Rom J Leg Med [31] 65-70 [2023]
DOI: 10.4323/rjlm.2023.65

THE SPECIFIC ASPECTS OF XENOBIOTICS POSTMORTEM REDISTRIBUTION IN FORENSIC TOXICOLOGY

Emilija Kostic[1,*], Dimitrije Pancic[1], Maja Vujovic[1, 2], Goran Ilic[1, 2]

[1]*University of Nis, Faculty of Medicine,* [2]*Institute of Forensic Medicine, Nis, Serbia*

Abstract: Forensic toxicology analyses are performed to be used as evidence in legal proceedings. In analytical toxicology, the terms 'sample' and 'specimen' refer to a portion of body fluid or tissue collected in previously defined conditions. Specimens should be collected after death as soon as possible to avoid possible putrefaction. Great care is needed to select the sampling site and how to collect and use the appropriate sample container. A concept of postmortem redistribution (PMR) of xenobiotics, considered when interpreting the drug concentration after death, must exist. The organs involved in redistributing drugs into the blood are also called "drug reservoirs". Knowledge of toxicologists about the characteristics of different specimens, xenobiotics, their toxicokinetic parameters and the possibility for postmortem redistribution is of great importance for quick, accurate and reliable results of toxicological analysis.

Keywords: toxicology, postmortem, organs, toxicokinetics, xenobiotics.

INTRODUCTION

Forensic toxicology is a discipline which involves the detection, identification, and measurement of xenobiotics in biological and non-biological samples to reveal the causes of fatal intoxications. An accurate and correct interpretation of drug postmortem concentration is becoming increasingly crucial in forensic toxicology. Part of the case assessment includes a review of forensic documentation, which contain data on postmortem drug concentrations. Forensic toxicology analyses are performed to be used as evidence in legal proceedings [1, 2].

Conclusions about the level of medications found after death may be influenced by other factors, such as relevant information provided by a relative about the life and habits of the deceased, the entire procedure of medical examination and treatment, as well as judicial authorities from which meaningful information can be obtained at the scene.

Postmortem drug concentrations do not primarily reflect the concentrations at the moment of death because that concentration may vary depending on the site used for blood sampling and the period between the time of death and sampling time.

Although it is common to think of the human body as a static entity after death, here it is not the case. Accordingly, a concept of drug postmortem redistribution (PMR), considered when interpreting the concentration of drugs after death, must exist. Denying its importance can lead to wrong conclusions about the death cause.

The organs involved in drug redistribution into the blood are also called "drug reservoirs". Passive elimination of drugs from the reservoir such as lungs, the gastrointestinal tract, liver or heart muscle can begin immediately after death. Mechanisms involved in this process are diffusion through blood vessels and transparietal diffusion to surrounding organs [3].

The factors that make drugs susceptible to PMR can be attributed both to the drug properties and to the changes that begin in the body after death.

Characteristics of xenobiotics

Volume of distribution

The volume of distribution (Vd) is defined as the total amount of drug in the body divided by the plasma concentration (L/kg). Medications that bind very well to plasma proteins have a small Vd, equal to the plasma volume, while drugs highly distributed to muscles,

*Correspondence to: Emilija Kostic, University of Nis, Faculty of Medicine, Blvd. Dr Zorana Djindjica 81, Nis, Serbia, E-mail: emilija.kostic@medfak.ni.ac.rs

adipose tissue, and intracellular structures have a higher Vd. For example, 11-tetrahydrocannabinol is deposited in fat; digoxin and other cardiotonic glycosides in the heart; while the glucuronides secreted from the liver are higher in the concentration of glucuronides in gallbladder than in the blood (e.g. morphine) [14]. After cell death and following lysis, the drugs are released into plasma, resulting in increased postmortem concentration. Drugs with a Vd higher than 3 L/kg have the biggest potential to undergo PMR [4].

Lipophilicity and acidity of the medium (pKa)
It is essential to mention two important facts regarding PMR. Organic bases and lipophilic drugs concentrate in the internal organs (liver, lungs and myocardium). This allows a higher concentration gradient for passive diffusion after death. The cell environment becomes acidic after cell death, and following lysis, the alkaline drugs will be more easily distributed in the acidic environment where they are dissolved [5].

