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Stability during cooking of anthelmintic veterinary drug residues in beef

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Abstract

Anthelmintic drugs are widely used for treatment of parasitic worms in livestock but little is known about the stability of their residues in food under conventional cooking conditions. As part of the European Commission-funded research project ProSafeBeef, cattle were medicated with commercially available anthelmintic preparations, comprising 11 active ingredients (corresponding to 21 marker residues). Incurred meat and liver were cooked by roasting (40 min at 190°C) or shallow frying (muscle 8-12 min, liver 14-19 min) in a domestic kitchen. Raw and cooked tissues and expressed juices were analysed using a novel multi-residue dispersive solid phase extraction method (QuEChERS) coupled with Ultra Performance Liquid Chromatography-Tandem Mass Spectrometry. After correction for sample weight changes during cooking, no major losses were observed for residues of oxiclozanide, clorsulon, closantel, ivermectin, albendazole, mebendazole or fenbendazole. However, significant losses were observed for nitroxynil (78% in fried muscle, 96% in roast muscle), levamisole (11% in fried muscle, 42% in fried liver), rafoxanide (17% in fried muscle, 18% in roast muscle) and triclabendazole (23% in fried liver, 47% in roast muscle). Migration of residues from muscle into expressed cooking juices varied between drugs, constituting 0% to 17% (levamisole) of total residues remaining after cooking. With the exception of nitroxynil, residues of anthelmintic drugs were generally resistant to degradation during roasting and shallow frying. Conventional cooking cannot, therefore, be considered a safeguard against ingestion of residues of anthelmintic veterinary drugs in beef.

Keywords: anthelmintics; veterinary drug residues; QuEChERS; stability; cooking; beef; ProSafeBeef.

Introduction

A wide range of veterinary drugs are used in prophylaxis and treatment of endoparasitic infections and ectoparasitic infestations in domestic livestock. Anthelmintic drugs used to control nematode (roundworm), cestode (tapeworm) and trematode (flake) infections include various benzimidazole compounds (including fenbendazole, triclabendazole, mebendazole and albendazole), imidazothiazoles (particularly levamisole), macrocyclic lactones (including ivermectin) and a range of flukicidal compounds (including oxclozanide, rafoxanide, closantel, nitroxynil and clorsulon). Concerns over the toxicity of some of these drugs (McKellar and Scott 1990; Lankas and Peter 1992) led the European Union to establish Maximum Residue Limits (MRLs) for their residues in tissues of food-producing species (collated in European Commission 2010). Drugs, other than those listed in this legislation, are not licensed for use in food-producing animals and therefore their residues ought not to be present in food. The occurrence and determination of benzimidazole and macrocyclic lactone residues in biological matrices have recently been reviewed by Danaher et al. (2006; 2007). Food safety and the exposure of the consumer to chemical residues in food are of continuing concern and there is an ongoing need for new analytical techniques and information on the occurrence of potentially harmful residues of veterinary drugs in our food. ProSafeBeef (www.prosafebeef.eu) is a EU Sixth Framework Integrated Project involving 41 leading research and industrial organisations working in 18 different countries, aiming to advance beef safety and the diversification of the European beef sector. Under this project, a novel analytical method, based on state-of-the-art ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), was developed simultaneously to detect 38 anthelmintic drug residues in bovine tissues (Kinsella et al. 2010; Whelan et al. 2010). This technique was subsequently applied in a survey of retail beef across Europe to determine the prevalence of anthelmintic drug residues in meat available to the European consumer, and in a study of the stability of anthelmintic drugs in solution, tissue extracts and stored tissue to assist the analyst in accurate quantification of residues of these drugs in food. Results of both these studies will be published elsewhere. Studies of the attitudes of

1 consumers towards the safety of the meat they consume were also a major pillar of ProSafeBeef
2
3 research (Van Wezemaal et al. 2010). Should unacceptable residues of anthelmintic drugs find their
4
5 way into our foods, legislators, analysts and consumers alike will wish to know if such residues are
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7 destroyed when that food is cooked.
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13 Between 1995 and 2005, Rose and co-workers in the UK Central Science Laboratory demonstrated
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15 that residues of a range of veterinary drugs exhibit varying degrees of stability during cooking and,
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17 therefore, that cooking influences the level of risk posed by such residues (Rose et al. 1996). This
18
19 compound-dependent stability during cooking is supported by studies of nitrofurans residues by
20
21 Cooper and Kennedy (2007) and malachite green residues by Mitrowska et al. (2007). However, to
22
23 date, the assessment of the stability of anthelmintic drug residues in foods has been piecemeal and
24
25 limited to the effects of pasteurisation on the macrocyclic lactones, including ivermectin, in milk
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27 (Imperiale et al. 2009; Cerkvenik et al. 2001), and the effects of various cooking procedures on
28
29 ivermectin in porcine liver and bovine, porcine and salmon muscle (Rose et al. 1998; Slanina et al.
30
31 1989), levamisole in bovine and porcine muscle (Rose et al. 1995a; Hsu and Epstein 1993) and
32
33 oxfendazole in bovine liver (Rose et al. 1997).
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42 To complement the outcomes of the ProSafeBeef European retail beef survey and aid relevant
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44 authorities and the consumer in assessing their exposure to anthelmintic drug residues, the current
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46 study set out to determine the stability during cooking, by frying and roasting, of a wide range of
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48 anthelmintic residues in meat and liver of cattle treated with commercially available veterinary
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50 parasiticide preparations.
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Materials and methods

Materials and instrumentation

Reference standards and internal standards analysed in this study are listed in Table 1 along with their sources and abbreviated names. Standards were obtained from Sigma-Aldrich (Poole, UK), Witega Laboratorien Berlin-Adlershof (Berlin, Germany), Janssen Animal Health (Beerse, Belgium), Pfizer Animal Health (Sandwich, UK) and QuChem (formerly of Belfast, UK). Unless stated, all other chemicals were obtained from Sigma-Aldrich.

