5. Gentian violet

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Identity

International Non-proprietary Name (INN): gentian violet

Synonyms: See Table 5.1.

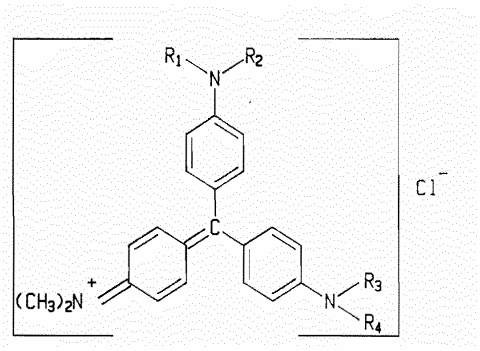
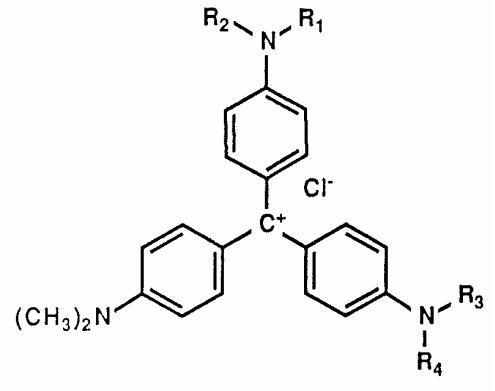
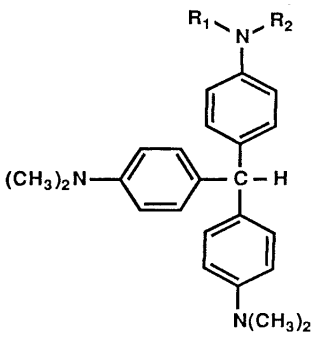
IUPAC Names: (4-[4,4-bis(dimethylamino)benzhydrylidene]cyclohexa-2,5-dien-1-yl-idene-dimethylammonium chloride);
Tris(4-(dimethylamino)phenyl)methylium chloride

Chemical Abstract Service Number: 548-62-9 (gentian violet)

Structural formula of main components: See Table 5.1.

The structural identity, some major physical-chemical properties and characteristics of the trimethylphenyl dye, gentian violet, its de-methylated derivatives, and its metabolite leucogentian violet are summarized in Table 5.1. All information provided in Table 5.1 is for the parent compound unless otherwise noted.

Table 5.1. Physical-chemical properties of gentian violet and its metabolite leucogentian violet

	Substance name	
	gentian violet	leucogentian violet
Structure	 	

	Substance name							
	gentian violet					leucogentian violet		
Derivatives		R_1	R_2	R_3	R_4		R_1	R_1
	Hexa- (Parent)	CH_3	CH_3	CH_3	CH_3	Parent	CH_3	CH_3
	Penta-	CH_3	CH_3	CH_3	H	Penta-	CH_3	H
	N′1-tetra-	CH_3	CH_3	CH_3	H			
	N″2-tetra-	CH_3	H	CH_3	H			
Depositor-supplied synonyms (PubChem)	Total number: 242 Examples: Andergon Aniline violet Axuris Badil Basic Violet 3 Brilliant Violet 58 Crystal Violet Gentiaverm Hexamethylpararosaniline chloride Meroxylan Meroxyl Methylrosalinide chloride Methyl Violet 10B Pyoktanin Vianin Viocid					Total number: 49 Examples: Leucocrystal Violet Leucomethyl green Leuco Crystal Violet 603-48-5 Crystal Violet leucobase 4,4',4''-Methylidynetris(N,N-dimethylaniline) C.I. Basic Violet 3, leuco Tris(p-dimethylaminophenyl)methane Tris(4-dimethylaminophenyl)methane ST057221		
Chemical Abstracts Registry Number	548-62-9 (parent) 603-47-4 (penta-) 84215-49-6 (N′1-tetra-) 89232-79-1 (N″2-tetra-)					603-48-5 (parent)		
PubChem-CID	11057					69048		
EINECS	208-953-6					210-043-9		
IUPAC	Tris(4-(dimethylamino)phenyl)methylium chloride					4,4′,4″-Methylidynetris(N,N-dimethylaniline)		
Molecular formula	$C_{25}H_{30}N_3Cl$					$C_{25}H_{31}N_3$		
Formula weight (g mol^{-1})	407.98					373.53		
Melting point (°C)	205					175–177		
Octanol/water partition coefficient: log $K_{o/w}$	1.172 at 25°C					Not found		
Solubility in water	50 g/L at 27°C					Not found		

NOTE: Structural formulas from McDonald, 1989.

Other information on identity and properties

Adsorption

Adsorption characteristics of synthetic dyes such as gentian violet were studied in order to find approaches to remove them from wastewaters. Due to low biodegradability of dyes, traditional processes are often not very effective in removing these dyes from wastewater. Adsorption on activated carbon has been found to be an effective process for dye removal; however, the use of commercially available products is very costly. Therefore, other, lower cost, alternatives have been examined, including (but not limited to) chemically treated sawdust (Garg, 2003), preparation of activated carbon using various products such as spent tea leaves (Bajpai and Jain, 2010, 2012), peanut shells (Zhang and Ou, 2013), waste apricot (Başar, 2006; Önal, 2006), and rice husk (Mohanty *et al.*, 2006).

Photodegradation

Triphenylmethane dyes are known to be quite light sensitive. Exposure of gentian violet to ultraviolet light results in demethylation, and in the presence of oxygen, oxidation. Hydrogen peroxide is an inexpensive oxidant, and has been used in the presence of suitable photo catalysts to decolorize synthetic dyes such as gentian violet. It has been reported that in the presence of NADPH and light, gentian violet was photo-reduced to the same triarylmethyl free radical that is formed by enzymatic reduction (Harrelson and Mason, 1982).

Binding to macromolecules

Gentian violet binds to macromolecules. It is known to bind to DNA. Liu *et al.*, (2013) investigated the binding of gentian violet to bovine haemoglobin. Binding of gentian violet to bovine haemoglobin changes spatial conformation of the bovine haemoglobin, leading to destabilization.

Industrial uses

Gentian violet and derivatives are used as a dye for various purposes, including as a dye for wood, leather, silk, nylon, paper and ribbon tapes, and also as a biological stain. It is also used in human medicine for use topically, and to inactivate *Trypanosoma cruzi* (the causative agent of Chagas disease) in blood collected for transfusion (Docampo and Moreno, 1990).

Sources of data

A collation and review of available data on gentian violet was provided by a national authority (Canada, 2013). For this document, published literature databases (Medline, CABI, Agricola and Toxnet) were searched for gentian violet using the terms: gentian violet, crystal violet, gentian [or crystal] violet toxicity, and gentian [or crystal] violet residues. Retrieved articles were assessed for relevance. References cited within the relevant articles were further assessed and reviewed where appropriate.

A supplementary search was conducting using the phrase "gentian [or crystal] violet" with the following primary search terms: adsorption, analytical method, aquaculture, biodegradation, depletion, detection, metabolism, photodegradation, and tissues.