Changes that take place in the organism after death

Cell death

Along with cell damage, different processes that result in cell death and the release of cell contents into extracellular fluids, including drugs, occur.

Influence of putrefaction on an organism

Decomposition of the body (putrefaction) can also cause changes in the concentration of the drug after death when the decay and destruction of soft tissues by bacteria and endogenous enzymes occur. The process of putrefaction varies depending on the environment and the body's condition.

One day of a cadaver at a high temperature can cause significant putrefaction, whereas weeks in the cold will not lead to significant changes in the cadaver and substance concentrations. The impact of putrefaction on different substances is different [6]. Many xenobiotics are stable in blood when stored under laboratory conditions. Instability has been observed in substances with an ester structure or the form of esters found in the body, such as heroin, cocaine, methylphenidate, substances with a sulfur atom (phenothiazines and olanzapine), easily oxidised and reduced substances (nitrobenzodiazepines and dihydropyridine calcium channel blockers) [6, 7].

Also, some bacteria can use some drugs and their metabolites as substrates, so it changes their concentration, while enterobacteria metabolise drugs and cause an increase in ethanol concentration [8]. Robertson and Dramer studied the biotransformation of nitro benzodiazepines: clonazepam, flunitrazepam, and nitrazepam in the presence of eight types of enterobacteria that are commonly present in the gastrointestinal tract after death. They found that numerous bacteria metabolise drugs, especially facultative anaerobes such as Bacillus fragilis and Clostridium perfringens [9]. The metabolic rate was reduced when they performed tests at a temperature of + 4°C, compared to temperatures from 22 to 37°C, emphasising the importance of preserving bodies at low temperatures.

Passive diffusion from the organs of the thoracic and abdominal cavities

Organs close to major blood vessels and the heart, such as the oesophagus, stomach, proximal duodenum, liver, myocardium and lungs, play a significant role in passive drug diffusion.

In 1992, Hilberg demonstrated passive drug diffusion by experimentally placing amitriptyline in the trachea of rats and then ligating the trachea. After 96 hours, he could still detect amitriptyline in the left liver lobe, and this result differed from the one with the unligated trachea, where amitriptyline was present for only 1.5 hours [10]. This suggests that there is a PMR of drugs. This is also important when interpreting drug levels, which may be falsely higher due to diffusion from surrounding organs.

Examples of xenobiotics subject to postmortem redistribution

Tricyclic antidepressants (TCA)

Tricyclic antidepressants are perhaps the most widely studied drugs concerning PMR and are even taken as a comparison control group [11]. They have a high Vd and are basic and highly lipophilic.

TCAs can be redistributed from the lungs and concentrated in the cavities of the left half of the heart through the venous pulmonary system. They can also bind to the myocardium, and from there, they can be released into the left and right heart cavities [12, 13].

Amphetamines

Amphetamine binds well to the myocardium tissue, and it is proven that it is subject to PMR [14]. This category includes: methamphetamine, 3,4-methylenedioxy-amphetamine (MDA) para-methoxyamphetamine (PMA), and 3,4-methylenedioxymethamphetamine (MDMA).

Cocaine

Cocaine binds to the myocardium. After death, it can be released into the heart cavities, so the concentration in the heart is higher than that in the

femoral vein, suggesting that it is undergoing PMR. [16]. However, its high metabolic rate (half-life 0.5-1.5h) makes it difficult to detect it in postmortem samples. Metabolites of cocaine are benzoylecgonine and methylecgonine. Cocaine is fast metabolised in a warm, alkaline environment, which explains its metabolism, which occurs even after death [15].

Some authors have recommended sampling of skeletal muscle or brain tissue because they can be more suitable for analysing cocaine in the body, and this especially applies to the brain tissue, whose composition is rich in lipids [16, 17]. It is not yet known whether or not taking brain samples will become a common practice in autopsies.

Morphine

Pharmacologically, morphine is expected to undergo PMR because of its Vd, 3-5 L/kg and its lipophilicity at physiological pH. There are consistent findings in animal models that morphine is subject to redistribution [18, 19].

However, there are contradictory data related to the PMR of morphine. In 40 cases of heroin-related deaths, a group of researchers found no significant difference in blood sample concentrations at admission and autopsy.

On the other hand, some studies have shown that morphine is subject to PMR and that there are apparent differences in morphine, morphine-6-glucuronide and morphine-6-glucuronide between samples from the centrally selected sampling sites compared to those from the peripheral ones [20].

