, and isomers of symphytine (standards of which are now available). Echium species contain an even wider range of PAs that are not included in the analytical method. The exclusion of several PAs is solely on account of the lack of availability of reference standards, alternative methods such as the sum parameter approach (Cramer *et al.*, 2013) mentioned earlier can detect and provide at least semi-quantitative information on these PAs.

Plant-based supplements

Results for the plant-based supplements are given in Table 5. The sample S14-010714 (Neem Oil) was not analysed as this product was labelled ‘for external use only’ and so was excluded from the survey.

Analysis of two Aloe vera gels (S14-010581 and S14-010644) was not completed as the matrix could not be passed through the SPE columns after addition of aqueous acid and centrifugation and so could not be tested.

Table 5. Summary results for the plant-based supplement category (µg/kg)

		Total	ECHI	INT	LYCO	LAS	LASNO	ERNO	ERU	HEL	HELNO	JAC	MC	MCNO	RET	RETNO	SENNO	SEN	SPHNO	SPY	SKIRK
S14-010569	Mixture	50	nd	nd	26	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	24	nd
S14-010570	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010571	Milk Thistle	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010576	Peppermint	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010578	Ginkgo Bilboa	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010579	Andrographis	130	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	99	nd	30	nd	nd
S14-010580	Ginger	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010581	Aloe vera	N/A																			
S14-010598	Barley Grass	117	nd	41	76	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010606	Fenugreek	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010615	Artichoke Leaf	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010619	Cayenne	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010620	Raspberry leaf	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010626	Evening Primrose	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010633	Fennel Seed	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010635	Gotu Kola & Turmeric	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010641	Slippery Elm	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010642	Ginger root	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010644	Aloe Vera	N/A																			
S14-010686	Elderberry	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010688	Marshmallow	344	53	157	102	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	31	nd	nd	nd	nd	nd
S14-010689	Papaya	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010697	Mixture	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010698	Dandelion	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010699	Wheat Grass	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010700	Agnus Castus	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 5 (continued). Summary results for the plant-based supplement category (µg/kg)

		Total	ECHI	INT	LYCO	LAS	LASNO	ERNO	ERU	HEL	HELNO	JAC	MC	MCNO	RETNO	RET	SENNO	SEN	SPHNO	SPY	SKIRK
S14-010706	Avena Sativa	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010713	Plantago	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010714	Neem oil	N/A																			
S14-010715	Wheat	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010716	Aloe vera	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010718	Chlorophyll	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010781	Garlic	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010782	Cranberry	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010786	Guarana	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010787	Ginseng	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010789	Liquorice root	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010790	Hawthorn berries	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010791	Beetroot	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010792	Saw palmetto	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010796	Maca	22	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	22	nd	nd	nd	nd	nd	nd	nd
S14-010797	Acai	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
s14-010802	Ginseng	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010803	Ginseng	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010810	Gingko Biloba	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010819	Garlic	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010820	Olive Leaf	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-101577	Sage	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = less than reporting limit of 20 µg/kg

LOQ = Limit of quantification

Key as Table 3

Of the 45 samples analysed successfully five contained PAs, again predominantly lycopsamine/intermedine. One sample (S14-010688) was analysed four times with different results, retrorsine *N*-oxide was measured at about 60 µg/ kg in two samplings but not found in two others. The average values are reported in Table 5.

One sample (S14-010796 Maca Powder) uniquely within this survey contained quantifiable monocrotaline *N*-oxide, the free base monocrotaline was detected in this sample but was not quantifiable.

Honeys

Results for the honey samples are given in Table 6. Honey samples (54) comprised 27 types that were blended or of unstated floral origin (including two honeydew samples), 14 manuka, 5 acacia, 4 borage, and single samples of thyme, black locust, sunflower and heather.

Table 6. Summary results for the honey category (µg/kg)

		Total	ECH	INT	LYC	LAS	LASNO	ERU	ERNO	HEL	HELNO	JAC	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SEPNO	SENK
S14-010594	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010595	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010601	Manuka	8.3	nd	1.7	6.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010602	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010603	Manuka	8.4	1.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.7	nd	2.3	nd	4.2
S14-010604	Acacia	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010605	Manuka	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010621	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010627	Blended	22.1	0.3	7.1	14.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010639	Manuka	3.1	0.5	1.0	1.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010660	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010661	Acacia	2.5	nd	0.8	1.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010662	Blended	0.5	0.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010663	Acacia	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010664	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010665	Heather	251.1	nd	31.0	213.3	nd	nd	0.6	nd	4.9	nd	nd	nd	nd	0.6	nd	0.6	nd	nd	nd	nd
S14-010666	Blended	17.2	nd	0.5	3.3	nd	nd	nd	nd	nd	nd	2.0	nd	nd	4.6	0.6	1.6	nd	3.8	0.6	nd
S14-010667	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010668	Black Locust	4.1	0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4	nd	1.7	nd	1.7
S14-010669	Manuka	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010670	Manuka	12.8	0.6	2.4	1.0	nd	nd	0.4	nd	nd	nd	1.4	nd	nd	0.6	nd	1.1	0.4	3.7	0.7	0.5
S14-010671	Blended	36.6	8.2	5.5	22.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.3	nd	nd	nd	nd
S14-010672	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010673	Acacia	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 6 (continued). Summary results for the honey category (µg/kg)