Commercially available parasiticide preparations were purchased from the following manufacturers: Levafas Diamond (Norbrook Laboratories Ltd.; Newry, UK), Curafluke 10% (Univet Ltd.; Cootehill, Ireland), Supaverm™ Oral Suspension (Janssen Animal Health; High Wycombe, UK), Valbazen® 10% (Pfizer Animal Health; Ringaskiddy, Ireland), Fasinex Super® 19.5% (Novartis Animal Health Ireland Ltd.; Waterford, Ireland), Deldrax® 34% (Intervet Ireland Ltd.; Dublin, Ireland) and Ivomec Super® Injection for Cattle (Merial Animal Health; Harlow, UK).

An Acquity UPLC® binary pump and sample management system (Waters Corporation, Manchester, UK) coupled to a Quattro Premier™ XE tandem mass spectrometer (Waters Corporation), both operating under MassLynx™ software, were used for sample analysis. The mass spectrometer operated under Electrospray Ionisation (ESI) with rapid polarity switching. Data acquisition was in Multiple Reaction Monitoring mode (MRM) with a total run time of 11 min. Data analysis was performed using Waters TargetLynx™ software. MS source settings were as follows: capillary voltage 3.0 kV (ESI+) and 2.2 kV (ESI-), source temperature 150°C, desolvation temperature 400°C, cone nitrogen gas flow 150 L h⁻¹, desolvation nitrogen gas flow 800 L h⁻¹. The UPLC system was equipped with an Acquity HSS T3 UPLC analytical column (100 × 2.1 mm) packed with HSS C₁₈ (1.8 μm) and an in-line filter unit (0.2 μm, 2.1 mm) (Waters Corporation),

1 heated to 60°C. A binary gradient mobile phase was applied at a flow rate of 0.6 mL min⁻¹, phase A
2 being 0.01% acetic acid in acetonitrile:water (10:90, v/v), phase B being 5 mM ammonium formate
3 in methanol:acetonitrile (75:25, v/v). The gradient profile was: (1) 0–0.5 min, 100% A, (2) 5 min,
4 50% A, (3) 7 min, 10% A, (4) 8.5 min, 10% A, (5) 8.51 min, 0% A, (6) 9.0 min, 0% A, (7) 9.01
5 min, 100% A, (8) 11 min 100% A. The UPLC weak wash was methanol:water (20:80, v/v) and
6 strong wash was methanol:propan-2-ol:water (80:10:10, v/v).
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18 A Testo 915-1 Universal probe thermometer was obtained from Testo AG (Lenzkirch, Germany).
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20 Tissues were minced in a domestic food processor before homogenisation in a SL2 laboratory
21 homogeniser (Silverson Machines Ltd., Chesham, UK). QuEChERS dispersive clean-up tubes (1.5
22 g MgSO₄ and 0.5 g C₁₈ in 50 mL centrifuge tubes) were obtained from UCT (Bristol, PA, USA).
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24 QuEChERS extraction salts, anhydrous MgSO₄ (4 g) and NaCl (1 g), were obtained from Sigma-
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26 Aldrich and pre-weighed in-house.
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35 [INSERT TABLE 1 ABOUT HERE]
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39 ***Production of incurred bovine tissues***

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42 Seven pasture-fed beef cattle (male, 16-17 months of age, approximately 450 kg body weight) were
43 treated with commercially available parasiticide preparations (comprising 11 active ingredients
44 corresponding to 21 marker residues) at the manufacturers' recommended doses and routes (sub-
45 cutaneous injection or oral drench) split half-and-half over two consecutive days (Table 2).
46
47 Administration was staggered over two days to encourage the formation of both parent and
48 metabolite drug residues in the tissues. Short withdrawal periods of one or two days were observed
49 after the second dose before cattle were slaughtered and *Longissimus dorsi* muscle (sirloin/filet
50 steak) and liver were sampled. Tissues were stored at -20°C prior to analysis.
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[INSERT TABLE 2 ABOUT HERE]

Cooking of incurred beef and liver

One portion of *L. dorsi* muscle and one portion of liver were selected from each medicated cow. After thawing to room temperature, adjoining pieces of muscle were sliced for frying (mean 185 g) and roasting (mean 300 g). A third adjoining slice was retained for analysis without cooking. Single slices of liver (mean 170 g) were taken for frying and adjoining uncooked slices retained for analysis without cooking. Excess juices and blood were removed from each sample using absorbent tissue before weighing immediately prior to cooking (or before mincing of uncooked samples). Cooked samples were re-weighed after cooling to room temperature and removal of excess juices. Cooked and raw samples were then individually minced in a domestic food processor and re-frozen at -20°C prior to analysis by LC-MS/MS. Cooking conditions were as follows.

Roasting. Muscle was roasted for 40 min at 190°C on the middle shelf of a pre-heated fan-assisted domestic oven, while wrapped in aluminium foil in an uncovered tray. All juices expressed during roasting were collected and weighed.

Frying. Muscle was fried for 4-6 min each side (depending on sample size, to give medium cooked meat) on a high heat setting on a domestic ceramic hob, in a minimal volume of sunflower oil (one teaspoon) to prevent burning. Small volumes of expressed juices were collected following frying of the majority of muscle samples. Liver was fried in approximately 10 mL sunflower oil, searing for 2 min each side on a high heat setting, then frying on medium heat for a further 10-15 min, depending on sample size, to give well done cooked liver in the centre of the portion. Significant amounts of juices were not expressed during frying of liver.