The explanation of the inconsistency between these studies probably lies in the variation in the selected sampling sites and variations in the content of water and hematocrit in blood. It is shown that blood samples with low hematocrit as well as high-water content contain the highest concentrations of morphine.

Samples for toxicological analyses

There are numerous challenges in the process of selection and collection of samples for toxicological analysis. The results' relevance directly depends on the type and characteristics of the samples.

In postmortem analysis, it is important to undertake systematic sampling. Great care is needed to select the sampling site and how to collect and use the appropriate sample container.

Postmortem specimens should be collected as soon as possible after death. Immediately after sampling, the samples should be stored refrigerated to minimise changes in substance concentration due to putrefaction.

Arterial or venous femoral blood, urine, vitreous humour, gastric contents, and organs (kidney, liver, and stomach) are the most important samples to collect [21].

Blood and urine are the most commonly used samples for analysis because blood drug concentration may provide the best estimation of the drug's pharmacological effect [22].

Appropriate quantitative analysis is often only possible by analysing plasma, serum or blood samples. Organs, or tissues, are of great importance when body fluids are not available and if the cadaver is decomposed. Tissues should be stored the same way as blood before analysis, but no preservatives should be added. To avoid cross-contamination between substances found near the deceased person and biological samples, they should be transported and stored separately [22].

Several other alternative specimens (e.g. thoracic or abdominal blood, blood clots, brain, cerebrospinal fluid, bile, spleen, bones, synovial fluid, oblique marrow, skeletal muscle) may be collected in certain circumstances.

Organs for postmortem toxicological analyses

Stomach content (including vomiting, gastric aspirates, and lavage fluid) and stomach

Taking all gastric contents and gastric wall sections for postmortem analysis is essential. The found tablets and capsules should be quickly isolated, dried and stored in another container to prevent their further decomposition under the action of gastric acid.

Characteristic odours should be noted, as some pesticides and solvents can be recognised based on odour. In the case of suspected intoxication of cyanide or phosphide gas, inhalation should be avoided as it may present a danger and risk of secondary poisoning (must work in a fume cupboard). The smell of cyanide is similar to that of almonds; the smell of halogenated hydrocarbons in cleaning liquids, the smell of toluene and xylene resembles glue, ethchlorvynol on carrots and organophosphorus insecticides on garlic [23].

A different colouration of the gastric contents may indicate the type of substance that caused the poisoning. The blue indicates the possible presence of methylcarbamate (methomyl), and the yellow indicates the presence of 4,6-dinitro-ortho-cresol (DNOC). Exact parts that are coloured differently should be taken for analysis. Nitrite poisoning can only be demonstrated in samples of gastric contents and not in blood. The presence of xenobiotics in gastric contents does not prove oral administration, especially

if the concentration is low. Xenobiotics distributed extracellularly will be found in a fluid that ultimately forms gastric secretion. Furthermore, the stomach will concentrate on basic xenobiotics due to ion trapping [21].

Redistribution from the gastrointestinal tract

Following the mechanism mentioned above, unabsorbed drugs in the stomach may be redistributed to the mediastinal blood vessels and surrounding organs. Through the bloodstream, the molecules quickly diffuse into the left and right half of the heart, the aorta, and the inferior vena cava. This diffusion can begin as early as a few hours after death, as with ethanol [39] and tricyclic antidepressants [24].

Substances can diffuse from the stomach to surrounding organs; mainly to the lower lobe of the left lung, the posterior left edge of the liver and to a lesser extent to the caudate lobe (when the corpse is in the supine position, the posterior part of the right lobe of the liver) [25]. Diffusion to the sites mentioned above of ethanol, amitriptyline, methanol and lithium is unequivocally known [25]. Airway contamination by regurgitation of drugs from the stomach can cause a redistribution of these drugs into the blood in the lungs and the blood in the heart cavities [26]. Such contamination may be due to inhalation during agony or passive relaxation of the esophagogastric sphincter [27]. This process can be facilitated by the manipulation of the corpse and the supine position of the corpse [28]. Airway contamination is associated with an increase in the concentration of the drug in the blood.