		Total	ECH	INT	LYC	LAS	LASNO	ERU	ERNO	HEL	HELNO	JAC	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SEPNO	SENK
S14-010674	Sunflower	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010675	Honeydew	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010676	Honeydew	22.0	1.1	3.9	17.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010677	Kamahi	20.8	0.5	2.0	18.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010678	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010679	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010680	Blended	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010681	Blended	4.4	nd	1.4	1.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.0	nd	0.4	nd	nd	nd	nd
S14-010684	Manuka	1.6	nd	0.4	0.7	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.5	nd	nd
S14-010685	Blended	0.3	0.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010692	Blended	25.8	4.7	1.4	2.0	nd	nd	nd	nd	nd	nd	0.4	nd	nd	14.5	nd	2.8	nd	nd	nd	nd
S14-010693	Manuka	41.2	13.6	14.0	9.4	nd	nd	nd	nd	0.4	nd	0.5	nd	nd	0.8	nd	0.4	nd	2.1	nd	nd
S14-010694	Manuka	1.0	nd	nd	0.5	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.5	nd	nd
S14-010695	Manuka	4.9	0.6	1.0	3.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010717	Manuka	17.4	nd	2.2	13.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.5	nd	0.4	nd	1.3	nd	nd
S14-010739	Blended	185.2	0.3	16.7	165.0	nd	nd	nd	nd	2.6	nd	nd	nd	nd	0.4	nd	nd	nd	nd	nd	nd
S14-010741	Thyme	9.7	5.9	1.2	2.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010742	Manuka	8.1	nd	0.3	0.9	nd	nd	nd	nd	nd	nd	0.7	nd	nd	0.5	nd	0.7	nd	4.1	0.6	nd
S14-010743	Blended	0.4	nd	nd	0.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010744	Manuka	6.1	nd	1.6	4.0	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.5	nd	nd
S14-010745	Manuka	25.3	4.4	4.5	16.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010771	Blended	15.4	nd	9.4	5.2	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.8	nd	nd
S14-010772	Blended	39.9	2.1	3.9	27.8	nd	nd	nd	nd	nd	nd	nd	nd	nd	2.1	nd	2.6	1.0	0.5	nd	nd
S14-010780	Blended	0.9	0.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-010784	Blended	8.2	nd	2.0	5.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4	nd	0.4	nd	nd	nd	nd
S14-010785	Acacia	< LOQ	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 6 (continued). Summary results for the honey category (µg/kg)

		Total	ECH	INT	LYC	LAS	LASNO	ERU	ERNO	HEL	HELNO	JAC	MC	MCNO	RET	RETNO	SEN	SENNO	SPY	SEPNO	SENK
S14-022697	Borage	117.5	1.4	68.4	47.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-022752	Borage	163.1	nd	85.8	77.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-022753	Borage	139.4	nd	82.1	57.3	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
S14-022754	Borage	110.2	nd	65.3	44.9	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

nd = less than reporting limit of 0.3 µg/kg

LOQ = Limit of quantification

Key as Table 3.

PAs were present in 35 of the 54 honey samples tested. Lycopsamine and / or intermedine were found in 30 samples, in many from New Zealand and in the UK borage honeys. These compounds accounted for about 80% by weight of the PA content of the honeys. 13 samples contained senecionine, frequently with retrorsine and seneciphylline free bases and/or *N*-oxides, five contained jacobine and three contained erucifoline. Three samples contained heliotrine.

ANNEX 1 – Herbal infusion ingredients

Sample code	Type	Ingredients
S14-010559	Peppermint	Peppermint
S14-010577	Fruit	Hibiscus 38.4%, Grapes 27.4%, Elderberries 25.6%, flavouring 4.6%, Blackcurrants 2.7%, Blueberries 1.4%
S14-010596	Mixture of plants	Organic chamomile, Lemon Balm, Lavender
S14-010600	Mixture of plants	Hibiscus, Dandelion root 20%, Liquorice root, Star Anise 10%, Burdock root 5%, Natural flavourings, Rosehip
S14-010607	Nettle fennel & peppermint	Nettle leaf 40%, Peppermint leaf 25%, Fennel seed 25%, Dandelion root, Licorice root, Aloe vera leaf concentrated natural extract
S14-010609	Fruit & hibiscus	Apple 45%,Hibiscus 30% ,Rosehip, Blackberry Leaves 7% ,Flavouring, Sunflower Petals, Raspberry Pieces 1%
S14-010611	Mixture of plants with borage	Strawberry leaf, Hibiscus flowers, Borage flowers
S14-010614	Mixture of plants with hibiscus	Hibiscus, Rosehip
S14-010618	Mixture of plants	Raspberry leaf, Nettle root, Alfalfa leaves, Rose petals, Ginger, Fenugreek, Fennel, Anise, Blessed thistle, Alfalfa, Calendula, Chamomile, Rooibos
S14-010622	Mixture of plants with borage	Fennel seed, Blessed Thistle, Borage, Fenugreek, Goats Rue
S14-010623	Mixture of plants	Four leaf ginseng, Dangshen, Apple, Ginseng panax, Tangerine peel, Fennel seed, Notoginseng flower, Chinese cinnamon, Dry Ginger, Cloves, Tianyeju
S14-010628	Green tea-based mixture of plants	Green tea, Rose petals, Natural flavors