The maximum internal temperature of each sample was measured on completion of cooking by inserting a digital penetration probe thermometer into the centre of the sample.

LC-MS/MS analysis

Each sample, raw and cooked, was analysed in triplicate as described below. Juices were analysed in duplicate. Different sample types were stored under identical conditions at all times and analysed in the same analytical batch to avoid time-dependent (stability) differences in residue concentrations. This analytical method is based on the QuEChERS-UPLC-MS/MS protocol developed by Kinsella et al. (2009; 2010) under the ProSafeBeef project.

Briefly, samples (10 g) were extracted by homogenisation in acetonitrile (12 mL). Phase separation was achieved by shaking homogenised samples with anhydrous MgSO_4 (4 g) and NaCl (1 g). Following centrifugation, the supernatant was cleaned-up using dispersive SPE by vortex mixing with anhydrous MgSO_4 (1.5 g) and C_{18} sorbent (0.5 g). Following centrifugation, a portion of the extract (1-3 mL) was mixed with dimethylsulphoxide (DMSO, 0.5-1 mL) and the acetonitrile evaporated under nitrogen at 50°C. The volumes of extract and DMSO employed were varied for a given analytical batch to keep the MS/MS signal within a linear range. Furthermore, highly concentrated samples were diluted with *L. dorsi* muscle or liver from untreated cattle, up to a total of 10 g, prior to homogenisation. Extracted matrix calibration standard curves were prepared by fortifying 10 g of *L. dorsi* muscle or liver from untreated cattle with standard mixtures prior to homogenisation and extraction. Final extracts in DMSO were injected (4 μL) onto the Acquity-Premier XE UPLC-MS/MS system described above. Ionisation polarities and internal standards applied are listed in Table 1. Calibration standards (n=5 to 7 depending on range of linearity) were injected in duplicate with acceptable regression coefficients (r^2) above 0.98 for analytes employing isotopic internal standards and 0.95 for those employing non-isotopic internal standards (Table 1). Analyte concentrations in samples were calculated by comparing the ratio of the response of the analyte's base peak to the internal standard response with the same ratio in calibration curve standards, followed by a correction for any sample dilution applied prior to extraction.

Results and discussion

Changes in anthelmintic drug residue concentrations during cooking are shown in Tables 3-5. Two cattle received medication containing levamisole – only data from that which received Levafas Diamond are presented. The concentrations detected in raw (uncooked) tissues were adjusted for sample weight changes resulting from the cooking procedures to give concentrations “expected after cooking”. These expected concentrations were then compared with the concentrations detected after cooking and a two-tailed, unpaired Student’s t-test applied to the triplicate analyses. Concentrations of the benzimidazoles are expressed as the sums of their parent compounds and/or metabolites as defined by Commission Regulation 37/2010 (European Commission 2010) which recently replaced Commission Regulation 2377/90 as an alphabetical listing of Maximum Residue Limits and marker residues for allowed pharmacologically active substances in foodstuffs of animal origin. Calculations of the concentrations of designated benzimidazole marker residues were as follows:

$$\text{Albendazole: } ABZ = (0.943 \times ABZ-SO) + (0.892 \times ABZ-SO_2) + (1.109 \times ABZ-NH_2-SO_2)$$

$$\text{Fenbendazole: } FBZ-SO_2 = FBZ-SO_2 + (1.107 \times FBZ) + (1.051 \times FBZ-SO)$$

$$\text{Mebendazole: } MBZ = MBZ + (1.244 \times NH_2-MBZ) + (1.014 \times OH-MBZ)$$

$$\text{Triclabendazole: } KetoTCB = KetoTCB + (0.916 \times TCB) + (0.878 \times TCB-SO) + (0.842 \times TCB-SO_2)$$

For those samples from which juices were expressed, mean residues detected in cooked tissue and juices were combined in absolute terms (μg residue in total cooked sample + μg residue in total cooked juices) to permit comparison with μg of residue in total uncooked sample (Tables 4-5). Replicate data ($n=3$) from tissues and juices cannot be paired to give replicate concentration data for combined cooked samples, therefore a t-test cannot be applied. A t-test could be applied only to triplicate analyses of the tissue concentrations without reference to juice concentrations.

[INSERT TABLES 3, 4 AND 5 ABOUT HERE]

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Maximum internal sample temperatures during cooking ranged from 55 to 96°C (Tables 3-5). In keeping with the differing sample sizes and cooking times, higher internal temperatures were achieved during roasting of muscle (mean 88°C) than frying (mean 65°C). Liver, fried thoroughly for up to 19 min, reached the highest temperatures (mean 91°C).

Major residue instability was evident only for nitroxylin which dropped by 96% in roast muscle and by 78% in fried muscle (Tables 4 and 5). Nitroxylin was not detected in uncooked liver. This disparity between nitroxylin concentrations in uncooked bovine muscle (531 µg kg⁻¹) and liver (<10 µg kg⁻¹) is in keeping with a previous report indicating that up to 98% of nitroxylin was metabolised in the liver of treated cattle (EMEA 1999). Our observed loss of nitroxylin in muscle is in agreement with the limited data of Ekström and Slanina (1982) who saw the complete destruction of nitroxylin in beef boiled for 2 h and a loss of up to 55% when fried.

Smaller, but significant, losses of levamisole (42% in fried liver, 11% in fried muscle), rafoxanide (17% in fried muscle, 18% in roast muscle) and triclabendazole (23% in fried liver, 47% in roast muscle) were also seen in the current study.

