

Branislav ŠIŠKA<sup>1</sup>, Robert TOMAN, Jozef GOLIAN,  
Michal BOŠIAK and Stefan KOVAC

## DISTRIBUTION OF DIAZINON AND SELENIUM IN VARIOUS TISSUES AFTER SINGLE AND COMMON INTRAPERITONEAL ADMINISTRATION

### DYSTRYBUCJA DIAZINONU I SELENU W WYBRANYCH TKANKACH PO POJEDYNCZYM PODANIU DOOTRZEWNOWYM

**Abstract:** The aim of this study was to evaluate the distribution of diazinon and selenium in various tissues of laboratory rats after single and common intraperitoneal administration. Rats in the age of 135 days were randomly divided into 4 groups. Each group consisted of 10 males. Animals in the first group were administrated with diazinon  $20 \text{ mg} \cdot \text{kg}^{-1}$  body weight intraperitoneally. Animals in the second group were administrated with selenium ( $\text{Na}_2\text{SeO}_3$ )  $2 \text{ mg Se} \cdot \text{kg}^{-1}$  body weight intraperitoneally in physiological solution. Animals in the third group were given a mixture of diazinon  $20 \text{ mg} \cdot \text{kg}^{-1}$  body weight and selenium  $2 \text{ mg Se} \cdot \text{kg}^{-1}$  body weight intraperitoneally in physiological solution. The fourth group served as a control group and was administrated only with the physiological solution. 24 hours after the administration of tested substances, animals were sacrificed and samples of livers, kidneys, muscles and fat tissue were taken during the autopsy. The amount of diazinon in tissues was determined using gas chromatography with mass spectrometry. The amount of selenium was determined using atomic absorption spectrometry. We detected significant increase of selenium amount in livers, kidneys and muscles after single selenium administration and also after common administration of both substances. On the other hand we detected significant decrease of selenium amount in muscles and fat tissue after single diazinon administration. We also observed slight accumulation of diazinon amount in samples of kidneys and muscles and significant increase of diazinon in fat tissue after single diazinon administration and also after common administration of both substances.

**Keywords:** diazinon, selenium, liver, kidney, muscle, fat, intraperitoneal administration

Diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) belongs to the group of organophosphate insecticides, used to control cockroaches, fleas and ants. It is also used to control a wide variety of sucking and leaf eating insects. It is used on rice, fruit trees, sugarcane, corn, tobacco, potatoes and on horticultural plants. Diazinon has veterinary use against fleas and ticks. Organophosphorus pesticides, including also diazinon have harmful effect on nervous system through the inhibition of

<sup>1</sup> Department of Food Hygiene and Safety, Faculty of Biotechnology and Food Sciences, Slovak Agricultural University in Nitra, Tr. A. Hlinku 2, 949 01 Nitra, Slovak Republic, email: branislav.siska@gmail.com

acetylcholinesterase. Acetylcholinesterase is an enzyme, which is necessary in process for controlling of nervous signals transfer. Lack of acetylcholinesterase causes accumulation of acetylcholine. Accumulation of this neurotransmitter on the connections between nerves and muscles causes uncontrolled muscular contraction and algospasmus, between nerves and glands causes continual secretion of these glands, while acetylcholine cumulation between certain nerve cells in a brain causes sensory behavior disorders [1–3]. Most frequently described symptoms of acute diazinon toxicity are headaches, nausea, vertigo, blurred vision, feeling of pressure in chest, respiration problems, muscular weakness or convulsions, diarrhea and vomiting. Typical symptoms of irritated nervous system are confuse, anguish, melancholy and insomnia. Symptoms of chronic poisoning are always connected with depression of cholinesterase activity [2, 4].

Diazinon could be frequently found in wide range of various fruit and vegetable species, including apples, pears, cereals, soya, strawberries, tomatoes, beans [2]. During a survey that was focused on monitoring of pesticides amounts in 21 various species of fruit were analyzed 150 samples for the presence of diazinon. Results of this study show that 90 % of analyzed samples did not contain any diazinon, 1 % of samples was very close to the detection limit, 8 % of samples were positive without exceeding of valid legislation limits for maximal content of diazinon and 1 % of all analyzed fruit samples contained diazinon in amount that was over the maximal legal limit for the presence of this pesticide [5].