Sample code	Type	Ingredients

S14-010629	Green tea-based mixture of plants	Green tea, Ginseng root, flavourings, Safflowers, freeze dried passion fruit granules
S14-010630	Mixture of plants with hibiscus	Hibiscus
S14-010631	Fruit	Apple pieces 57%, Rosehip peel 18.5%, Hibiscus 15.5%, Blueberries 3%, flavouring 3%, Blackcurrant leaves 2%, Cornflower petals 1%
S14-010634	Mixture of plants	Apple Pieces 66%, Rosehip peel 14%, Hibiscus 4.5% , Elderflower 3.5%, Liquorice root 3.5%, flavouring 2.5%, Cider vinegar 2%, Rose petals 1%
S14-010638	Nettle	Nettle
S14-010640	Mixture of plants	Ginger 38%, Dried Apple, Rosehip, Flavourings, Lemon Peel 3%, Lemon Grass 2%
S14-010645	Peppermint	Peppermint
S14-010646	Rooibos	Rooibos
S14-010648	Rooibos	Redbush 91%, Flavouring 8%, Natural vanilla flavouring 1%
S14-010650	Peppermint	Peppermint
S14-010652	Green tea & mint	Green tea 60%, Peppermint 40%
S14-010653	Ginseng	Not provided
S14-010655	Camomile	Camomile
S14-010657	Camomile	Camomile
S14-010659	Peppermint	Peppermint
S14-010687	Fennel (instant)	Glucose, fennel extract 6%

Sample code	**Type**	**Ingredients**

S14-010690	Fruit	Hibiscus, Blackberry leaves, Apple pulp, Flavourings: Strawberry 7.5%, Raspberry 3%, Cranberry 0.5%, Red fruit 0.5%; Raspberry fibres 5%, Strawberry fibres 5%, Apple, Citric acid, Rosehip, Cranberry fibres 0.5%
S14-010704	Mixture of plants	Rose hips, Blackcurrant fruit, Lavender flowers
S14-010705	Mixture of plants with hibiscus	Bai Mu Dan white tea, Hibiscus, Cornflowers, Rose hips; Lychee, Goji Berry and Cabernet flavors
S14-010707	Mixture of plants (instant)	Glucose, herbal extracts (fennel, camomile, anis) 7.5%
S14-010710	Mixture of plants	Chamomile, Oatstraw, Linden flower, Skullcap, Catnip, Lemon verbena, Fennel, Wild lettuce, Nutmeg, Calendula
S14-010711	Mixture (instant)	Melissa, Camomile, Linden flower.
S14-010712	Mixture of plants	Aniseed, Fennel, Caraway, Lemon balm
S14-010722	Rooibos & vanilla	Rooibos Tea (*Aspalathus linearis*), Madagascan Bourbon Vanilla extract, Natural flavouring
S14-010723	Rooibos	Rooibos Tea
S14-010737	Ginger	Ginger root, Liquorice root, Cinnamon, Cloves
S14-010738	Camomile	Camomile
S14-010755	Mixture of plants	Nettle, Fennel & Peppermint
S14-010756	Mixture of plants	Cinnamon, cardamom, ginger, cloves, peppercorns
S14-010757	Mixture of plants with hibiscus	Apple pieces 37%, Rosehip peel 33%, Hibiscus 19%, Citrus peel 4.5%, flavouring 3%, Goji berries 1.5%, Acai 0.3%
S14-010759	Rooibos	Rooibos, 4.6% Macadamia nut 3.5%, flavouring 0.5%, Safflower petals
S14-010761	Comfrey	Common Comfrey (*Symphytum officinale*)
S14-010762	Mixture of plants	Cinnamon, chamomile, Valerian and other natural herbs, natural orange flavouring