A small loss of levamisole from fried bovine muscle (11%) was observed. Rose et al. (1995a) saw no loss of levamisole during frying of incurred porcine muscle or in boiling water at 100°C. Roasting bovine muscle in the current study led to a 17% loss of levamisole in the meat but this was wholly accounted for by the residue detected in the expressed juices (Table 5). Rose et al. (1995a) also demonstrated a loss of levamisole during roasting of porcine muscle but expressed juices were not analysed in that instance. Hsu and Epstein (1993) suggested that levamisole was stable under ordinary cooking conditions, although a 26% decrease was evident during broiling of porcine muscle. The present study, however, clearly demonstrated a 42% loss of levamisole during frying of incurred bovine liver (not studied by Rose). Given that levamisole migrated into expressed juices during roasting of muscle to a greater degree than any other residue in this study (17% of

1 total levamisole residues post-cooking were detected in the juices; Table 5), it is conceivable that
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3 this 42% loss may be accounted for, at least in part, by juices expressed during frying which then
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5 evaporated in the pan and were thus unavailable for analysis. Levamisole residues remaining in the
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7 pan following evaporation of expressed juices may still be ingested if the consumer chooses to
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9 prepare sauces or gravy in the same pan.
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15 Rafoxanide (Tables 3-5) was seen to decrease by 17-18% in cooked muscle and by 12% in fried
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17 liver (although this was not statistically significant). Caldow et al. (2009) demonstrated that
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19 rafoxanide was generally stable during storage, although degradation in fortified tissue and low
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21 concentration solvent solution was evident after one week storage at room temperature in daylight.
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23 The significance of a minor reduction (less than one fifth) in residues of a drug to which relatively
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25 low EU Maximum Residue Limits have been assigned ($30 \mu\text{g kg}^{-1}$ bovine muscle and $10 \mu\text{g kg}^{-1}$
26
27 bovine liver; Table 2) is debatable. Consumers want their meat to be residue-free on the
28
29 supermarket shelf, and on their plate, and will view such partial degradation of residues during
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31 cooking as no safeguard to their health or the perceived quality of their food. Furthermore, it
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33 cannot be discounted that residues of any compound lost during cooking are in fact being converted
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35 to unidentified products with equal or greater toxicity.
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44 Triclabendazole was the only benzimidazole compound which exhibited instability during cooking
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46 when expressed as the sum of its metabolites as defined by Commission Regulation 37/2010
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48 (European Commission 2010). Total triclabendazole fell by 23% in fried liver and by 47% in roast
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50 muscle. This two-fold difference in stability (cooking temperatures were comparable) may be due
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52 in part to the different starting metabolite concentrations in the uncooked tissues. TCB-SO₂ is the
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54 predominant metabolite in muscle, followed by TCB, while the reverse is the case in liver (Tables 3
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56 and 4). However, in both tissues TCB-SO₂ exhibited higher percentage losses than TCB during
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58 cooking, leading to a substantially greater reduction in total triclabendazole residues in muscle than
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60 in liver. Total triclabendazole did not fall significantly when muscle was fried – the treatment