Diazinon does not belong to the substances that are typical for its high cumulative ability in animal tissues and it is not found in the food of animal origin very frequently. However, certain amounts of this substance could be found in a fat tissue in some cases. This tissue has certain ability to cumulate diazinon during relatively short time period. This observation was found also in the cattle. Diazinon was applied on the skin of tested animals in the form of a spray. Till 14 days, substance was completely excreted from the organism of tested animals. Application of diazinon on the skin of these animals caused also the presence of this substance in their milk during 24 hours after application [6, 7].

Residuals of this pesticide are commonly found in a sheep fat in Great Britain. In the year of 1999, food authorities in this country revealed presence of diazinon residuals in 20 samples of kidney fat. Amounts of diazinon varied from  $21 \mu\text{g} \cdot \text{kg}^{-1}$  to  $150 \mu\text{g} \cdot \text{kg}^{-1}$  [8].

Selenium is a typical biogenous element, which is necessary for all living organisms. However, selenium could act also as a toxic element, especially when it is present in organism in the surplus. Due to this fact selenium was considered to be strictly toxic and scientists did not deal with the benefits of selenium. Selenium essentiality was scientifically proven for the first time in 1957 [9]. Selenium is widely used in various industries, mostly in glass industry and electronic industry and it has been frequently used also as a part of inorganic pigments [10].

The selenium content in plants is affected by the content and availability of this element in soil in which they are grown and also varies from country to country, while the mineral composition of flesh also reflects the feeding patterns of livestock [11]. The most solid source of selenium in human nutrition is a food of animal origin, especially eggs, meat and sea food. Lower levels of selenium are usually in cereal crops and the lowest levels of this element are present in fruit and vegetable [12–14].

Elementary selenium does not have any function in living organisms and it is not absorbed by gastrointestinal tract of humans and animals. All of the known effects of selenium are accomplished through the specific selenoenzymes, or selenoproteins as are for example selenocysteine, selenomethionine, or some other selenium compounds. Selenoenzymes protect cells against oxidative damage and act also the important role in the metabolic processes of the living organism through the changing of thyroid gland hormone thyroxine to its biological active form triiodothyronine. It has been proven that various selenium compounds have also protective effect against certain kinds of a cancer. In spite of long term research, the mechanism of selenium caused protective effect against cancer diseases is still not cleared [15–17]. Humans are probably not so sensitive for effect of increased amounts of selenium in comparison with animals. Irritation of eyes, nose and throat, digestive difficulties, vomiting, increased body temperature, sleepiness, psychoneurological symptoms, convulsion and death as the result of interrupted respiration are typical symptoms of acute selenium poisoning. Chronic toxicity of selenium compounds is usually linked with airways inflammation, pulmonary oedema, hemorrhage, dermatitis, dedentition, arthritis, depilation, headaches, psychoneurological symptoms and paralysis. [10, 18, 19]. A lot of studies have mentioned that effects of various toxic compounds are dependent not only on their dose but also on mutual interactions with other compounds [20–22].

## Material and methods

### Experimental animals

Experiment took place in the accredited breeding and experimental laboratory in the Department of Veterinary Disciplines of the Slovak Agricultural University. Laboratory rats (*Rattus norvegicus* sp.) had been chosen as the experimental animal. Animals were fed with the special feed pellets for laboratory mice and rats *ad libitum* and they were also given drinking water. Rats were housed in plastic cages and exposed to 12-h light : 12-h dark cycle, at room temperature of 18–22 °C.

### Chemicals

Diazinon, purity 99 %, was obtained from Sigma-Aldrich, USA. Sodium selenate(IV), purity 98 %, was purchased from the same company.

### Animal treatment schedule

Rats in the age of 135 days (weighting approximately 410 g) were randomly divided into 4 groups. Each group consisted of 10 males. Animals in the first group were administrated with diazinon (Sigma, USA) 20 mg · kg<sup>-1</sup> body weight intraperitoneally in physiological solution. Animals in the second group were administrated with selenium in the form of Na<sub>2</sub>SeO<sub>3</sub> (Sigma, USA) 2 mg Se · kg<sup>-1</sup> body weight intraperitoneally in physiological solution and animals in the third group were given a mixture

of diazinon (Sigma, USA)  $20 \text{ mg} \cdot \text{kg}^{-1}$  body weight and  $\text{Na}_2\text{SeO}_3$  (Sigma, USA)  $2 \text{ mg Se} \cdot \text{kg}^{-1}$  body weight intraperitoneally in physiological solution. The fourth group served as a control group and was administered only with the physiological solution. 24 hours after the administration of tested substances, animals were sacrificed and samples of livers, kidneys, muscles and fat tissue were taken during the autopsy.