Residues in food and their evaluation

Conditions of use

Gentian violet has been used as an antifungal and antiparasitic agent for treatment of fish, and also as a topical antiseptic, antibacterial and antifungal compound for treatment of skin and eye infections in livestock. It could also be used for treatment of *Ichthyophthirius multifiliis*, which causes 'white spot disease' in freshwater fish. Gentian violet is not currently authorised for use in aquaculture in most developed countries. However, because of its antibacterial and antifungal properties, and its similarities with malachite green, there is a potential for it to be used in aquaculture to mitigate bacterial or fungal infections in some countries (e.g. Khoshkho and Matin, 2013). Fish products imported to a number of countries, including Canada, EU member states and the United States of America have occasionally tested positive for gentian violet or its metabolite, leucogentian violet.

Gentian violet was previously used in poultry feeds to inhibit the growth of mould and fungus; however, several countries have withdrawn approval or registration of this use (FDA, 1991a; CFIA, 1992). It is currently prohibited from use in food producing animals in the United States of America (FDA, 1991b; Davis *et al.*, 2009). There are currently no approved veterinary drug products containing gentian violet available in the United States of America.

Australia conducted a special review of gentian violet in 1994 and subsequently: cancelled the clearance certificates for crystal (gentian) violet with effect from 1 November 1994; cancelled the MRLs for crystal (gentian) violet, with effect from 1 November 1994; and cancelled all registrations and relevant approvals of veterinary products containing crystal (gentian) violet, effective 1 November 1994 (APVMA, 1994). Heberer (2009) reported that "according to EU law, zero tolerance applies to all residues of malachite green (MG) and gentian violet in food for human consumption, as both compounds are not registered for use as veterinary drugs with food producing animals".

In Canada, gentian violet is approved as a topical preparation for use in food producing animals (Health Canada, 2013). Current indications include topical therapy for ringworm, treatment of pink eye and topical treatment of skin wounds. However, the use of gentian violet in animal feeds to prevent mould growth is prohibited in Canada (CFIA, 1992). Gentian violet (1% solution) is approved for use in human medicine for topical use (Health Canada, 2013).

At the time of preparation of this monograph, some EU member states have established an action limit of 0.5 µg/kg for residues of gentian violet in internationally traded food consignments (DEFRA, 2007). Canada has set an action level of ≥0.5 µg/kg for leucogentian violet and/or gentian violet (in presence of leucogentian violet) for compliance purposes.

Pharmacokinetics and metabolism

Metabolism in micro-organisms

Biological decolourization of gentian violet has been widely reported. Some examples are presented below. A detailed review is beyond the scope of this monograph.

Intestinal bacteria

McDonald and Cerniglia (1984) studied the metabolism of gentian violet from human, rat, and chicken intestinal microflora, human faecal samples, and 12 pure anaerobic bacteria cultures representative of those found in the human gastro-intestinal tract. Incubations were carried out under anaerobic and aerobic conditions. All pure cultures and mixed intestinal microflora reduced gentian violet to leucogentian violet. Gentian violet and leucogentian violet were identified in the incubation mixtures using high performance liquid chromatography (HPLC) and mass spectrometry with electron ionization. *Escherichia coli* and *Salmonella typhimurium* possessed little ability to reduce gentian violet under either anaerobic or aerobic conditions. Gentian violet at a concentration of 2.67 µg/ml of incubation medium was not toxic and did not inhibit bacterial growth when compared with control incubations.

Fungi

Some ligninolytic (nitrogen-limited) fungi have been found capable of decolorizing synthetic dyes. Ligninolytic cultures of the white rot fungus *Phanerochaete chrysosporium* were shown to metabolize gentian violet to N-demethylated metabolites catalyzed by lignin peroxidase. Non-ligninolytic (nitrogen-sufficient) cultures also degrade gentian violet, suggesting that there is an additional mechanism by which degradation occurs (Bumpus and Brock, 1988). Ganesh *et al.* (2011) reported decolourization of gentian violet by *Aspergillus* sp. CB-TKL-1. Addition of glucose or arabinose (2%) and sodium nitrate or soyapeptone (0.2%) enhanced the decolourization ability of the culture. Analysis also showed that the decolourization occurs in a stepwise pattern, and N-demethylation appears to be the dominating mechanism in decolourization.

Microsomes

The *in vitro* metabolism of gentian violet in microsomes isolated from livers of hamsters, guinea pigs, chickens, four strains of mice and three strains of rats has been investigated (McDonald *et al.*, 1984a; McDonald, 1989). All three demethylated metabolites of gentian

violet (Table 5.1) were produced, with mice microsomes producing less demethylated products than the other species. Microsomes from guinea pigs produced less of the N′1 tetra-metabolite and more of the N″2 tetra- metabolite than the other species examined. Sex differences in de-methylation were not apparent among the species. Leucogentian violet was not mentioned by the authors, and therefore its potential fate in these studies is unknown.

Gentian violet was metabolized by rat liver microsomes under a nitrogen atmosphere while supplemented with NADPH to give a single-line electron spin resonance (ESR) spectrum, and considered to be the tri-(p-dimethylaminophenyl) methyl radical. Removal of the NADPH-generating system, gentian violet or using heat denatured microsomes resulted in no ESR spectrum. This one-electron reduction to produce a carbon centred free radical was inhibited approximately 50% by metyrapone, and also by an atmosphere of carbon monoxide, suggesting the involvement of cytochrome P-450 (Harrelson and Mason, 1982).

Metabolism in laboratory animals

Rats

One Fischer 344 female rat was given 0.84 mg [phenyl-U-^{14}C]-gentian violet (5.68 µCi) (94.8% gentian violet and 5.2% pentamethylpararosaniline) twice daily for three days, and faeces were collected between 48 and 72 hours after the first dose to examine gentian violet metabolism. Samples were extracted with diethyl ether, with metabolite identification using HPLC. Leucogentian violet accounted for 67% of the radioactivity in the ether extract, and a minimum of 11% of the total radioactivity present in the 48 to 72 h faeces collection (McDonald and Cerniglia, 1984; McDonald, 1989).

The depletion kinetics and metabolism of gentian violet in single- and multiple-dosing regimens have also been studied (McDonald *et al.*, 1984b; McDonald, 1989). Six F344 rats (3 male, 3 female) were housed individually in metabolism cages and given a single dose by gavage of [^{14}C]gentian violet (94.8% gentian violet and 5.2% pentamethylpararosaniline). The males and females received 4.8 mg (3.1 µCi) and 5.2 mg (9.26 µCi) gentian violet/kg, respectively, and were killed 2, 4, 14 or 24 hours later. Urine, faeces, liver, kidney, muscle, testes or ovaries, and a fat sample were collected and radioactivity measured (Tables 5.2 and 5.3). Half-lives of 14.5 and 14.4 h were calculated following a single dose for the liver and kidney, respectively, for males, and 17.0 and 18.3 hours, respectively, for females.