Sample code	**Type**	**Ingredients**

S14-010763	Mixture of plants	Cinnamon 27%, Chicory Root, Rosehip, Liquorice 18%, Orange Peel, Flavouring, Spearmint 2.5%
S14-010764	Liquorice	Liquorice root
S14-010765	Camomile	Camomile
S14-010767	Lemon Balm	Lemon balm
S14-010768	Camomile	Camomile flowers
S14-010773	Fennel	Sweet fennel seed 50%, Wild fennel seed 45%, Fennel leaf 5%
S14-010774	Mixture of plants	Eleuthero ginseng, chamomile, B vitamins
S14-010775	Mixture of plants	Aniseed, Fennel, Camomile, Caraway, Thyme
S14-010777	Mixture of plants	Siberian ginseng root, Codonopsis root and Panax ginseng root 80%, Hibiscus, Cinnamon, Licorice, Cloves
S14-010778	Lemon Balm	Lemon Balm
S14-010793	Fennel	Fennel
S14-010794	Mixture of plants	Hibiscus flower, Cherry fruit 4%, Rosehip fruit, Apple fruit, Raspberry leaves, Blackcurrant fruit, Liquorice root, Anise Fruit, Cherry natural aroma
S14-010804	Mixture of plants with hibiscus	Rosehip 50%, Hibiscus 50%
S14-010808	Green tea-based mixture of plants	Green tea 74%, Ginkgo leaf 15%, Licorice, Peppermint leaf, Cinnamon, Fennel seeds, Natural vanilla and caramel flavour 1%
S14-010809	Rooibos	Rooibos Tea (*Aspalathus linearis*), Natural Oil of Bergamot
S14-010812	Nettle	Nettle
S14-010813	Green tea-based mixture of plants	Green tea, Natural flavouring 5%, Siberian ginseng root 5%

Sample code	**Type**	**Ingredients**

S14-010815	Mixture of plants	Fennel, Lemon Balm, Peppermint, Raspberry leaves, Lime flower, cumin
S14-010816	Mixture of plants	Lemon Balm - Herb (Herba Melissae), Peppermint herb, Chamomile flower, Fennel fruit.
S14-010817	Mixture of plants	Aniseed 40%, Fennel seed 20%, Cardamom seed 15%, Licorice root, Coriander seed, Celery seed
S14-010821	Fruit	Dried apple pieces, Hibiscus, Rosehip, Cornflower petals, Natural dried currants, Elderberries, Natural flavors.
S14-010822	Peppermint	Peppermint
S14-010823	Liquorice	Liquorice root

SUMMARY

The BfR methods have been adapted as necessary and applied to measure the PA content of teas and honeys with results in broad agreement with those obtained and reported by the BfR. A wide range of ingredients were present in many of the herbal infusions and these could have contributed to the PA contamination.

The sampling of teas is significant for PA analysis. In this work tea bags were mixed well into a composite but were taken from a limited number of packets.

The results are consistent with the data provided by the BfR (Bodi *et al.*, 2014) for 274 tea samples including black, green, and several single and mixed herb types. The BfR reported a high (57%) incidence of contamination of 41 fennel infusions. Five fennel samples were analysed in the survey reported here. The BfR found 100% contamination of melissa (16 samples) and rooibos (24 samples) infusions. This survey did not include melissa types but 4 out of 6 rooibos infusions contained high levels of PAs.

Both surveys (BfR (Bodi *et al.*, 2014) and that reported here) confirmed that the N-oxide forms were present at higher concentrations than the free base, that certain PAs, notably monocrotaline and its N-oxide were absent, and that lycopsamine, echimidine and senecionine N-oxide were the major contaminating PAs.

The analysis of plant-based supplements has been hampered by a lack of validation or even method development and testing as no published procedures are available for plant extracts and tinctures prepared in water, alcohol or vegetable oils, or for naturally liquid or gummy samples such as Aloe vera gels. No data for plant-based supplements has yet been reported by other sources

Results reported by the BfR for 87 honey samples showed a lower level and incidence of contamination of honey from German and Austrian beekeepers with higher levels and incidence in honey from Southern European, Central America and South America. The results reported here complement this data in confirming the higher contamination of honey from countries having a warmer climate and in particular a relatively high frequency of low level contamination of manuka honeys, and very high levels of borage PAs in honey from bees foraging on borage.

GLOSSARY OF TERMS

Alkaloid - a (usually) naturally occurring compound containing a basic nitrogen atom. Many alkaloids have physiological activity in humans, animals and other creatures.

BMDL10 - The 'Benchmark Dose' at which a response to a toxin will occur in 10% of subjects with 95% confidence.

C18 - An SPE sorbent material with a non-polar hydrocarbon nature that can bind PAs and allow other material to be separated from them.

Calibration standards - solutions with known and different levels of authentic PAs used to measure the PA levels in samples by comparison of responses.

CONTAM - Panel on Contaminants in the Food Chain of the European Food Safety Authority (EFSA).

EFSA - European Food Safety Authority - an official EU authority that advises Member States on food safety issues and commissions surveys for the calculation of consumer exposure to toxins by its Panel on Contaminants in the Food Chain (CONTAM).

Free base - the form of the PA in which there is no oxygen molecule attached to the nitrogen atom of the basic ring structure.

LC-MS/MS - An analytical detection and quantification method based on separation of compounds by Liquid Chromatography and their measurement by two sequential Mass Spectrometric measurements in tandem (MS/MS).

LOD - limit of detection - the lowest level of a compound for which a response can be seen in the analytical system without confirmation of its identity.

LOQ - limit of quantification - the lowest level of a compound that can be measured with confidence regarding its identity and level.