### Determination of diazinon and selenium

The amount of diazinon in tissues was determined using gas chromatography with mass spectrometry and the amount of selenium was determined with atomic absorption spectrometry. The amount of diazinon was not analyzed in the second group (selenium treated group) because diazinon was not applied to laboratory animals in this experimental group.

### Statistical analysis

Basic statistical characteristics – arithmetic mean, standard deviation and variation coefficient were calculated for the amount of analyzed substance of each group. Obtained data were then processed in order to determine statistical significance of the results. The F-test two sample for variances was used to compare the population variances. The Student's t-test (two sample assuming equal variances) was finally used for establishment of statistical significance.

### Results and discussion

No deaths were observed in any of groups of experimental animals. However, animals from diazinon treated group approximately 12 hours after the administration of diazinon showed typical symptoms connected with depression of cholinesterase activity and did not react on external stimuli. The same behaviour of experimental animals was observed also after common administration of diazinon and selenium.

Table 1 presents distribution of selenium in different tissues that were obtained after single intraperitoneal administration of selenium. According to our findings, the highest level of selenium was found in the kidneys of experimental rats, lower amount of selenium was detected in livers of experimental rats and the lowest amount of selenium was in muscles and in samples of fat tissue. The amount of selenium in tissues of control animals was always lower, in comparison with tissues of experimental animals. The increasing of selenium amount in tissues of livers, kidneys and muscles in experimental animals was even statistically significant. Our observations (in control group and in experimental group of animals as well) are in accordance with a known fact that selenium amount is naturally highest in the kidney and lowest in the muscle [23]. These findings are easily observable from Fig. 1.

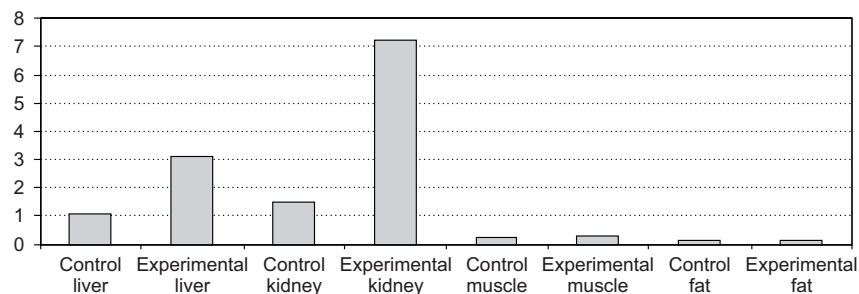


Fig. 1. Amount of selenium in different tissues after single selenium administration [mg · kg<sup>-1</sup>]

Table 1

Amount of selenium in different tissues after single selenium administration

| Tissue | Amount of selenium [mg · kg <sup>-1</sup> ] |                              |                        |                              |
|--------|---|------------------------------|------------------------|------------------------------|
|        | Control<br>X ± SD                           | Variation coefficient<br>[%] | Experimental<br>X ± SD | Variation coefficient<br>[%] |
| Liver  | 1.085 ± 0.262                               | 24.164                       | 3.105 ± 1.09*          | 35.116                       |
| Kidney | 1.527 ± 0.239                               | 15.65                        | 7.235 ± 3.995*         | 55.218                       |
| Muscle | 0.25 ± 0.027                                | 10.995                       | 0.304 ± 0.061*         | 19.931                       |
| Fat    | 0.137 ± 0.048                               | 35.267                       | 0.122 ± 0.062          | 50.94                        |

X – arithmetic mean, SD – standard deviation, \* p < 0.05.

Table 2 together with Figure 2 presents results of selenium amount in different tissues after single intraperitoneal administration of diazinon. Results show that amount of selenium in muscles and fat tissue is lower in comparison with control group. Depression of selenium amount in tissues of muscles and fat is statistically significant. This fact could be probably connected with a response of organism on pathological condition [24]. Diazinon that was administrated to the organism presumably caused a mobilization of selenium from tissues of muscle and fat. This could happen in order to protect organism of experimental animal against injury [9].