Table 5.2. Concentrations following a single oral dose of [phenyl-U-^{14}C]-gentian violet in rats

Time after dose (h)	Gentian violet equivalents (mg/kg)				
	Liver	Kidney	Muscle	Testis/ovary	Fat
			Males		
2	2.52 ±0.75	0.48 ±0.11	0.05 ±0.01	0.03 ±0.01	0.12 ±0.05
4	3.51 ±0.79	0.47 ±0.04	0.05 ±0.01	0.02 ±0.02	0.12 ±0.03
14	1.71 ±0.15	0.22 ±0.01	0.05 ±0.02	0.04 ±0.01	0.50 ±0.1
24	0.99 ±0.14	0.13 ±0.01	0.02 ±0.01	0.02 ±0.01	0.66 ±0.07
36	0.76 ±0.12	0.10 ±0.01	0.03 ±0.02	0.02 ±0.01	0.72 ±0.14
			Females		
2	1.37 ±0.28	0.48 ±0.11	0.05 ±0.01	0.03 ±0.01	0.13 ±0.01
4	2.84 ±0.41	0.52 ±0.11	0.15 ±0.01	0.02 ±0.02	0.42 ±0.09
14	1.22 ±0.19	0.23 ±0.05	0.13 ±0.05	0.04 ±0.01	2.07 ±0.36
24	1.11 ±0.23	0.21 ±0.06	0.16 ±0.10	0.02 ±0.01	3.30 ±0.45
36	0.69 ±0.15	0.14 ±0/02	0.05 ±0.01	0.02 ±0.01	2.92 ±0.77

NOTES: Values are means ±1 SD for 3 rats.

Table 5.3. Excretion following a single oral dose of [phenyl-U-^{14}C]-gentian violet in rats

Time after dose (h)	Excretion (µCi)	
	Urine	Faeces
	Males	
2	0.045	0.001
4	0.064	0.009
14	0.25	3.76
24	0.33	11.10
36	0.29 (2.2%)	9.55 (72.9%)
	Females	
2	0.025	0.001
4	0.017	0.011
14	0.11	4.39
24	0.33	5.14
36	0.20 (2.2%)	5.91 (63.8%)

NOTES: Numbers in (parentheses) indicate% of dose.

For a multiple-dosing experiment, 8 male and 8 female F344 rats were housed individually in metabolism cages and administered 14 doses at 12 h intervals with [^{14}C]gentian violet using a mixture of 94.8% gentian violet and 5.2% pentamethylpara-rosaniline (McDonald *et al.*, 1984b). The total dose for males and females was 3.5 (140 µCi) and 5.69 (79.72 µCi) mg/kg, respectively. The rats were killed 2 h after receiving the final dose. Urine, faeces, liver, kidney, muscle, testes or ovaries and a fat sample were collected and radioactivity measured (Table 5.4).

Table 5.4. Disposition and excretion of multiple oral doses of [phenyl-U-^{14}C]-gentian violet in F344 rats

Sample	Gentian violet residues (mg equivalents/kg)	
	Male	Female
Liver	4.0 ±0.6	3.7 ±0.8
Kidney	0.7 ±0.1**	2.9 ±1.7**
Muscle	0.09 ±0.03*	0.6 ±0.5*
Gonad	0.08 ±0.04	3.67 ±0.76
Fat	3.2 ±0.4**	20.2 ±5.8**
Urine	3.18 (2.2%)	1.29 (1.6%)
Faeces	92.02 (65.5%)	58.04 (72.8%)

NOTES: Values are means ±1SD for seven male and eight female rats. Asterisks indicates a significant sex difference (* = P <0.02; ** = P<0.01, by student T test). Numbers in (parentheses) indicate% of dose.

Two female bile duct-cannulated rats were orally dosed with 300 µg (3.27 µCi) or 840 µg (9.16 µCi) [phenyl-U-^{14}C]-gentian violet (94.8% gentian violet and 5.2% pentamethylpararosaniline) and bile collected for 24 and 28 h, respectively (McDonald *et al.*, 1984b; McDonald, 1989). The percentages of the oral dose collected from the 2 rats were 6.4% and 5.7% after 24 and 28 h, respectively. The authors concluded that orally administrated gentian violet cation (which could be combined with a hydroxyl ion in the small intestine) with a molecular weight of 372 was absorbed to a greater extent than had been reported for other triphenylmethane dyes. It was speculated that leucogentian violet, which is produced under anaerobic conditions by intestinal bacteria, may be absorbed and accumulate in the fat (McDonald *et al.*, 1984b; McDonald, 1989).

Mice

Twenty-four (12 male, 12 female) B6C3F$_1$ mice were housed 3 per metabolism cage and administered [phenyl-U-^{14}C]-gentian violet (94.8% gentian violet and 5.2% pentamethylpara-rosaniline) by oral gavage every 12 hours for 7 days (McDonald *et al.*, 1984b; McDonald, 1989). The total dose for males and females was 5.6 (19.55 µCi) and 7.1 (19.55 µCi) mg gentian violet/kg, respectively. The mice were killed 2 h after receiving the final dose. Urine, faeces,

liver, kidney, muscle, testes or ovaries and a fat sample were collected during the study and radioactivity measured (Table 5.5). The data show that gentian violet residues accumulated in the adipose tissue, although a major portion (64–73%) excreted in faeces.

Similar to rats, gentian violet residues in mice accumulated in the adipose tissue, with more accumulation in females than in males. The percentages of gentian violet radioactivity administrated and excreted in the faeces of rats and mice were very similar, while a larger percentage of gentian violet was excreted in the urine of mice than that of rats.

Table 5.5. Disposition and excretion of multiple oral doses of [phenyl-U-14C]-gentian violet to mice

Sample	Gentian violet residues (mg equivalents/kg)	
	Male	Female
Liver	17.8 ±2.6**	10.7 ±3.4**
Kidney	1.6 ±0.1**	2.7 ±0.8**
Muscle	0.6 ±0.4**	1.3 ±0.7**
Gonad	0.49 ±0.08	3.66 ±1.08 (1)
Fat	14.3 ±3.0**	24.1 ±7.0**
Urine	1.16 (5.9%)	1.58 (8.1%)
Faeces	12.89 (65.9%)	13.17 (67.4%)

NOTES: (1) Mean of 8 mice. Values are means ±1 SD for 12 male and female mice. Asterisks indicate a significant sex difference (* = P <0.02; ** = P <0.01 by student T test). Numbers in (parentheses) indicate% of total dose.

Metabolism in food producing animals

Chickens

Eighteen (9 male, 9 female) Hubbard × Hubbard adult broiler breeders were fed a single dose of [^{14}C]gentian violet (31.78 µCi) of 6.82 mg/bird (Olentine, Gross and Burrows, 1980). Prior to dosing, the birds had received a diet containing 1 kg unlabelled gentian violet/1000 kg feed for 30 days. One female and one male were randomly selected and killed after 8, 24, 48, 120, 168, 240, 336, 432 and 504 h following the administration of the radiolabelled dose of gentian violet. Blood samples were taken after 1, 4 and 8 h and at time of slaughter. Liver, muscle, kidney, skin, fat and eggs were collected and analysed for radioactivity. Analysis of the blood [^{14}C] activity data indicated half-lives of 1.43 and 1.68 h for males and females, respectively. Results of the analysis of the tissue samples collected for radioactive residues are presented in Table 5.6. All the eggs collected over the first 144 h had low but detectable levels of radioactivity, with one collected after 456 h having detectable levels of radioactivity.