MOE - Margin of Exposure - an assessment of the dose at which a small but measurable adverse effect of a toxin is first observed and the level of exposure to the toxin.

Matrix - The material extracted from a sample along with the PA and not entirely removed by SPE. This material can affect the LC-MS/MS signal and therefore

calibration standards are prepared in a matrix similar to the sample but known to be free of PAs (matrix matched standard).

Measurement uncertainty - a statistically derived measure of the error about a result.

N-oxide - the form of the PA in which the nitrogen atom of the basic ring structure is oxidised (has an oxygen atom attached to it). This is the most prevalent form.

PAs - Pyrrolizidine alkaloids - natural toxins found in specific but many plant families. Chemically they are esters of a cyclic dialcohol with one or two acids attached to a nitrogen-containing basic ring structure.

SCX - strong cation exchange - An SPE sorbent material with an ionic nature that can bind alkaloids and allow other material to be separated from them.

SPE - Solid phase extraction - a means of separating PAs from other materials extracted from the sample using a cartridge filled with sorbent material such as C18 or SCX.

z-scores - a measure of the performance of a laboratory participating in a test involving analysis of a sample of unknown composition.

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Appendix 3

Risk assessment for pyrrolizidine alkaloids (PAs)

PAs are known to have caused acute and chronic hepatic veno-occlusive disease (HVOD) in humans through consumption of herbal products and contaminated grain crops. The acute disease is associated with high mortality. The lowest known dose associated with HVOD in humans is reported to be 15 µg/kg body weight (b.w.) per day over a period of about 6 months. The 1,2-unsaturated PAs are genotoxic, and those that have been tested in experimental animals have been shown to be carcinogenic, particularly in the liver. Based on the known mode of action via binding to DNA, it is likely that all 1,2-unsaturated PAs have the potential to be carcinogenic. Different PAs are likely to differ in their carcinogenic potency, but data are not available to allow identification of relative potency factors. Epidemiological studies on cancer in humans have not been performed but based on their genotoxicity and on their carcinogenicity in experimental animals it is considered that they have the potential to be carcinogenic in humans (EFSA, 2011).

Both the European Food Safety Authority (EFSA, 2005) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (FAO/WHO, 2006) have concluded that advice on compounds that are genotoxic and carcinogenic should be based on estimated Margins of Exposure (MOEs). MOEs are calculated by dividing a benchmark dose lower confidence limit (BMDL) derived from animal or human data by the estimated exposure value. A $BMDL_{10}$ of 237 µg/kg b.w. per day for an increased incidence of liver haemangiosarcomas by riddelliine in female rats has been calculated as the reference point for exposure to PAs.

The EFSA Scientific Committee proposed that a MOE of 10,000 or higher, based on a $BMDL_{10}$ from an animal study, would be of low concern from a public health point of view (EFSA, 2005) The MOEs are presented in the tables below expressed as one significant figure in order to avoid an undue impression of precision.

(i) PAs in varieties of tea

The mean concentrations of the PAs measured in the tea samples tested, are given in Table 1. In summary, the data presented for black and green tea samples were as follows:

Black tea - < LOQ for 31 out of 39 samples, then 31 - 1170 µg/kg

Green tea - < LOQ for 7 out of 9 samples, then 227 and 485 µg/kg

Earl Grey - < LOQ for 3 out of 4 samples, one sample at 44 µg/kg

Instant tea - all 3 samples < LOQ

Table 1: Concentrations of PAs in varieties of dry tea leaf used in the exposure assessments

Type of Tea	Mean* concentration of PAs (µg/kg)
Mixed herbal infusions (peppermint, camomile, lemon balm, rooibos, hibiscus, fennel, nettle, liquorice and mixture with borage)**	321
Babies' teas***	196
Borage and comfrey†	51,377
Black teas††	63
Green tea	95

NB: The limit of quantification (LOQ) for each PA was 20 µg/kg.

* For the exposure assessment when the sum of PAs in tea samples was reported as < LOQ then no PAs were above the individual LOQ values, a single LOQ value of 20 µg/kg was used in calculating the mean. When some PAs were present above the LOQ, the mean value was calculated using the sum of the quantified PAs and zero for unquantified PAs (i.e. a lower bound approach).

**- < LOQ for 28 out of 59 samples; rest of samples ranged between 20 and 2,881 µg/kg.

*** - < LOQ for 2 out of 9 samples; rest of samples ranged between 20 to 539 µg/kg.

† - 2 samples: 50,245 and 52,508 µg/kg

†† - Earl Grey and instant tea sample results were included in the black tea group

Risk assessment

Exposure to PAs from teas are shown in Table 2, estimated from the mean concentration data reported in Table 1, with consumption data from the National Diet and Nutrition Survey (NDNS) (Bates *et al*., 2014; Bates *et al.,* 2016; Roberts *et al.,* 2018).