PMR refers to the entire upper gastrointestinal tract, not just the stomach. However, it is influenced by some physical factors such as the concentration of the drug in the stomach contents, the amount of stomach contents, the corpse temperature and the time after death and sampling.

Liver

The liver should always be taken for analysis, if available. The deep right lobe is commonly chosen to avoid contamination by diffusion of xenobiotics from stomach content into the left lobe.

The gallbladder should not be collected with the liver.

The liver is an important sample because it is a major metabolic organ and an important reservoir of many xenobiotics (e.g. alkaloids, tricyclic antidepressants, substances that bind extensively to plasma proteins - lipophilic, and substances susceptible to the first-pass metabolism in the liver) [29-31].The liver is the organ with the most information regarding xenobiotic concentrations. In some cases (drugs deposited in the liver), qualitative analysis of certain xenobiotics is more accessible than from blood.The liver as a sample for toxicological analysis also has a few disadvantages. Quantitative relationships between liver and blood concentrations are unavailable in most xenobiotics, and high lipid and protein content may interfere with the toxicological analysis.

The ratio of xenobiotic concentrations in the liver and the blood is an important parameter for determining whether or not a substance is subject to redistribution. A ratio lower than 5 suggests a low propensity for PMR; A ratio exceeding 20–30 suggests significant PMR [32].

Redistribution from the liver

PMR from the liver is a complex process because it includes various mechanisms. Drugs isolated from the liver at the time of death can be redistributed through the hepatic veins to the inferior vena cava and then to the right ventricle, pulmonary blood vessels, or peripheral venous blood [25]. It can result in a reduction in the concentration of drugs in the lobes of the liver [33]. However, this process is not as intense and does not occur immediately after death as lung redistribution. Also, drugs can be redistributed directly to surrounding organs. The anatomical position of the liver and the stomach, proximal duodenum, and gallbladder makes the liver suitable for PMR, but redistribution through the liver's blood vessels is more important [25].

Opposite redistribution is also possible because the liver is the site of the redistribution of drugs from the gastrointestinal tract. The most significant part of the lower surface of the left lobe of the liver is very close to the stomach, while the proximal part of the duodenum is located opposite the right lobe of the liver and gallbladder. Finally, xenobiotics present at the time of death can enter the liver parenchyma, either by passive diffusion or through the portal vein.

Kidney

Kidney tissue can be important in the absence of urine, so the kidney must be taken for analysis in this case. It is used for ethylene glycol analysis (structure damage documented histologically) [34]. Also, different metals tend to concentrate in the kidneys, so kidney tissue is the optimal sample for analysis [35]. Unfortunately, it is useful only for qualitative analysis.

Lungs

High concentrations of various xenobiotics are commonly found in lung tissue, especially after intoxication after inhalation or intravenous route of

administration. Depending on the characteristics of xenobiotics, concentrations in the lungs can be higher than in the liver.

When we suspect solvent abuse or anaesthetic-mediated death, besides lungs, a brain sample must be collected for toxicological analysis.

In paraquat intoxication-related deaths, lungs should always be analysed. Paraquat primarily accumulates in the lungs, which remains even when the blood level decreases [36].

Redistribution from the lungs

In vivo, the lungs receive blood from the right ventricle, and thus, more drugs accumulate there, especially weak lipophilic bases with pKa values greater than 8, such as imipramine, amitriptyline, methadone, and chlorpromazine [37]. PMR from the lungs begins in the first two hours after death, causing an increase in drugs in the heart ventricles and blood vessels of the chest, and this redistribution appears to be more intense than the one in the gastrointestinal tract [38]. The following two mechanisms are possibly involved in this redistribution. Firstly, drugs can be redistributed through the blood vessels of the lungs. The increase in drug levels, primarily in the pulmonary veins and then in the arteries, can be explained by the fact that diffusion through thinner venous walls happens faster [39]. Moreover, drugs located in the pulmonary parenchyma and blood vessels can be redistributed directly to the surrounding tissue, including the blood vessels of the chest and heart chambers [40].

The intensity of PMR from the lungs can be explained by the huge surface of the alveoli, their thin diffusion membrane and the excellent vascularisation. This redistribution may coexist with PMR from the gastrointestinal tract, and it may be challenging to determine the primary mechanism of redistribution of orally administrated drugs [10].