Table 5.6. Calibrated residue concentration of [^{14}C]gentian violet in tissues of broiler breeder chickens

Tissue	Sex	Concentration of ^{14}C-gentian violet (µg equivalents/kg) at time-points (h) after administration (1)(2)								
		8	24	48	120	168	240	336	432	504
Muscle	M	ND	ND	ND	ND	ND	ND	ND	ND	ND
	F	45 (164)	ND	ND	ND	ND	ND	ND	ND	ND
Skin	M	19 (44)	ND	ND	ND	ND	ND	ND	ND	ND
	F	38 (52)	26 (37)	ND	ND	ND	ND	ND	ND	ND
Fat	M	16 (18)	ND	ND	ND	ND	ND	ND	ND	ND
	F	126 (143)	ND	ND	ND	ND	ND	ND	ND	ND
Liver	M	81 (302)	71 (258)	ND	ND	ND	ND	ND	ND	ND
	F	313 (876)	30 (106)	37 (92)	18 (55)	ND	ND	ND	ND	ND
Kidney	M	234 (977)	96 (282)	57 (235)	48 (199)	16 (58)	17 (58)	17 (63)	ND	ND
	F	319 (1188)	101 (374)	94 (221)	36 (109)	30 (121)	58 (196)	16 (65)	17 (72)	ND

NOTES: (1) Values in (parentheses) represent dry weight concentration. (2) ND = not detectable.

In a GLP compliant study, five groups of Cornish-White Rock broiler chickens (5 male and 5 females per group) were given [phenyl-U-^{14}C]-gentian violet (94.3% pure, specific activity of 37.3 mCi/mmol) by capsule 3 times daily for 7 days (McDonald, 1985). The dosing level was equal to 15 mg gentian violet per kg feed, or 1.72 and 1.45 mg gentian violet/kg bw

for males and females, respectively. The chickens were allowed access to regular feed and water ad libitum. Chickens were killed 6, 24, 48, 120 or 240 h after the last treatment, and samples of liver, kidney, gizzard, breast, thigh, heart and skin taken for total ^{14}C residue determination. Samples of excreta were collected from each chicken in the 240-h depletion group beginning 16 h after receiving the first capsule and then at 24 h intervals for total ^{14}C determination. Total residues (Tables 5.7 and 5.8), determined by combustion analysis and liquid scintillation counting, were calculated on the basis of the hexamethylpararosaniline cation (molecular weight 373) rather than on the molecular weight of the chloride (408). Highest residue concentrations were detected in the liver of males at the 6 h collection time. In females, liver and kidney contained almost equally high residue concentrations at 6 h withdrawal, but at subsequent withdrawal periods the highest concentrations were reported in liver. The depletion of total residues was bi-phasic in each tissue, with graphical half-lives in the range of 59 to 215 h for the second phase (Table 5.9). The depletion half-lives were consistent between the sexes, with the longest half-life of the second phase occurring in the liver. The authors concluded that the tissue residues had not reached equilibrium at cessation of dosing.

Table 5.7. Total residue concentrations (µg/kg; mean ±SD) of [phenyl-U-^{14}C]-gentian violet equivalents for male chicken tissues

Tissue	Withdrawal period				
	6 h	24 h	48 h	120 h	240 h
Liver	169.1 ±112.6	44.6 ±8.0	38.0 ±15.4	34.7 ±20.0	20.9 ±12.2
Kidney	78.7 ±18.4	30.4 ±4.9	18.6 ±5.7	9.8 ±2.3	3.8 ±0.6
Gizzard	33.6 ±22.2	7.8 ±3.6	4.4 ±1.6	2.6 ±1.1	0.89 ±0.48
Breast	11.4 ±5.9	4.4 ±1.0	3.2 ±1.5	1.2 ±0.4	0.48 ±0.61
Thigh	18.7 ±10.8	6.5 ±1.9	4.1 ±2.2	1.7 ±1.3	0.73 ±0.24
Heart	27.7 ±13.2	5.0 ±0.9	2.7 ±1.3	2.1 ±0.6	1.1 ±0.6
Skin	45.3 ±12.8	19.3 ±4.9	12.6 ±2.5	10.6 ±2.7	6.1 ±2.6

Table 5.8. Total residue concentrations (µg/kg; mean ±SD) of [phenyl-U-^{14}C]-gentian violet equivalents for female chicken tissues

Tissue	Withdrawal Period				
	6 h	24 h	48 h	120 h	240 h
Liver	73.9 ±20.9	60.4 ±31.8	31.4 ±9.9	19.0 ±7.7	12.8 ±10.1
Kidney	73.3 ±15.1	33.6 ±13.2	15.3 ±2.2	11.7 ±9.7	2.9 ±1.3
Gizzard	21.0 ±10.8	10.1 ±4.9	4.4 ±2.3	1.6 ±0.9	0.45 ±0.25
Breast	5.9 ±3.2	4.3 ±1.3	2.4 ±1,3	0.61 ±0.49	0.27 ±0.26
Thigh	7.6 ±2.3	5.8 ±2.9	2.6 ±1.3	2.2 ±1.7	0.41 ±0.32
Heart	17.5 ±4.1	7.1 ±3.1	2.7 ±0.8	2.7 ±2.6	0.87 ±0.30
Skin	18.2 ±8.8	18.9 ±6.2	12.7 ±4.0	9.4 ±3.9	3.4 ±1.3

For the determination of metabolites, tissues and excreta were extracted with acidic methanol, partitioned, and cleaned up and subjected to chromatography on a Bondapak C_{18} reversed phase column prior to liquid scintillation counting. The chromatographic conditions for tissue extracts were such that parent gentian violet, its demethylated derivatives, and leucogentian violet (see Table 5.1) eluted at 25.2 (parent gentian violet), 21.6, 17.9, 16.7 and 23.5 (leucogentian violet) minutes, respectively. The metabolite profile identified at the 6 h collection time-point is presented in Table 5.10. These metabolites were not present in samples taken past the 6 h withdrawal period; however some other unidentified metabolites were present at subsequent time-points.

Table 5.9. Graphical half-lives (hours) for biphasic depletion of total residues of [phenyl-U-^{14}C]-gentian violet from chicken tissue

	Phase 1	Phase 2	
	$t_{½}$ (1)	$t_{½}$ (2)	Correlation
	Males		
Liver	2.7	215	-0.971
Kidney	6.7	84.2	-0.999
Gizzard	5.0	82.7	-0.999
Breast	5.3	71.3	-0.993
Thigh	6.4	79.1	-0.984
Heart	4.9	146	-0.994
Skin	6.9	179	-0.989
	Females		
Liver	38.2	153	-0.978
Kidney	8.9	77.3	-0.973
Gizzard	11.1	59.0	-0.996
Breast	26.1	63.5	-0.958
Thigh	27.0	68.8	-0.958
Heart	9.1	110	-0.928
Skin	— (3)	98.6	-0.987

NOTES: (1) Calculated using the difference between the 6- and 24-hour data points, and corresponding points on the phase 2 curves. (2) Calculated by least squares using the 48-, 120- and 240-hour data points. (3) Data did not allow calculation of half-life.

Table 5.10. Concentrations of metabolites and gentian violet (µg/kg) of [phenyl-U-^{14}C]-gentian violet measured in chicken tissue (6-hour depletion)

Tissue	N'1-tetra- + N''2-tetra-		Penta-		Parent drug (gentian violet)	
	male	female	male	female	male	female
Liver	5.7	0.64	2.4	0.26	2.3	0.26
Kidney	2.8	2.9	2.4	2.6	2.1	2.8
Gizzard	8.0	0.35 (1)	6.6	0.66	20.3	1.8
Breast	0.09	0.11	0.08	0.25	0.22	0.54
Thigh	0.28	0.09	0.06	0.15	0.11	0.31
Heart	0.83	0.61	1.2	0.82	1.1	1.3
Skin	n.a.	0.15	n.a.	0.21	n.a.	0.29

NOTES: (1) A portion of this sample was lost to spillage. n.a. = Values not available.