For toddlers, the mean and high level exposures for PAs in teas ranged from 0.0037 to 8.4 and 0.015 to 24 µg/kg b.w./day respectively. For adults, the mean and high level exposures for PAs in teas ranged from 0.0041 to 1.8 and 0.013 to 7.1 µg/kg b.w./day respectively. The highest exposure for both age groups is for consumption of borage and comfrey infusions. It is notable that possible high level exposure of toddlers from borage and comfrey infusions is in excess of the lowest level of PAs at which hepatic veno-occlusive disease has been reported.

There were few toddlers consuming herbal infusions, borage and comfrey infusions and thus the statistical reliability of these estimates is uncertain. The exposure to PAs from green tea in toddlers was not estimated because only one consumer was reported in the NDNS. As a guide, estimates based on less than 60 consumers should be treated with extreme caution.

The MOEs (Table 2) for black teas and green teas are larger than 10,000, indicating low concern. The MOEs for the herbal infusions sampled are less than 10,000 in most instances, therefore indicating a concern for cancer, for all consumers, except mean adult consumers. This is particularly the case for borage and comfrey infusions, for which the MOEs are very low.

Table 2: Consumption of and exposure to PAs in varieties of tea for UK population groups

Age Group	No of consumers	Mean tea consumption* (g/kg b.w./day)	97.5 percentile tea consumption (g/kg b.w./day)*	Mean PA exposure (µg/kg b.w./day)	MOE at mean exposure	97.5 percentile exposure (µg/kg b.w./day)	MOE at 97.5 percentile exposure
Babies' herbal infusions							
Toddlers aged 1½ to 3 years	4	0.16	0.48	0.032	7406	0.094	2521
Herbal infusions (peppermint, camomile, lemon balm, rooibos, hibiscus, fennel, nettle, liquorice and mixture with borage)							
Toddlers aged 1½ to 3 years	4	0.16	0.48	0.052	4558	0.15	1,580
Adults aged 19+ years	125	0.034	0.14	0.011	21,544	0.044	5,386
Borage and comfrey infusions							
Toddlers aged 1½ to 3 years	4	0.16	0.48	8.4	28	24	10
Adults aged 19+ years	125	0.034	0.14	1.8	132	7.1	33
Black teas, including earl grey							
Toddlers aged 1½ to 3 years	62	0.059	0.24	0.0037	64,050	0.015	15,800
Adults aged 19+ years	1,540	0.074	0.21	0.0047	50,426	0.013	18,231
Green tea							
Adults aged 19+ years	58	0.043	0.14	0.0041	57,804	0.013	18,230

* Consumption of tea is reported as dry leaves. A conversion factor of 1% (assuming 10 g of tea leaf makes 1000 mL of infusion) was applied when converting consumption as tea infusion to dry leaf (Food Standards Agency, 2002).

(ii) PAs in plant-based supplements

Of 45 types of plant-based supplements that were analysed, PAs were reported in five; exposure was estimated for these five based on the available dosage information and a lower bound approach (individual PAs below the LOQ were treated as zero). The average body weights of 78.1 kg for UK adults (aged 19 years and above) and 14.6 kg for toddlers (aged 1.5 to 3 years) recorded in the NDNS Rolling Program (Bates *et al*., 2014; Bates *et al.,* 2016; Roberts *et al.,* 2018) were used to express exposure on a body weight basis.

Marshmallow

PAs in a sample of marshmallow rapid release capsules were recorded at a level of 344 µg/kg. The product label specifies that two capsules contain 2,500 mg of marshmallow root extract and indicates a dosage for adults of two capsules three times daily as well as a warning “not intended for use by persons under the age of 18”. Therefore, exposure has only been estimated for adults, at the specified dosage of 7,500 mg per person per day.

Barley Grass Powder

PAs in a sample of barley grass powder were recorded at a level of 117 µg/kg. The label indicates a dosage for adults of 5-10 g daily and for children, half the adult amount. The label also indicates that “higher levels may be taken ….”. The specified doses of 10 g per day for an adult and 5 g per day for a toddler have been used for the exposure assessment in Table 3. Exposures would clearly be higher if larger amounts are taken.

Mixture of Organic Andrographis & Holy Basil (blended with other whole herbs)

PAs in a sample of a mixture of organic herbs were recorded at a level of 50 µg/kg. The label indicates that each capsule contains 400 mg of organic herbs and suggests a dosage for adults of two capsules three times daily, i.e. 2,400 mg per person per day. It is assumed that toddlers would be unlikely to consume this product since the capsules would be difficult for them to swallow.

Maca Powder

PAs in a sample of maca powder were recorded at a level of 22 µg/kg. The label recommends a dosage of 10 to 15 g daily and for children, half the adult amount. The label also suggests: “amounts that work best for you”; which indicates that consumers may use higher doses than recommended. The higher specified doses of 15 g per day for an adult and 7.5 g per day for a toddler have been used for the exposure assessment in Table 3. Exposures would clearly be higher if larger amounts are taken.

Organic Andrographis

PAs in a sample of organic Andrographis were recorded at a level of 130 µg/kg. The label indicates that each capsule contains 500 mg of organic Andrographis and suggests a dosage for adults of two capsules twice daily, i.e. 2,000 mg per person per day. It is assumed that toddlers would be unlikely to consume this product since the capsules would be difficult for them to swallow.