1 which used the shortest time and lowest temperatures. In this case the predominant TCB-SO₂
2 metabolite concentration fell slightly but was off-set by an increase in TCB concentration. Of the
3 four triclabendazole metabolites analysed, TCB-SO exhibited the largest percentage reductions
4 during cooking, but it comprised only a small proportion (approximately 2%) of total
5 triclabendazole residues.
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16 Minor statistically significant reductions (6%) in total albendazole and total mebendazole residues
17 were evident in roast muscle, but these can be considered negligible in the context of food safety.
18 Analysis of the metabolites of albendazole suggests a shift towards higher ABZ-NH₂-SO₂
19 concentrations when liver and muscle are fried, but this was not evident when muscle was roasted
20 (Tables 3-5). Analysis of the metabolites of mebendazole suggested no shift in their relative
21 concentrations during cooking with the exception of NH₂-MBZ which doubled its concentration in
22 liver during frying, leading to a considerable 86% increase in total mebendazole residues. This
23 change was not replicated in cooked muscle in which the starting concentration of mebendazole
24 residues was 10 fold lower than in liver. The source of this elevated NH₂-MBZ in fried liver was
25 not transformation of MBZ or OH-MBZ, and must therefore be attributed to other unidentified
26 hepatic metabolites of mebendazole.
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44 Rose et al. (1997) postulated the formation of an amine of oxfendazole (FBZ-SO) when cooking
45 incurred bovine liver and concluded that, while cooking did not destroy the residues, it may affect
46 the equilibrium between FBZ-SO, FBZ-SO₂, FBZ and other unidentified fenbendazole metabolites
47 in incurred tissue. The current study supports this assertion. FBZ-SO is a very minor metabolite in
48 muscle and no significant change in total fenbendazole residues or shift in the relative
49 concentrations of its metabolites was evident following cooking. Similarly, no significant drop in
50 total fenbendazole residues was seen following frying of incurred liver although the concentration
51 of FBZ-SO₂ did fall by 80%.
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1 The finding that ivermectin is stable under normal cooking conditions is in agreement with Rose et
2 al. (1998) who fried, microwaved and boiled medicated cattle and pig tissues, and Cerkvenik et al.
3
4 al. (1998) who fried, microwaved and boiled medicated cattle and pig tissues, and Cerkvenik et al.
5
6 (2001) who saw no degradation of ivermectin in milk pasteurised at 80°C or boiled at 100°C. Rose
7
8 et al. (1998) and Slanina et al. (1989) observed ivermectin losses of up to 50% when bovine muscle
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10 was minced before boiling or frying. In the former study, the majority of the missing residue was
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12 detected in the cooking fluids which the latter study did not analyse.
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18 In addition to ivermectin, the flukicides oxclozanide, closantel and clorsulon exhibited no
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20 instability under cooking in the present study. However, statistically significant increases (27-29%)
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22 were apparent for oxclozanide and clorsulon after frying of muscle (Table 4). As postulated above
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24 for NH₂-MBZ, these increases may arise from transformation of other unidentified metabolites
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26 during cooking, although it is difficult to explain why they were not replicated in roast muscle or
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28 fried liver.
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34 Migration of drug residues into expressed juices during cooking is one aspect of studies such as this
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36 which is difficult to control and to assess accurately. The availability of expressed juices is sample
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38 and operator-dependent, being affected by many factors including the fat and water contents of the
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40 tissue, cooking temperature, the physical treatment of the tissue (slicing, squeezing) and resting
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42 time post-cooking. Furthermore, the fate of any residues which do migrate into cooking juice is
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44 dependent upon consumer choice – the choice to discard juices or use them in sauces or gravy or to
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46 baste the meat. Culinary practices do not always fit neatly with analytical laboratory practices.
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49 Nevertheless, the evidence of the current study suggests that migration of anthelmintic drug
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51 residues into juices during roasting or shallow frying of beef or bovine liver is not a major
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53 consideration. With the exception of levamisole (17% from roast muscle), residues in expressed
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55 juices constituted only 8% or less of the total residues remaining after cooking (Tables 4 and 5).
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58 The extent of drug residue migration into cooking juices is known to be dependent upon the
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1 compound (<2% for nicarbazin; Tarbin et al. 2005) and method of cooking (20% for
2 sulphamethazine from microwaved pig meat, but 54% when pressure cooked; Rose et al. 1995b).
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9 In summary, with the exception of nitroxynil, residues of anthelmintic drugs in beef and bovine
10 liver were generally resistant to degradation during roasting and frying. Apparent increases in
11 residue concentrations during cooking may be due to conversion of unidentified metabolites into the
12 specified marker residues. Consequently, conventional domestic cooking cannot be considered a
13 safeguard against ingestion of residues of commonly used anthelmintic veterinary drugs in beef.
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23 How such findings impact upon the consumer of beef and their perception of beef quality is another
24 aspect of the research conducted under the ProSafeBeef project. Verbeke et al. (2007) described
25 how consumers of food do not differentiate significantly between different types of risk within a
26 particular food group. For example, in the mind of the consumer dioxins in poultry, hormones in
27 beef and antibiotics in pork were conflated and considered to be equivalent. Furthermore, risks in
28 one food group can be erroneously attributed to another – for example, the belief that bovine
29 spongiform encephalopathy (BSE) was a cause for concern in poultry (Verbeke 2001). A recent
30 ProSafeBeef project study of European consumer perceptions of beef safety demonstrated that,
31 rather than fearing the separate risks of contamination of their food by either “bugs”
32 (microbiological risks) or “drugs” (chemical risks), consumers applied an overarching criterion such
33 that safe beef was perceived as beef that is not bad for consumers’ health (“It shouldn’t be making
34 me ill”; Van Wezemaal et al. 2010). Consumers may thus apply the well-understood public advice
35 on bacterial risks (store the meat properly and cook it thoroughly) believing that their vigilance will
36 also afford a degree of protection against the health risks of chemical contaminants. The present
37 study and much of the scientific literature demonstrate that such action affords the consumer little
38 or no such protection against veterinary drug residues. This study adds weight to the general
39 principle that veterinary drug residues in food are resistant to degradation under conventional
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1 cooking and supports the approach of minimising and controlling the use of veterinary medicines at
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4 source to safeguard both the health of the consumer and the profitability of food producers.
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Table 1. Reference and internal standard abbreviations, sources, ESI modes and applied internal standards.

Standard	Abbreviation	Source	ESI mode	Internal standard
Clorsulon	CLORS	Sigma-Aldrich	-	DNC-D8
Closantel	CLOSAN	Sigma-Aldrich	-	TBCP
Nitroxynil	NITROX	Sigma-Aldrich	-	TBCP
Oxyclozanide	OXYCLOZ	Sigma-Aldrich	-	TBCP
Rafoxanide	RAFOX	Sigma-Aldrich	-	DNC-D8
Levamisole	LEVAM	Sigma-Aldrich	+	LEVAM-D5
Ivermectin B1a	IVER	Sigma-Aldrich	+	SELA
Albendazole	ABZ	Sigma-Aldrich	+	ABZ-D3
Albendazole Sulphoxide	ABZ-SO	Witega	+	ABZ-SO-D3
Albendazole Sulphone	ABZ-SO ₂	Witega	+	ABZ-SO ₂ -D3
Albendazole 2-Amino-Sulphone	ABZ-NH ₂ -SO ₂	Witega	+	ABZ-D3
Fenbendazole	FBZ	Sigma-Aldrich	+	FBZ-D5
Fenbendazole-Sulphoxide	FBZ-SO	Sigma-Aldrich	+	FBZ-SO-D5
Fenbendazole-Sulphone	FBZ-SO ₂	Witega	+	FBZ-SO ₂ -D5
Mebendazole	MBZ	Sigma-Aldrich	+	TBZ-D4
Hydroxy-Mebendazole	OH-MBZ	Janssen Animal Health (gift)	+	OH-MBZ-D3
Amino-Mebendazole	NH ₂ -MBZ	Janssen Animal Health (gift)	+	TBZ-D4
Triclabendazole	TCB	Sigma-Aldrich	+	TCB-D3
Triclabendazole-Sulphoxide	TCB-SO	Witega	-	TBCP
Triclabendazole-Sulphone	TCB-SO ₂	Witega	-	TBCP
Keto-Triclabendazole	Keto-TCB	Witega	-	SALI
Levamisole-D5	LEVAM-D5	QuChem (custom synthesis)	+	*
Albendazole-D3	ABZ-D3	Witega	+	*
Albendazole Sulphoxide-D3	ABZ-SO-D3	Witega	+	*
Albendazole Sulphone-D3	ABZ-SO ₂ -D3	Witega	+	*
Fenbendazole-D5	FBZ-D5	QuChem (custom synthesis)	+	*
Fenbendazole-Sulphoxide-D5	FBZ-SO-D5	QuChem (custom synthesis)	+	*
Fenbendazole-Sulphone-D5	FBZ-SO ₂ -D5	QuChem (custom synthesis)	+	*
Thiabendazole-D4	TBZ-D4	QuChem (custom synthesis)	+	*
Triclabendazole-D3	TCB-D3	Witega	+	*
Hydroxy-Mebendazole-D3	OH-MBZ-D3	Witega	+	*
Selamectin	SELA	Pfizer Animal Health UK (gift)	+	*
Salicylanilide	SALI	Sigma-Aldrich	-	*
2,2-Thiobis(4-chlorophenol)	TBCP	Sigma-Aldrich	-	*
Dinitrocarbanilide-D8	DNC-D8	Witega	-	*