Insoluble residue in tissue, as determined by combustion analysis, represents a substantial portion of the total residue in most tissues at all depletion times (Table 5.11). The chromatographic conditions for excreta were such that parent gentian violet and each demethylated metabolite (see Table 5.1) eluted at 13.0 (parent gentian violet), 9.7, 7.1, 7.1 and 19.3 (leucogentian violet) minutes, respectively. The metabolic profile identified in excreta from the 240 h depletion group is presented in Table 5.12. De-methylated products were identified, but gentian violet was the predominant excretion product. Inconclusive evidence was obtained for the presence of leucogentian violet in tissues and excreta (McDonald, 1985).

Table 5.11. Insoluble residue (µg/kg) of [phenyl 1-U-^{14}C]-gentian violet cation equivalents for pooled, solvent-extracted chicken tissue

	Withdrawal period				
	6 h	**24 h**	**48 h**	**120 h**	**240 h**
			Females		
Liver	27.4	33.0	18.8	34.8	9.06
Kidney	24.5	12.6	6.42	2.75	0.08
Gizzard	8.42	6.51	1.33	0.74	0.20
Breast	2.38	3.09	0.59	0.22	0.04
Thigh	1.19	1.27	0.97	0.74	0.21
Heart	6.22	0.53	1.61	1.06	0.24
Skin	0.15	6.92	3.68	2.29	0.78
			Males		
Liver	105.1	22.6	20.8	17.1	1.06
Kidney	51.8	12.7	0.82	0.49	2.49
Gizzard	23.2	2.41	1.18	1.79	0.17
Breast	2.05	1.10	0.76	0.31	0.01
Thigh	8.85	2.07	0.62	0.56	0.51
Heart	5.95	1.52	1.15	0.98	0.68
Skin	16.8	4.37	5.71	5.61	3.32

Table 5.12. Quantitation by [^{14}C] of metabolites in excreta of chicken from the 240-h depletion group

	Collection Day							
	Day 3		**Day 5**		**Day 7**		**Day 9**	
	Total	**(%) [(2)]**	**Total**	**(%) [(2)]**	**Total**	**(%) [(2)]**	**Total**	**(%) [(2)]**
				Female #57				
N′1-tetra- , N″2-tetra-	14.4	(7.9)	14.4	(8.1)	14.2	(8.0)	58	(12.2)
Penta-	29.6	(16.2)	30.8	(17.1)	30.1	(16.8)	63	(13.3)
Parent	112.4	(61.8)	109.8	(62.3)	110.0	(62.0)	165	(34.7)
Leucogentian Violet	2.7	(1.5)	0.0	(0)	0.0	(0)	0	(0)
				Male #34				
N′1-tetra-, N″2-tetra-	23.6	(9.7)	20.6	(9.3)	20.5	(9.5)	169	(13.9)
Penta-	46.4	(19.0)	40.6	(18.3)	42.3	(19.0)	127	(10.5)
Parent	134.2	(55.0)	124.6	(56.2)	127.9	(57.0)	143	(11.8)
Leucogentian Violet	2.6	(1.1)	0.0	(0)	0.0	(0)	0	(0)

NOTES: (1) Total = amount excreted in faeces for the day in ng. (2) Portion of total radioactivity in chromatogram.

As part of method development for determination of gentian violet, its demethylated metabolites and leucogentian violet, gentian violet residues were measured in livers and muscle from chickens treated with a standard broiler diet containing 30 mg/kg gentian violet for 3 weeks (Roybal *et al.,* 1990). Feed containing gentian violet was withdrawn 3 h prior to slaughter. Mean results from 10 analyses of residue-incurred chicken liver were 31 µg/kg gentian violet (coefficient of variation (CV) 9.7%), 34 µg/kg pentamethyl metabolite (CV 8.8%), and 40 µg/kg tetramethyl metabolite(s) (CV 5.0%), for an average value of 105 µg/kg total residues (CV 5.7%); no leucogentian violet was found. Subsequent work on method development for detection of gentian violet residues in chicken tissues indicated that the method used could influence the recovery of gentian violet residues, in particular leucogentian violet (Munns *et al.,* 1990). However, when the method is optimized, leucogentian violet represented the major residues in chicken fat when gentian violet is fed to chicken in feed.

Tissue residue depletion studies

Radiolabelled residue depletion studies

There were no radiolabel residue depletion studies for evaluation in fish treated with gentian violet.

Residue depletion studies with unlabelled drug

Limited tissue residue depletion data are available for gentian violet. Where depletion and pharmacokinetics and metabolism were investigated in the same study, they are reported under the Pharmacokinetics and Metabolism section. No absorption or depletion data for topical administration in terrestrial species were available. Since gentian violet use in aquaculture for food is not authorized in many countries, reliable data to inform the exposure regime are not readily obtainable; however, anecdotal reports suggest that gentian violet is applied using similar exposure protocols to malachite green (100 mg malachite green/kg/min).

Atlantic salmon

Chan *et al.* (2012) exposed 90 Atlantic salmon (approx. 100 g, <12 month old) to 1 µg gentian violet /L as a bath in a tank with continual flushing. The calculated exposure was 100 µg gentian violet/L-minute. Flush rates in the tank were adjusted to achieve a gentian violet exposure of 100 µg/L-minute for 5 h, then returned to normal flush rate, at which the gentian violet concentration in the tank was <0.1 mg/kg. The concentration of gentian violet in the tank was negligible (<0.01 µg/kg) 24 h after addition of the gentian violet. Thirty-five fish were held in a separate tank as controls. Fish were sampled at 1, 7, 14, 28, 63 and 91 days post-dosing, with tissues stored at -20°C until analysis. Samples were analysed either for total residues (by oxidizing leucogentian violet back to gentian violet) or for the parent and the leucogentian violet separately, using LC-MS/MS. Data obtained by both methods were comparable; however, total gentian violet was measured with better precision. Gentian violet was rapidly metabolized to leucogentian violet within 24 h post-dosing. The mean leucogentian violet and gentian violet concentrations on Day 1 post-dosing were 134 ±36 µg/kg and 2.4 ±0.0 µg/kg, respectively (ratio of leucogentian violet:gentian violet = 56:1). Gentian violet was not detected (LOD 2 µg/kg) by 14 days post-dosing. Leucogentian violet was detected at all times post-treatment, with 8 µg/kg detected on Day 91 post-treatment.

Table 5.13. Concentrations of gentian violet and leucogentian violet in muscle of catfish exposed to 100 ng/ml gentian violet in water for 1 hour

Withdrawal period	gentian violet (µg/kg)	leucogentian violet (µg/kg)
Pre-treatment	< LOD	0.0 ±0.1
1 hour	0.5 ±0.1	11.7 ±1.8
2 hour	0.8 ±0.3	16.8 ±2.2
4 hour	<LOD	15.9 ±4.3
7 hour	<LOD	15.5 ±3.6
1 day	<LOD	15.1 ±3.1
2 days	LOD	13.5 ±3.3
5 days	0.3 ±0.2	9.4 ±3.3
8 days	<LOD	9.7 ±2.8
15 days	<LOD	5.7 ±2.2
22 days	LOD	3.3 ±0.5
33 days	<LOD	2.8 ±0.9
51 days	LOD	1.5 ±0.6
79 days	<LOD	3.1 ±0.5

NOTES: Method limit of detection (LOD): 0.2 µg/kg for gentian violet. Values are mean and SD of single determinations of five fish at each sampling point.