Risk assessment

For toddlers, the estimated exposures for PAs in the two plant-based supplements recommended for this age group (barley grass powder and maca powder) were 0.011 and 0.040 µg/kg b.w./day. For adults, the estimated exposures for PAs in five plant-based supplements ranged from 0.0015 to 0.033 µg/kg b.w./day.

The MOEs (Table 3) are less than 10,000 for toddlers consuming Barley grass powder, indicating a concern. For adults, the MOE was less than 10,000 for the marshmallow capsules also indicating a concern. For the mixture of organic herbs, organic andrographis and the maca powder, the MOEs were higher, but it should be noted that in the case of maca powder the product label suggested that larger amounts could be taken, and it is similarly likely that users of other supplements could take more than the amount specified on the label, under the assumption that the product is good for them in some way.

Table 3: Exposure assessment for PAs in plant-based supplements for UK population groups

Age Group	Consumption (mg/kg b.w./day)	Exposure (µg/kg b.w./day)	MOE
Marshmallow capsules			
Adults 19+ years	96	0.033	7,174
Barley grass powder			
Toddlers (1½ to 3 years)	342	0.040	5,925
Adults 19+ years	128	0.015	15,800
Mixture of organic herbs capsules			
Adults 19+ years	31	0.0015	154,248
Organic andrographis capsules			
Adults 19+ years	26	0.003	71,191
Maca powder			
Toddlers (1½ to 3 years)	514	0.011	21,454
Adults 19+ years	192	0.0042	56,429

(iii) PAs in honey

The levels detected in most of the samples were very low (29 samples contained measurable levels of PAs at concentrations of < 50 µg/kg). Samples of borage honey (110 - 163 µg/kg), one sample of blended honey (185 µg/kg) and one sample of heather honey (251 µg/kg) had slightly higher levels. Exposure to the PAs from honey was estimated from the results of the survey of honey samples together with consumption data from the NDNS (Bates *et al.,* 2014; Bates *et al.,* 2016; Roberts *et al.,* 2018). Consumption data for specific forms of honey were not available therefore consumption data for all types recorded in NDNS were used in the exposure assessment.

Risk assessment

Exposure estimates were derived for mean and high-level consumers using the overall mean concentration data for all honeys sampled, and also the separate mean concentrations for non-borage honey and for borage honey (Table 4). The first of these scenarios represents a situation in which the consumer selects a range of honeys, whereas the second and third are more relevant for the consumer who always eats a particular type of honey. For toddlers, the mean and high level exposures for PAs in honeys ranged from 0.0038 to 0.032 and 0.011 to 0.088 µg/kg b.w./day, respectively. For adults, the mean and high level exposures for PAs in honeys ranged from 0.0016 to 0.014 and 0.0071 to 0.059 µg/kg b.w./day, respectively. The highest exposure for both age groups was associated with the consumption of borage honey.

For toddlers, the MOEs for both mean and high level consumption of borage honey are below 10,000 indicating a concern. The MOEs calculated in the scenario in which borage honey is not consumed do not indicate a concern except in the instance of high consuming toddlers. It should be noted that the number of toddlers consuming honey was below 60, suggesting the exposure estimates may not be statistically reliable. For adults, the MOE for the high level consumption of borage honey was below 10,000 also indicating a concern.

Table 4: Consumption of and exposure to PAs in varieties of honey for UK population groups

Age Group	No of consumers	Mean consumption (g/kg b.w./day)	97.5 percentile consumption (g/kg b.w./day)	Mean exposure (µg/kg b.w./day)	MOE at mean exposure	97.5 percentile exposure (µg/kg b.w./day)	MOE at 97.5 percentile exposure
54 samples of all types of honey with (mean value PAs* = 25 µg/kg)							
Toddlers aged 1½ to 3 years	32	0.24	0.67	0.0060	39,500	0.017	13,941
Adults aged 19+ years	254	0.10	0.44	0.0026	91, 154	0.011	21,454
50 samples of all types of honey excluding borage mean value PAs* = 16 µg/kg)							
Toddlers aged 1½ to 3 years	32	0.24	0.67	0.0038	62,368	0.011	21,454
Adults aged 19+ years	254	0.10	0.44	0.0016	148,250	0.0071	33,380
4 samples of borage honey with mean value PAs = 133 µg/kg)**							
Toddlers aged 1½ to 3 years	32	0.24	0.67	0.032	7,406	0.088	2,693
Adults aged 19+ years	254	0.10	0.44	0.014	16,929	0.059	4,016

* The sum of PAs in some honey samples was reported as nd (i.e. no PAs were above the LOQ), a single LOQ value of 0.3 µg/kg was used in calculating the mean. When some PAs were present above the LOQ, the mean value was calculated using the sum of the quantified PAs and zero for undetected PAs (i.e. lower bound approach).