Table 2. Parasitocidal preparations administered to beef cattle (~450 kg body weight) and EU Maximum Residue Limits (MRL). NA = none assigned.

Brand name	Manufacturer	Active ingredients (% w/v)	Dosage (mg kg ⁻¹ body weight)	Total dose (mL)	Route	Days before slaughter	MRL bovine muscle (µg kg ⁻¹)	MRL bovine liver (µg kg ⁻¹)
Fasinex Super® 19.5%	Novartis	Triclabendazole 12% Levamisole HCl 7.5%	12 7.5	45	Oral	2	225 10	250 100
Supaverm™ Oral Suspension	Janssen	Mebendazole 7.5% Closantel 5%	15 10	90	Oral	1	NA 1000	NA 1000
Ivomec Super®	Merial	Clorsulon 10% Ivermectin 1%	2 0.2	9	Injection (subcut.)	2	35 20 (action limit)	100 100
Levafas Diamond	Norbrook	Oxyclozanide 6% Levamisole HCl 3%	15 7.5	112.5	Oral	1	20 10	500 100
Deldrax® 34%	Intervet	Nitroxylnil N- ethylglucamine 34%	10	13.5	Injection (subcut.)	2	400	20
Curaf Luke 10%	Univet	Fenbendazole 10% Rafoxanide 10%	11.25 11.25	50.6	Oral	1	50 30	500 10
Valbazen® 10%	Pfizer	Albendazole 10%	10	45	Oral	1	100	1000

Table 3. Effect of frying on concentration of anthelmintic drug residues in bovine liver. Concentration data are mean \pm standard error (n=3). P value indicates a statistically significant difference between expected and observed concentrations after cooking. Expected concentrations are corrected for weight loss during cooking. ND = none detected (estimated assay Limit of Detection accounting for sample dilution).

LIVER residue	Weight before (g)	Weight after (g)	Max. internal temp. ($^{\circ}$ C)	μ g kg $^{-1}$ uncooked	μ g kg $^{-1}$ expected after frying	μ g kg $^{-1}$ observed after frying	Residues % change during frying
Levamisole	143.0	103.7	91.3	2928.6 \pm 65.0	4038.5 \pm 89.6	2332.6 \pm 43.2 (P<0.0001)	-42
Oxyclozanide	143.0	103.7	91.3	4014.7 \pm 243.1	5536.2 \pm 335.2	5861.0 \pm 90.4	+6
Closantel	133.4	99.2	92.4	5652.8 \pm 881.3	7601.7 \pm 1185.1	7654.9 \pm 692.2	+1
Nitroxynil	209.5	133.8	96.1	ND (<10)	ND (<10)	ND (<10)	*
Clorsulon	187.5	144.9	88.7	1470.2 \pm 19.3	1902.5 \pm 24.9	1727.3 \pm 105.5	-9
Ivermectin	187.5	144.9	88.7	487.2 \pm 35.2	630.4 \pm 45.6	485.9 \pm 61.6	-23
Rafoxanide	165.5	113.6	88.0	3160.2 \pm 334.8	4604.0 \pm 487.7	4056.3 \pm 171.1	-12
ABZ	154.2	108.0	93.7	1112.2 \pm 20.0	1588.0 \pm 28.6	1368.6 \pm 39.8 (P<0.02)	-14
ABZ-SO	154.2	108.0	93.7	290.2 \pm 6.3	414.3 \pm 8.9	256.6 \pm 25.4 (P<0.005)	-38
ABZ-SO ₂	154.2	108.0	93.7	1403.9 \pm 108.8	2004.5 \pm 155.4	1854.8 \pm 89.4	-8
ABZ-NH ₂ -SO ₂	154.2	108.0	93.7	720.3 \pm 21.4	1028.4 \pm 30.6	1211.9 \pm 43.2 (P<0.03)	+18
SUM ABZ	154.2	108.0	93.7	2324.8 \pm 78.4	3319.3 \pm 111.9	3240.4 \pm 99.7	-2
MBZ	133.4	99.2	92.4	22.4 \pm 1.0	30.2 \pm 1.4	33.2 \pm 2.0	+10
OH-MBZ	133.4	99.2	92.4	73.2 \pm 2.1	98.4 \pm 2.8	99.7 \pm 7.3	+1
NH ₂ -MBZ	133.4	99.2	92.4	297.8 \pm 25.4	400.4 \pm 34.2	833.5 \pm 142.8 (P<0.05)	+108
SUM MBZ	133.4	99.2	92.4	467.0 \pm 32.4	628.0 \pm 43.6	1171.2 \pm 174.3 (P<0.04)	+ 86
FBZ	165.5	113.6	88.0	9243 \pm 998	13466 \pm 1454	13623 \pm 251	+1
FBZ-SO	165.5	113.6	88.0	1791.1 \pm 90.8	2609.3 \pm 132.3	525.6 \pm 21.0 (P<0.0001)	-80
FBZ-SO ₂	165.5	113.6	88.0	1029.4 \pm 76.0	1499.7 \pm 110.7	1637.3 \pm 111.9	+9
SUM FBZ	165.5	113.6	88.0	13144 \pm 1085	19149 \pm 1581	17270 \pm 387	-10
TCB	186.3	138.0	89.7	2911.0 \pm 43.9	3929.9 \pm 59.3	3160.4 \pm 183.2 (P<0.02)	-20
TCB-SO	186.3	138.0	89.7	ND (<50)	ND (<50)	ND (<50)	*
TCB-SO ₂	186.3	138.0	89.7	1162.9 \pm 85.5	1569.9 \pm 115.4	988.6 \pm 53.1 (P<0.02)	-37
Keto-TCB	186.3	138.0	89.7	383.8 \pm 34.2	518.2 \pm 46.2	453.7 \pm 133.0	-12
SUM TCB	186.3	138.0	89.7	4029.5 \pm 171.7	5439.8 \pm 231.8	4181.0 \pm 246.9 (P<0.03)	-23