Channel catfish

One study exposed channel catfish (*Ictalurus punctatus*) to gentian violet under simulated aquaculture farming conditions (Thompson *et al.*, 1999). The uptake of gentian violet was determined by placing fish in water containing 100 µg gentian violet/L of water for 1 h. The fish were then transferred to gentian violet-free water for 79 days to study the depletion of gentian violet residues. Fillets of 5 fish per sampling time were analysed for gentian violet and leucogentian violet. Gentian violet was rapidly (approximately 2 h) converted to leucogentian violet. Mean leucogentian

violet residues were approximately 17 and 3 µg/kg after 2 h and 79 days, respectively (Table 5.13).

The concentrations of gentian violet in muscle from catfish that were exposed in an aquarium to concentrations of 10 or 100 µg gentian violet/L for 1 h and then placed in clean water for 24 h, were 0.4 and 0.8 µg/kg, respectively (Doerge *et al.*, 1996). The corresponding concentrations of leucogentian violet were 44 and 118 µg/kg, respectively. Online LC-APCI/MS was used for confirmation of gentian violet and leucogentian violet residues.

Eels

Wild eels caught in river waters downstream of municipal sewage treatment plant effluents discharge in Germany were tested for gentian violet residues (Schuetze, Herberer and Juergensen, 2008). Using solid-phase extraction with LC-MS/MS, 35 of the 45 samples tested were positive for leucogentian violet. The range for the gentian violet and leucogentian violet combined residues was 0.06 to 6.7 µg/kg fresh weight of tissue. The maximum concentration of gentian violet detected was 0.35 µg/kg. The predominant residue was leucogentian violet, with only trace levels of gentian violet detected (residue ratios varied from 10:1 to 20:1).

Methods of analysis for residues in tissues

General

Analytical methods for detecting gentian violet and leucogentian violet, either alone or together with other triphenylmethane dyes, have been published by a number of authors (Table 5.14). Most of these methods focus on detection and quantitation of gentian violet/leucogentian violet residues in seafood products.

Two strategies are currently followed for gentian violet and leucogentian violet determination in tissue. The first is based on the measurement of each molecule separately, and the second is based on measurement of the molecules together after conversion of gentian violet and leucogentian violet by chemical oxidation. Gentian violet and leucogentian violet are generally extracted from tissue with an acetonitrile buffer mixture and then purified on solid-phase extraction (SPE) cartridges. HPLC coupled to a UV or fluorescence detector has been reported, but methods based on these technologies have never achieved the performances of MS-based methods. Post-column oxidation (e.g. with lead dioxide) of leucogentian violet to gentian violet is often reported when UV is used as a detector. Due to the rapid metabolism of gentian violet into leucogentian violet, and the persistence of leucogentian violet residues in fish tissue, a method utilizing detection of leucogentian violet may be preferred for monitoring of residues in fish.

Chickens

Roybal *et al.*, (1990) examined residues of gentian violet, its demethylated metabolites (pentamethyl and tetramethyl), and leucogentian violet in chicken tissue. The analytes were extracted from tissue with acetonitrile/buffer and partitioned into methylene chloride. Polar lipids were removed on an alumina column followed by partitioning into methylene chloride from a citrate buffer. The compounds of interest were isolated on a disposable carboxylic acid cation exchange column and then eluted with 0.02% HCl in methanol. Gentian violet, its demethylated metabolites and leucogentian violet were determined by HPLC using isocratic elution from a cyano column with a buffered mobile phase and amperometric electrochemical detection. Average recoveries of gentian violet and leucogentian violet from commercially purchased chicken liver fortified with 20 µg/kg of each compound were 92% (CV 7.6%) and 86% (CV 8.1%), respectively. Mean recoveries of gentian violet, leucogentian violet, the pentamethyl metabolite and one of the tetramethyl metabolites from control chicken liver fortified with 20 µg/kg of each compound were 80% (CV 8.8%), 76% (CV 3.9%), 83% (CV 7.2%) and 76% (CV 10.5%), respectively. Data are also presented to show

applicability of the method to muscle tissue.

Munns *et al.,* (1990) analysed leucogentian violet residues in chicken fat obtained from birds that were treated with 30 mg/kg of gentian violet in feed. The fat tissue was extracted with methylene chloride, and leucogentian violet on separation from the fat with 1N HCl was protonated and re-extracted into methylene chloride, and after evaporation dissolved in ACN-water before being subjected to LC with an electrochemical detector. Average recovery for leucogentian violet was 84% with a CV of 13% for 5 µg/kg fat. Gentian violet and its oxidized metabolites were not detected in the fat tissue. Recovery of leucogentian violet was influenced by several factors within the analytical procedure, such as temperature and volume in the last evaporation step, and needed to be carefully controlled.

Aquaculture

A number of published articles describe analytical methods for residues of gentian violet in aquaculture products (Andersen *et al.,* 2009; Chen and Miao, 2010; Doerge *et al.,* 1996; Dowling *et al.,* 2007; Li and Kijak, 2011; Rushing, Webb and Thompson, 1995; Rushing and Hansen, 1997; Rushing and Thompson, 1997; Thompson *et al.,* 1999; Wu *et al.,* 2007; Xie *et al.,* 2013; Xu *et al.,* 2012).

Catfish fillets were blended and then homogenized with aqueous hydroxylamine, p-TSA and 0.1M ammonium acetate, followed by extraction with acetonitrile. The acetonitrile was partitioned with water, methylene chloride and diethylene glycol mixture. The bottom layer was concentrated and chromatographed on an alumina cartridge. The leucogentian violet and gentian violet residues were eluted from the cartridges and subjected to liquid chromatography with a UV-visible detector set at 588 nm. The method is capable of analyzing residues of gentian violet and leucogentian violet to 1 µg/kg level (Rushing, Webb and Thompson, 1995). The method was later modified by so that gentian violet, leucogentian violet, malachite green and leucomalachite green could be simultaneously determined (Rushing and Thompson, 1997). An analytical method for the confirmation of residues of gentian violet and leucogentian violet in catfish was also developed using a post-column oxidation colorimetric electrochemical cell, a UV-VIS diode array detector and a fluorescence detector (Rushing and Hansen, 1997). In a method to analyse gentian violet, leucogentian violet, malachite green and leucomalachite green in salmon tissue, tissue was extracted with pH 3 McIlvaine buffer with clean up on a Bakerbond strong cation exchange solid phase extraction cartridge, and analysed by LC-MS/MS (Dowling *et al.,* 2007).