According to some research [10], higher the concentration of drugs in the blood from the heart cavities than in the myocardium implies that diffusion occurred from the lungs through the pulmonary veins. On the other hand, a higher concentration of drugs in the myocardium indicates that direct diffusion from the stomach or lungs through the myocardium has occurred. PMR of drugs from the lungs to the liver was also noted.

The anatomical boundary between the abdominal and thoracic cavities is the diaphragm. However, if diffusion across this barrier were the only source of this redistribution, higher concentrations of drugs in the liver in contact with the diaphragm would be expected, but this is not the case. Pleural and peritoneal fluids are considered mediums for exchanging drugs between these two cavities' organs and walls [40].

In conclusion, the significance of visceral organs as samples for toxicological analyses is enormous. Knowledge of toxicologists about the properties of different specimens used for clinical and forensic analysis, characteristics of xenobiotics, such as pharmacokinetic parameters, and the possibility for PMR is essential for quick, accurate and reliable results of postmortem toxicological analysis.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Langman LJ, Kapur BM. Toxicology: then and now. Clin Biochem. 2006; 39: 498–510.
2. Kennedy M. Interpreting postmortem drug analysis and redistribution in determining cause of death: a review. PMI. 2015; 7:55-62.
3. Pélissier-Alicot A-L, Gaulier J-M, Champsaur P, Marquet P. Mechanisms underlying postmortem redistribution of drugs: a review. J Anal Toxicol. 2003; 27: 533–544.
4. Hilberg T, Rogue S, Mørland J. Postmortem Drug Redistribution—Human Cases Related to Results in Experimental Animals. Forensic Sci. 1999; 44(1): 3-9.
5. Yarema MC, Becker CE. Key Concepts in Postmortem Drug Redistribution. Clin. Toxicol. 2005; 43: 235–241.
6. Drummer OH. Postmortem toxicology of drugs of abuse. Forensic Sci Int. 2004; 142: 101–113.
7. Peters FT. Stability of analytes in biosamples-an important issue in clinical and forensic toxicology? Anal Bioanal Chem. 2007; 388: 1505–1519.
8. Chung H, Choe S. Challenges in forensic toxicology. Aust J Forensic Sci. 2019; 51(6): 665–673.
9. Robertson MD, Drummer OH. Postmortem Distribution and Redistribution of Nitrobenzodiazepines in Man. J Forensic Sci. 1998; 43(1):9-13.
10. Hilberg T, Bugge A, Beylich K-M, Mørland J, Bjørneboe A. Diffusion as a mechanism of postmortem drug redistribution: An experimental study in rats. Int J Leg Med. 1992; 105: 87–91.
11. Hilberg T, Ripel Å, Slørdal L, Bjørneboe A, Mørland J. The Extent of Postmortem Drug Redistribution in a Rat Model. J Forensic Sci. 1999; 44(5):956-62.
12. Bailey DN, Shaw RF. Concentrations of Basic Drugs in Postmortem Human Myocardium. J. Toxicol. Clin. Toxicol. 1982; 19(2): 197–202.
13. Moriya F, Hashimoto Y. Redistribution of Basic Drugs into Cardiac Blood from Surrounding Tissues During Early-Stages Postmortem. J Forensic Sci. 1999; 44(1):10-16.
14. Barnhart FE, Fogacci JR, Reed DW. Methamphetamine—A Study of Postmortem Redistribution. J Anal Toxicol. 1999; 23(1): 69–70.
15. Baselt RC. Stability of cocaine in biological fluids. J Chromatogr. 1983; 268(3):502-505.
16. Spiehler VR, Reed D. Brain Concentrations of Cocaine and Benzoylecgonine in Fatal Cases. J Forensic Sci. 1985; 30(4):1003-1011.