Table 2

Amount of selenium in different tissues after single diazinon administration

| Tissue | Amount of selenium [mg · kg <sup>-1</sup> ] |                              |                        |                              |
|--------|---|------------------------------|------------------------|------------------------------|
|        | Control<br>X ± SD                           | Variation coefficient<br>[%] | Experimental<br>X ± SD | Variation coefficient<br>[%] |
| Liver  | 1.085 ± 0.262                               | 24.164                       | 1.222 ± 0.25           | 20.481                       |
| Kidney | 1.527 ± 0.239                               | 15.65                        | 1.615 ± 0.213          | 13.194                       |
| Muscle | 0.25 ± 0.027                                | 10.995                       | 0.218 ± 0.03*          | 13.812                       |
| Fat    | 0.137 ± 0.048                               | 35.267                       | 0.062 ± 0.022*         | 35.5                         |

X – arithmetic mean, SD – standard deviation, \* p < 0.05.

On the other hand, the amount of selenium after common administration of selenium and diazinon decreased only in samples of fat tissue however, this depression was not

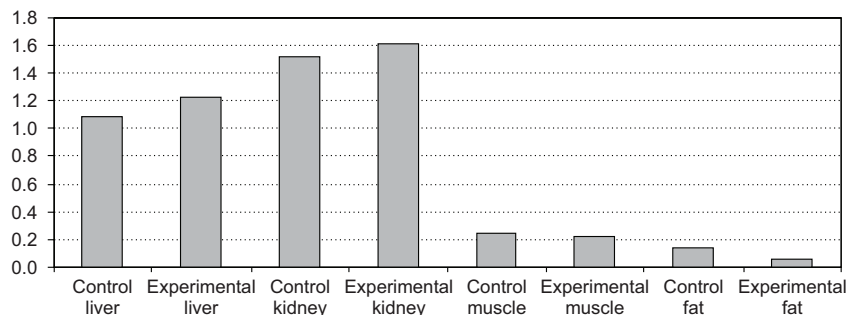


Fig. 2. Amount of selenium in different tissues after single diazinon administration [mg · kg<sup>-1</sup>]

statistically significant. In this experimental group, we observed significant increase of selenium amount in samples of livers, kidneys and muscles. It means, in our opinion, that dose of selenium was high enough to protect organism against injury [9] and selenium in tissues was not mobilized to increase its status, as it is usual in pathological conditions [24]. Selenium amount in tissues was in accordance with group of animals that was administrated only with single selenium – highest amount of selenium was in kidneys, lower amount of this element was livers and the lowest amount of selenium was in the samples of muscles and fat tissue. These observations are visible in Table 3 and Fig. 3.

Table 3

Amount of selenium in different tissues after common administration

| Tissue | Amount of selenium [mg · kg <sup>-1</sup> ] |                              |                        |                              |
|--------|---|------------------------------|------------------------|------------------------------|
|        | Control<br>X ± SD                           | Variation coefficient<br>[%] | Experimental<br>X ± SD | Variation coefficient<br>[%] |
| Liver  | 1.085 ± 0.262                               | 24.164                       | 3.098 ± 0.779*         | 25.159                       |
| Kidney | 1.527 ± 0.239                               | 15.65                        | 6.451 ± 1.966*         | 30.479                       |
| Muscle | 0.25 ± 0.027                                | 10.995                       | 0.322 ± 0.032*         | 9.908                        |
| Fat    | 0.137 ± 0.048                               | 35.267                       | 0.098 ± 0.107          | 109.67                       |

X – arithmetic mean, SD – standard deviation, \* p < 0.05.

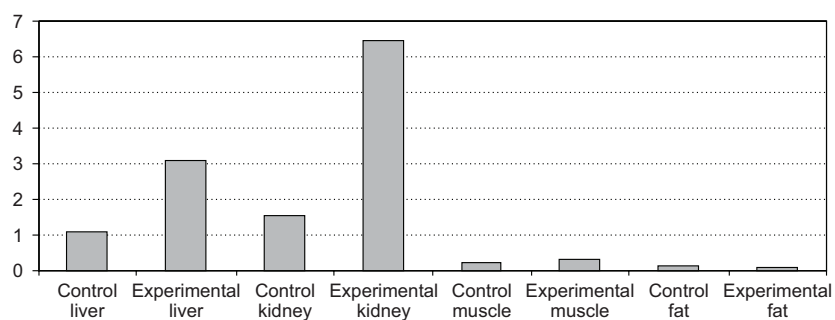


Fig. 3. Amount of selenium in different tissues after common administration [mg · kg<sup>-1</sup>]