A number of multi-residue methods have been reported for residues of the trimethyl-phenyl dyes in aquaculture products. A multi-residue method for determination of gentian violet, leucogentian violet, malachite green and leucomalachite green in a number of fish makes use of McIlvaine buffer and acetonitrile for extraction, followed by partitioning with dichloromethane and clean-up on basic alumina and OASIS MCX SPE column (Wu *et al.,* 2007). Detection and quantification use LC-ESI-MS/MS with the selected reaction monitoring mode. The LODs and LOQs were in the 0.02 and 0.13 µg/kg range, respectively, with recoveries ranging from 80 to 115% for 0.25–10 µg/kg tissue. In another method for the determination and confirmation of residues of gentian violet, leucogentian violet, malachite green, leucomalachite green, brilliant green and leucobrilliant green in fish tissue at ≤1 µg/kg, residues were extracted with ammonium acetate buffer and acetonitrile, followed by clean up using dichloromethane portioning and solid-phase extraction, with analyses by liquid chromatography with visible detection (Andersen *et al.*, 2009).

Table 5.14. Summary of approaches and performance of analytical methods for gentian violet (GV) and leucogentian violet (LGV).

Matrix & source	Extraction	Clean up	Oxidation	Detection	LOD and LOQ (µg/kg)	Recovery (%)
Catfish muscle Andersen *et al.,* 2009	Ammonium acetate buffer, HAH, p-TSA & ACN	dichloromethane partitioning & solid phase extraction (SPE)	DDQ	LC-VIS & LC-MS with ND-APCI for confirmation	0.07 & 0.18, respectively, by LC=VIS 0.07 & 0.21 by LC-MS	GV: 84.4 for 2 µg/kg LGV: 90.6 for 0.25–10 µg/kg
Catfish muscle Chen and Miao, 2010	McIlvaine buffer, p-TSA, TMPD, ACN, NaCl	MCX SPE columns		LC with DAD & fluorescence detectors. Confirmation by LC-MS/MS	LOD for GV - 0.26 LOD for LGV - 0.09	GV: 101.4, 92.2 & 97.7 for 1, 2 & 10 µg/kg, respectively LGV: 86.5, 85.9 & 80.6 for 1, 2 & 10 µg/kg, respectively
Catfish muscle Doerge *et al.,* 1996	Aqueous hydroxyl-amine hydrochloride, 0.05M pTSA, 0.1M ammonium acetate, ACN, basic alumina Partitioned with water, methylene chloride, diethylene glycol		PbO_2 post-column	LC & UV-VIS at 588 nm. Confirmation using LC-MS (APCI)	GV & LGV LOD ≤1 pg	
Whole salmon Dowling *et al.,* 2007	ACN:McIlvaine buffer	Cation exchange solid phase extraction cartridge		LC-ESI-MS/MS	0.35 and 0.8, respectively for GV 0.17 and 0.32, respectively for LGV	GV: 81 to 111 for 1, 1.5 & 2.0 µg/kg LGV:- 77 to 103 for 1, 1.5 & 2.0 µg/kg
Shrimp Li and Kijak, 2011	ACN, pH 6 McIlvaine buffer-MeOH – ACN, 20 µL TMPDA	LC-Polymer based stationary phase, washed with a set of buffers/ solvents and then chromatographed on a phenyl column	NA	LC/MS/MS	LOQs for GV & LGV were 1 and 7.5 µg/kg, respectively	GV & LGV were 96 and 102 for 1–20 and 7.5–150 µg/kg, respectively
Grass carp, shrimp & shellfish Long *et al.,* 2009	20% hydroxylamine hydrochloride & ammonium acetate buffer	ACN, methyl alcohol-ammonium acetate buffer, MI SPE	Lead oxide	HPLC, tungsten light @ 588 nm	GV LOD: 0.11 carp, 0.12 shrimp & 0.13 shellfish GV LOQ: 0.19 carp, 0.20 Shrimp & 0.22 shellfish LGV LOD: 0.14 carp, 0.13 shrimp & 0.12 shellfish LGV LOQ: 0.24 carp, 0.22 shrimp & 0.21 shellfish	GV: 97.2–101.2 for 1–2 µg/kg carp; 93.8–96.3 for 1–2 µg/kg shrimp & 95.0–95.7 for 1.5–2 µg/kg shellfish LGV: 91.8–95.4 for 1–2 µg/kg carp, 93.3–95.2 for 1–2 µg/kg shrimp & 93.6–95.6 for 1.5–2 µg/kg shellfish
Chicken fat Munns *et al.,* 1990	Methylene chloride	Partition extraction with 1N HCl & methylene chloride, ACN	NA	LC with EC detection		LGV- 83.9, 82.8 & 77.7 for 5.05 , 10.1 & 20.2 µg/kg fat, respectively

Matrix & source	Extraction	Clean up	Oxidation	Detection	LOD and LOQ (µg/kg)	Recovery (%)
Chicken liver Roybal *et al.,* 1990	ACN/buffer pH 4.5	Alumina column with ACN & methylene chloride partition, carboxylic acid cation exchange in 0.02% HCl in methanol		LC with EC detection		GV- 80.7, 92 LGV- 76, 86 Penta- 83 N″2-tetra - 76
Catfish Rushing, Webb and Thompson, 1995	Aqueous hydroxyl-amine hydrochloride, 0.05M pTSA, 0.1M ammonium acetate, ACN, basic alumina. Partitioned with water, methylene chloride, diethylene glycol	Stacked alumina/PRS cation exchange solid phase extraction cartridge ACN/buffer elution of GV & LGV from PRS cartridge	PbO_2 post-column	LC & UV-VIS at 588 nm	LOQ: 1 for GV and LGV	GV: 92.7±1.8, 95.0±2.2 & 93±2 for 20, 10 & 1 µg/kg, respectively LGV: 83.1±1.2, 78.4±4 & 84±8 for 20, 10 & 1 µg/kg, respectively
Catfish, trout Rushing and Hansen, 1997	As above	As above	EC cell	LC & EC cell & diode array and fluorescence detectors	LOQ: 10 µg for LGV-fluorescence detection	
Catfish, trout Rushing and Thompson, 1997	As above	As above	PbO_2 post-column	LC & UV-VIS at 588 nm	Trout tissue: 1.8 and 3.0 for GV 0.3 and 0.5 for LGV Catfish tissue: 0.6 and 1.0 for GV 0.3 and 0.5 for LGV	87 for 5 to 20 µg GV /kg 71 for 5 to 20 µg LGV/kg 88 for 10 µg GV/kg 73 for 10 µg LGV/kg
Eels Schuetze, Herberer and Juergensen, 2008		SPE cartridges		LC-MS/MS	GV: LOD 0.01 LOQ 0.02 LGV: LOD 0.005 LOQ 0.01	GV 30 LGV 98–105
Salmon & shrimp Tao *et al.,* 2011	ASE McIlvaine buffer/ACN/hexane	OASIS MCX SPE column		LC-ESI-MS/MS	LOD: GV, salmon & shrimp 0.012 & 0.009, respectively LOD: LGV, salmon & shrimp 0.009 & 0.007, respectively LOQ: GV salmon & shrimp 0.13 & 0.11, respectively LOQ: LGV salmon & shrimp 0.10 & 0.09, respectively	Salmon, GV: 101.6, 97.3 & 91.7 for 0.1, 0.5 & 1 µg/kg, respectively LGV: 89.2, 102.9 & 93.7 for 0.1.0.5 & 1 µg/kg, respectively Shrimp, GV: 86.2, 88.4 & 102.5 for 0.1, 0.5 & 1 µg/kg, respectively LGV 95.3, 86.8 & 89.1 for 0.1, 0.5 & 1 µg/kg, respectively
Catfish Thompson *et al.,* 1999	ACN/buffer pH 4.5, basic alumina, centri-fuged, partitioned water, methylene chloride and diethylene glycol	Stacked alumina/PRS cation exchange solid phase extraction cartridge, ACN/buffer elution of GV & LGV	Supleco LC-CN, mobile phase 60% ACN, 40% water, 0.05 M ammonium acetate buffer, PbO_2 post-column	UV-VIS at 588 nm	LOD GV: 0.2	Not identified