17. Moriya F, Hashimoto Y. Postmortem Stability of Cocaine and Cocaethylene in Blood and Tissues of Humans and Rabbits. J Forensic Sci. 1996; 41(4):612-616.
18. Klein GKAJ, Gideon Koren And. Postmortem Redistribution of Morphine in Rats. Ther Drug Monit. 1992; 14(6):461-463.
19. Sawyer WR, Forney RB. Postmortem disposition of morphine in rats. Forensic Sci Int. 1988; 38(3-4):259-273.
20. Moriya F, Hashimoto Y. Distribution of Free and Conjugated Morphine in Body Fluids and Tissues in a Fatal Heroin Overdose: Is Conjugated Morphine Stable in Postmortem Specimens? J Forensic Sci. 1997; 42(4):736-740.
21. Skopp G. Preanalytic aspects in postmortem toxicology. Forensic Sci Int. 2004; 142(2-3):75-100.
22. Dinis-Oliveira RJ, Carvalho F, Duarte JA, Remião F, Marques A, Santos A, Magalhães T. Collection of biological samples in forensic toxicology. Toxicol Mech Methods. 2010; 20: 363–414.
23. Karch SB, Drummer O. Karch's pathology of drug abuse. CRC Press; 2008.
24. Hilberg T, Bugge A, Beylich K-M, Ingum J, Bjørneboe A, Mørland J. An Animal Model of Postmortem Amitriptyline Redistribution. J Forensic Sci. 1993; 38(1):81-90.
25. Pounder DJ, Fuke C, Cox DE, Smith D, Kuroda N. Postmortem Diffusion of Drugs from Gastric Residue. Am J Forensic Med Pathol. 1996; 17(1):1-7.
26. Marraccini JV, Carroll T, Grant S, Halleran S, Benz JA. Differences Between Multisite Postmortem Ethanol Concentrations as Related to Agonal Events. J Forensic Sci. 1990; 35(6):1360-1366.
27. Moriya F, Hashimoto Y. Postmortem diffusion of tracheal lidocaine into heart blood following intubation for cardiopulmonary resuscitation. J Forensic Sci. 1997; 42(2):296-299.
28. Pounder DJ, Smith DRW. Postmortem Diffusion of Alcohol from the Stomach. Am J Forensic Med Pathol. 1995; 16(2): 89-96.
29. Luckenbill K, Thompson J, Middleton O, Kloss J, Apple F. Fentanyl postmortem redistribution: preliminary findings regarding the relationship among femoral blood and liver and heart tissue concentrations. J Anal Toxicol. 2008; 32: 639–643.
30. Cantrell FL, Lee Cantrell F, Vance C, Schaber B, McIntyre I. Fatal Fluoxetine Intoxication with Markedly Elevated Central Blood, Vitreous, and Liver Concentrations. J Anal Toxicol. 2009; 33(1):62-64.
31. Jenkins AJ, Caplan YH, editors. Drug Testing in Alternate Biological Specimens. Humana Press; 2008.
32. Karch SB, Drummer O. Karch's pathology of drug abuse. CRC Press; 2008.
33. Pohland RC, Bernhard NR. Postmortem Serum and Tissue Redistribution of Fluoxetine and Norfluoxetine in Dogs Following Oral Administration of Fluoxetine Hydrochloride (Prozac®). J Forensic Sci. 1997; 42(5): 812-816.
34. Takahashi S, Kanetake J, Kanawaku Y, Funayama M. Brain death with calcium oxalate deposition in the kidney: clue to the diagnosis of ethylene glycol poisoning. Leg Med. 2008; 10: 43–45.
35. Yilmaz O. Cadmium and lead levels in human liver and kidney samples obtained from Bursa Province. Int J Environ Health Res. 2002; 12: 181–185.
36. Dinis-Oliveira RJ, Remião F, Carmo H, Duarte JA, Navarro AS, Bastos ML, Carvalho F. Paraquat exposure as an etiological factor of Parkinson's disease. Neurotoxicology. 2006; 27: 1110–1122.
37. Macintyre AC, Cutler DJ. The potential role of lysosomes in tissue distribution of weak bases. Biopharm Drug Dispos. 1988; 9(6): 513-526.
38. Miyazaki T, Kojima T, Yashiki M, Wakamoto H, Iwasaki Y, Taniguchi T. Site Dependence of Methamphetamine Concentrations in Blood Samples Collected from Cadavers of People Who Had Been Methamphetamine Abusers. Am J Forensic Med Pathol. 1993; 14(2): 121–124.
39. Pounder DJ, Owen V, Quigley C. Postmortem Changes in Blood Amitriptyline Concentration. Am J Forensic Med Pathol. 1994; 15:224–230.
40. Hilberg T, Mørland J, Bjørneboe A. Postmortem release of amitriptyline from the lungs; a mechanism of postmortem drug redistribution. Forensic Sci Int. 1994; 64(1): 47-55.