Table 4. Effect of frying on concentration of anthelmintic drug residues in bovine muscle. Tissue concentration data are mean \pm standard error (n=3). Juice concentration data are means (n=2). NA = not available. ND = none detected (estimated assay Limit of Detection accounting for sample dilution). P value indicates a statistically significant difference between expected and observed concentrations after cooking.

MUSCLE residue	Weight before (g)	Weight after (g)	Weight juices (g)	Max. internal temp. (°C)	$\mu\text{g kg}^{-1}$ uncooked	$\mu\text{g kg}^{-1}$ expected after frying	$\mu\text{g kg}^{-1}$ observed after frying	$\mu\text{g kg}^{-1}$ juices	% of total fried residue in juices	Residue % change during frying (juices included)
Levamisole	189.6	151.2	0	57.0	108.1 \pm 0.9	135.5 \pm 1.2	121.3 \pm 0.3 (P<0.0003)	NA	NA	-11
Oxyclozanide	189.6	151.2	0	57.0	547.5 \pm 14.9	686.5 \pm 18.7	868.6 \pm 22.1 (P<0.004)	NA	NA	+27
Closantel	149.2	117.5	3.4	67.3	2089.8 \pm 74.7	2653.6 \pm 94.8	2499.9 \pm 95.0	684.8	<1	-5
Nitroxynil	248.1	196.0	4.0	66.9	531.4 \pm 7.2	672.7 \pm 9.2	142.9 \pm 9.7 (P<0.0001)	238.5	3	-78
Clorsulon	151.1	119.5	3.9	70.2	289.2 \pm 10.3	365.7 \pm 13.0	470.0 \pm 28.3 (P<0.03)	ND(<150)	0	+29
Ivermectin	151.1	119.5	3.9	70.2	14.4 \pm 0.6	18.1 \pm 0.7	15.6 \pm 0.9	NA	NA	-14
Rafoxanide	124.2	88.0	3.6	68.6	1813.3 \pm 49.1	2559.2 \pm 69.4	2106.8 \pm 125.0 (P<0.04)	133.3	<1	-17
SUM ABZ	227.6	170.5	0	55.0	156.9 \pm 5.2	209.4 \pm 6.9	225.0 \pm 2.6	NA	NA	+7
MBZ	149.2	117.5	0	67.3	0.89 \pm 0.05	1.13 \pm 0.06	1.01 \pm 0.06	NA	NA	-10
OH-MBZ	149.2	117.5	0	67.3	8.75 \pm 0.11	11.1 \pm 0.1	8.34 \pm 0.31 (P<0.002)	NA	NA	-25
NH ₂ -MBZ	149.2	117.5	0	67.3	23.1 \pm 0.7	29.3 \pm 0.9	30.0 \pm 0.3	NA	NA	+2
SUM MBZ	149.2	117.5	0	67.3	38.5 \pm 0.9	48.9 \pm 1.1	46.8 \pm 0.1	NA	NA	-4
FBZ	124.2	88.0	3.6	68.6	265.3 \pm 7.8	374.5 \pm 11.0	340.0 \pm 4.0 (P<0.05)	7.75	<1	-9
FBZ-SO	124.2	88.0	3.6	68.6	5.57 \pm 0.19	7.86 \pm 0.26	2.53 \pm 0.09 (P<0.0001)	ND (<2)	0	-68
FBZ-SO ₂	124.2	88.0	3.6	68.6	154.6 \pm 1.6	218.2 \pm 2.3	209.5 \pm 13.6	24.9	<1	-4
SUM FBZ	124.2	88.0	3.6	68.6	454.2 \pm 9.6	641.1 \pm 13.6	588.5 \pm 17.5	33.4	<1	-8
TCB	205.5	158.5	3.9	72.0	292.4 \pm 9.7	379.1 \pm 12.6	617.0 \pm 27.9 (P<0.002)	16.2	<1	+63
TCB-SO	205.5	158.5	3.9	72.0	72.1 \pm 4.0	93.5 \pm 5.2	23.1 \pm 1.6 (P<0.0003)	ND (<50)	0	-75
TCB-SO ₂	205.5	158.5	3.9	72.0	2674.4 \pm 165.3	3467.4 \pm 214.3	2953.6 \pm 175.9	55.6	<1	-15
Keto-TCB	205.5	158.5	3.9	72.0	52.6 \pm 0.4	68.2 \pm 0.5	96.7 \pm 3.3 (P<0.001)	ND (<50)	0	+42
SUM TCB	205.5	158.5	3.9	72.0	2635.5 \pm 149.2	3417 \pm 193.5	3169.1 \pm 169.2	61.6	<1	-7