Matrix & source	Extraction	Clean up	Oxidation	Detection	LOD and LOQ (μg/kg)	Recovery (%)
Grass carp, eel, salmon, shrimp & shellfish Wu *et al.,* 2007	McIlvaine buffer & ACN	Partitioning with dichloro-methane, basic alumina and OASIS MC-X SPE column		LC-ESI-MS/MS in SRM mode	GV: eel 0.06 & 0.10 salmon 0.10 & 0.16 shrimp 0.04 & 0.08 shellfish 0.06 & 0.10 carp 0.04 & 0.07 LGV: eel 0.04 & 0.07 salmon 0.04 & 0.06 shrimp 0.04 & 0.08 shellfish 0.03 & 0.07 carp 0.03 & 0.05	GV: 87.2–103.7 for 0.25–10 μg/kg eel, 92.6–115.7 for 0.25–10 μg/kg salmon, 90–111 for 0.25–10 μg/kg shrimp, 92.1–108.6 for 0.25–10 μg/kg shellfish, 87.2–101.0 for 0.25–10 μg/kg carp LGV: 91.4–103.4 for 0.25–10 μg/kg eel, 88.5–108 for 0.25–10 μg/kg salmon, 94.3–106.6 for 0.25–10 μg/kg shrimp, 96.8–107.2 for 0.25–10 μg/kg shellfish & 81.6–96.7 for 0.25 – 10 μg/kg carp

NOTES: GV = gentian violet; LGV = leucogentian violet.

A multi-residue method making use of molecularly imprinted polymers (MIPs) for the selective detection and binding of analytes was developed using HPLC coupled with a diode array detector, for the analysis of gentian violet and leucogentian violet in carp, shrimp and shellfish (Long *et al.*, 2009). The LOQs were in the 0.2 µg/kg range, with a recovery of greater than 95%. A method was developed to determine 21 veterinary drugs in shrimp using an online SPE automated sample cleanup prior to LC-MS/MS analysis (Li and Kijak, 2011). The recoveries for gentian violet and leucogentian violet were 96 and 102% over the linear ranges of 1–20 and 7.5–150 µg/kg shrimp, respectively.

Additional methods

A method has been reported for residues in catfish muscle which does not require the oxidation of the leucogentian violet to gentian violet and can detect residues at ≤1 ng/g (Chen and Miao, 2010). Residues were extracted with pH 3 McIlvaine buffer and acetonitrile, with clean up by a polymeric strong cation-exchange column, followed by HPLC with a diode array and fluorescence detectors and confirmed by MS/MS. An automated method for the determination of gentian violet, leucogentian violet, malachite green and leucomalachite green in shrimp and salmon by LC-MS/MS uses accelerated solvent extraction and auto solid-phase clean-up (Tao *et al.*, 2011). The recoveries of gentian violet, leucogentian violet, malachite green and leucomalachite green at spiked levels of 0.1 to 1.0 µg/kg averaged from 82.1 to 102.9%, with the relative standard deviation less than 14.6%.

Estimation of daily intake

There are limited data on detection of residues of gentian violet in terrestrial food-producing animals, especially when the animals are treated with topical drug products. In limited research studies, poultry fed gentian violet have been shown to contain residues (both gentian violet and leucogentian violet) in their tissues, with highest residues observed for leucogentian violet in fat. However, residue monitoring data for residues of gentian violet and leucogentian violet were not available from consistent or on-going monitoring programmes for terrestrial food-producing animals. Useful information on frequency of occurrence and concentrations of residues can primarily be obtained from monitoring activities, or from well conducted studies under field conditions. Given the limited data available from well conducted studies in food animals, many conclusions cannot be made from those data. The limited information available via monitoring activities permits only a very limited assessment due to the largely random nature of the sampling procedures, as well as the limited species involved.

Residues of gentian violet and leucogentian violet have been reported in fish products, both in wild fish and those from aquaculture. Gentian violet and leucogentian violet residues were detected in 35 of 45 tissues of wild eels caught in waters where effluents from municipal sewage treatment plants discharged. The range for the gentian violet and leucogentian violet combined residues was 0.06 to 6.7 µg/kg fresh weight of tissue. The maximum concentration of gentian violet detected was 0.35 µg/kg. The predominant residue was leucogentian violet, with only trace amounts of gentian violet detected (residue ratios varied from 10:1 to 20:1) (Schuetze, Herberer and Juergensen, 2008). Twenty samples of salmon or shrimp from the market in China were analysed for gentian violet and leucogentian violet using a method with a LOQ of 0.1 µg/kg fresh tissue (Tao *et al.*, 2011). Three samples (15%) were positive; one sample contained 1.2 µg gentian violet/kg and 2.5 µg leucogentian violet/kg fresh tissue, and two other samples contained only leucogentian violet at 0.43 and 0.7 µg/kg.

Residue monitoring results for gentian violet and leucogentian violet conducted on domestic and imported aquaculture products in Canada by the Canadian Food Inspection Agency (Canada, 2013) and in the United States of America by the US FDA (FDA, 2008, 2014)

are summarized in Table 5.15. While the proportions of seafood samples testing positive ranged from 0 to 4.4% over the years, mean residues of gentian violet and leucogentian violet detected in these samples were low (<3 ppb). However, residues as high as 26.9 µg/kg have been reported by the US FDA (2014) in imported fish products.

Table 5.15. Detection of gentian violet and leucogentian violet in aquaculture products monitored by Canada and the United States of America

Monitoring country	Year	Samples tested (No.)	No. of samples positive (%)	Residue level (µg/kg) [1] in positive samples		Positive sample types
				Mean ±SD	Range	
Canada	2008/09	135	6 (4.4%)	2.48 ±2.32	0.64–5.60	Tilapia, salmon, shrimp
	2009/10	484	0 (0%)	—	—	—
	2010/11	542	11 (2.0%)	1.92 ±1.69	0.50–4.30	Tilapia, perch, shrimp, milkfish, catfish
	2011/12	396	2 (0.5%)	2.23 ±2.02	0.80–3.65	Bass, prawn
	2012/13	269	3 (1.1%)	3.06 ±2.07	0.98–5.12	Perch, dried fish maw
USA [2]	2004	622	0 (0%)	—	—	—
	2005	536	0 (0%)	—	—	—
	2006	588	0 (0%)	—	—	—
	2007	686	3+ (not confirmed) (0.4%) [3]	?	2.5–26.9	Eel, catfish, shrimp

NOTES: (1) Residues for Canada are sum of gentian violet and leucogentian violet. Note that in Canada the presence of leucogentian violet is considered to be an indication of intentional use of the drug in aquaculture. (2) Sources: FDA, 2008, 2014. (3) Based on the description in FDA (2014), the values are probably an underestimate of % samples positive.

Maximum Residue Limits

MRLs could not be recommended by the Committee, as it was not considered appropriate to establish an ADI. The Committee also noted that there was limited information on residues.

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