** All samples had detectable levels

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Appendix 4

Comments from Manufacturers and Brand Owners

WHITTARD Statement

January 2016

Of the 9 samples taken from our product range, 5 contained low traces of PA's. However, these samples were taken almost 2 years ago and all affected products are now discontinued and beyond shelf life.

Nonetheless, we are aware of the potential for PA contamination to occur and be detected in food products, partly because test methods have improved making it possible to look for PA's where once they could not be found.

So in partnership with our three tea and infusion suppliers, we have been taking steps to achieve a steady reduction.

We know that PA's affect herbs and tea due to weeds that naturally contain them growing close by, so weed control is key. Pollen can also transfer PA's. Work to develop weed control measures has already commenced: species that present risk have been identified, and suppliers are being upskilled in this respect. It will take many years to educate and train suppliers all over the world, especially if weeds are to be reduced without increasing the use of chemical pesticides. But already our key partners are making real progress in this area.

In addition to controlling the growth of implicated weeds close to herbs and tea, steps are also being taken to remove weeds from the harvest with improved sorting. To help gauge the success of this, our suppliers are testing their own samples regularly to establish PA levels and ensure levels are reducing.

One thing that we would like to stress, is that PA contamination is not a result of negligence or malpractice. It is a new and naturally occurring issue that has only been understood for the last couple of years, and in that time the tea industry has been quick to learn about the issue and prepare action plans. Also, scientific advice suggest that the levels being detected should not pose a risk to adults based on normal levels of consumption.

UKTIA Statement

June 2019

- The UK and EU tea and herbal infusion industry take the presence of Pyrrolizidine Alkaloids (PAs) very seriously and are actively working with the supply chain to reduce their presence.
- PAs exist due to the presence of tiny fragments of weeds, seeds and pollen during cultivation.
- The results published within the FSA Study were from retail products obtained around the start of 2014 (raw material harvested in 2012/ 2013), **which are no longer on the market.**
- Black, green and herbal teas are safe to consume as part of a varied diet, where a number of different beverage types are consumed*.
- Although the FSA Study shows high levels of PAs in Borage and Comfrey herbs, neither of these are used by the UKTIA membership in any products.
- UKTIA members continuously monitor the PA levels in their products through sampling and testing.

Since first being highlighted in July 2013 by the German Federal Institute for Risk Assessment (BfR) as a potential area of concern for tea and herbal infusions, Pyrrolizidine Alkaloids (PAs) have remained an area of focus for the industry.

The United Kingdom Tea & Infusions Association (UKTIA) and Tea and Herbal Infusions Europe (THIE) members are fully aware of the issue and are dedicating significant time and effort to establish measures to minimise the presence of PAs and to better understand possible sources throughout the supply chain.

EU and UK trade association members have put measures in place to ensure that PA levels in teas and herbal infusions are as low as reasonably achievable (the ALARA standard). One of the main tools utilised by the members is the '**Codex Code of Practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed*:***

http://www.fao.org/input/download/standards/13794/CXP_074e_2014.pdf

In July 2018, THIE published a new code of practice for our sector:

http://www.thie-online.eu/fileadmin/inhalte/Publications/THFI/2018-07-12_THIE_Code_of_Practice_PA_in_TEA-HFI_ISSUE_1.pdf

The industry has undertaken many actions to date

In the UK

- Industry has communicated about the presence of PAs to producers/suppliers and actions are being taken to manage the presence of PAs effectively.
- **Recent industry results continue to show a significant reduction in PA levels**, compared with those found in the older products featured in the FSA Survey.
- Letters have been sent to the Tea Boards around the world to make them and their members aware of the issue.
- Industry is actively engaging with FSA in order to establish a full understanding of PAs.
- UKTIA Members have provided data for submission to the European Food Safety Authority (EFSA) database and the European industry (THIE) PAs database to assist with additional data analysis.

At a European level

- To ensure open dialogue, the European industry met with the European Commission, in November 2015, to discuss a comprehensive overview on PAs, which detailed measures being taken by the industry to minimise the presence of PAs.

Monitoring PAs in Tea and Herbal Infusions Products

UKTIA members are continuously testing their products to ensure that the measures put in place remain effective. Data collected for samples harvested through 2016, including raw material samples and results from UKTIA studies on it members retail products in 2017 and 2018, shows continued significant reduction in PA levels compared to the results presented by the FSA (raw materials harvested in 2012/

2013). For both teas and herbal infusions, maximum results were substantially lower than reported in the FSA survey. The industry continues to monitor retail products and raw materials.

Sampling and analysis methods for detection and measurement of PAs in teas and herbal infusions are still developing. This highlights the need for more robust, standardised sampling and test methods to address the challenge of variability within the current analytical capability. UKTIA welcomes the EU Commission's ongoing work in this area.

The United Kingdom Tea & Infusions Association and its members continue to establish stronger communication channels with suppliers (countries of origin) raising awareness about Pyrrolizidine Alkaloids and possible measures to help minimise their presence.

*Federal Institute for Risk Assessment (2013)
https://www.bfr.bund.de/cm/349/pyrrolizidine-alkaloids-in-herbal-teas-and-teas.pdf