Table 5. Effect of roasting on concentration of anthelmintic drug residues in bovine muscle. Tissue concentration data are mean \pm standard error (n=3). Juice concentration data are means (n=2). NA = not available. ND = none detected (estimated assay Limit of Detection accounting for sample dilution). P value indicates a statistically significant difference between expected and observed concentrations after cooking.

MUSCLE residue	Weight before (g)	Weight after (g)	Weight juices (g)	Max. internal temp. ($^{\circ}$ C)	μ g kg $^{-1}$ uncooked	μ g kg $^{-1}$ expected after roasting	μ g kg $^{-1}$ observed after roasting	μ g kg $^{-1}$ juices	% of total roasted residue in juices	Residue % change during roasting (juices included)
Levamisole	278.1	190.4	31.2	87.0	108.1 \pm 0.9	157.8 \pm 1.3	131.7 \pm 1.5 (P<0.0003)	164.1	17	+1
Oxyclozanide	278.1	190.4	31.2	87.0	547.5 \pm 14.9	799.6 \pm 21.8	728.2 \pm 31.6	ND (< 25)	0	-9
Closantel	314.2	206.5	49.7	92.9	2089.8 \pm 74.7	3179.7 \pm 113.6	3447.7 \pm 117.5	20.0	<1	+9
Nitroxylnil	311.6	198.9	68.3	92.8	531.4 \pm 7.2	832.6 \pm 11.4	34.3 \pm 0.9 (P<0.0001)	ND (<10)	0	-96
Clorsulon	314.7	211.4	54.1	84.2	289.2 \pm 10.3	430.6 \pm 15.3	463.2 \pm 41.8	117.5	6	+15
Ivermectin	314.7	211.4	54.1	84.2	14.4 \pm 0.6	21.4 \pm 0.9	26.9 \pm 2.9	ND (<5)	0	+26
Rafoxanide	262.9	169.3	45.0	87.6	1813.3 \pm 49.1	2815.8 \pm 76.3	2313.6 \pm 114.9 (P<0.03)	17.8	<1	-18
SUM ABZ	325.1	224.5	35.0	82.0	156.9 \pm 5.2	227.1 \pm 7.5	195.1 \pm 3.6 (P<0.02)	112.7	8	-6
MBZ	314.2	206.5	49.7	92.9	0.89 \pm 0.05	1.36 \pm 0.07	0.93 \pm 0.02 (P<0.004)	0.23	6	-28
OH-MBZ	314.2	206.5	49.7	92.9	8.75 \pm 0.11	13.3 \pm 0.2	13.4 \pm 0.5	3.36	6	+6
NH ₂ -MBZ	314.2	206.5	49.7	92.9	23.1 \pm 0.7	35.1 \pm 1.1	29.1 \pm 0.8 (P<0.01)	11.8	9	-9
SUM MBZ	314.2	206.5	49.7	92.9	38.5 \pm 0.9	58.6 \pm 1.3	50.6 \pm 1.2 (P<0.02)	18.3	8	-6
FBZ	262.9	169.3	45.0	87.6	265.3 \pm 7.8	412.0 \pm 12.1	386.9 \pm 7.0	12.3	1	-5
FBZ-SO	262.9	169.3	45.0	87.6	5.57 \pm 0.19	8.64 \pm 0.29	21.5 \pm 1.4 (P<0.0009)	13.7	14	+190
FBZ-SO ₂	262.9	169.3	45.0	87.6	154.6 \pm 1.6	240.1 \pm 2.5	250.3 \pm 3.7	39.4	4	+9
SUM FBZ	262.9	169.3	45.0	87.6	454.2 \pm 9.6	705.3 \pm 14.9	701.2 \pm 5.9	67.3	2	+2
TCB	302.2	203.1	44.9	88.3	292.4 \pm 9.7	435.0 \pm 14.5	475.3 \pm 46.8	2.87	<1	+9
TCB-SO	302.2	203.1	44.9	88.3	72.1 \pm 4.0	107.3 \pm 6.0	8.50 \pm 0.88 (P<0.0001)	ND (<2)	0	-92
TCB-SO ₂	302.2	203.1	44.9	88.3	2674.4 \pm 165.3	3979.3 \pm 246.0	1869.8 \pm 65.5 (P<0.002)	18.5	<1	-53
Keto-TCB	302.2	203.1	44.9	88.3	52.6 \pm 0.4	78.2 \pm 0.6	68.2 \pm 3.5 (P<0.05)	ND (<20)	0	-13
SUM TCB	302.2	203.1	44.9	88.3	2635.5 \pm 149.2	3921.5 \pm 222.0	2085.4 \pm 94.3 (P<0.002)	18.2	<1	-47