Table 4 and Figure 4 shows diazinon amount in tissues after single administration of diazinon. Amount of diazinon in samples of livers were in all samples below the detection limit. Significant increase of diazinon was detected only in samples of fat tissue. Diazinon does not belong to substances that are characteristic by cumulation in animal organism. However, it is known, that fat tissue is able to cumulate diazinon in certain cases for relatively short period of time [6]. Our finding of diazinon amount in fat ( $3.717 \text{ mg} \cdot \text{kg}^{-1}$ ) is therefore equivalent to dose that were administrated to organism.

Table 4

Amount of diazinon in different tissues after single diazinon administration

| Tissue | Amount of diazinon [ $\text{mg} \cdot \text{kg}^{-1}$ ] |                              |                                   |                              |
|--------|---|------------------------------|-----------------------------------|------------------------------|
|        | Control<br>$X \pm \text{SD}$                            | Variation coefficient<br>[%] | Experimental<br>$X \pm \text{SD}$ | Variation coefficient<br>[%] |
| Liver  | UDT   | —                            | UDT                               | —                            |
| Kidney | UDT   | —                            | $0.072 \pm 0.036$                 | 49                           |
| Muscle | UDT   | —                            | $0.067 \pm 0.041$                 | 62                           |
| Fat    | UDT   | —                            | $3.717 \pm 3.749^*$               | 100                          |

UDT – under the detection limit, X – arithmetic mean, SD – standard deviation, \*  $p < 0.05$ .

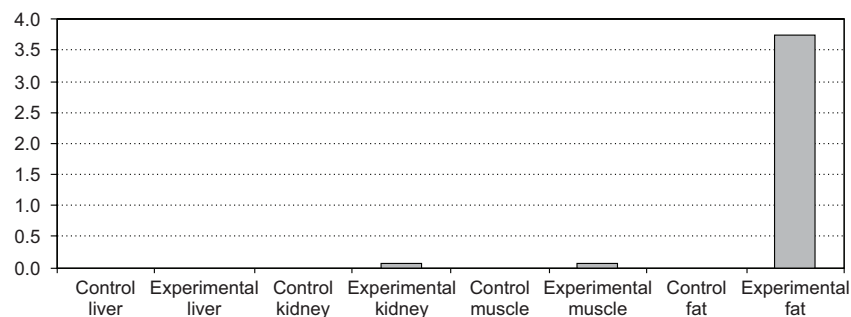


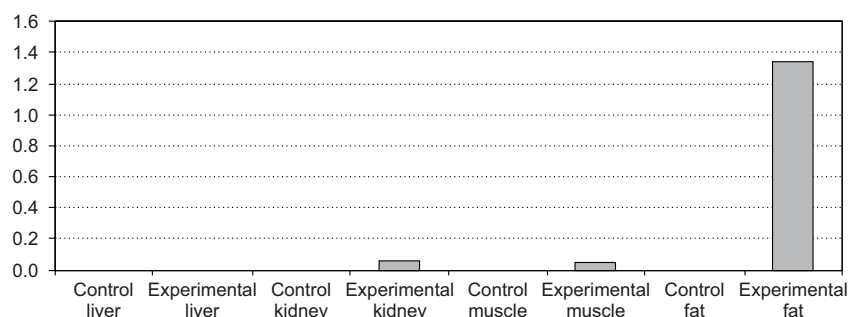
Fig. 4. Amount of diazinon in different tissues after single diazinon administration [ $\text{mg} \cdot \text{kg}^{-1}$ ]

Almost the same results were also obtained after common administration of selenium and diazinon. Results from this experimental group are presented in Table 5 and Fig. 5. The only one significant result was the increase of diazinon amount in the samples of fat tissue. However, this increase was not as high as in the previous group after single administration of diazinon. This could be caused by coadministration of selenium because selenium could act a role of protective element against diazinon. This important role of selenium was many times proven in other researches [25–27]. However, mechanisms of interaction between diazinon and selenium are still not cleared and further studies are still required

Table 5

Amount of diazinon in different tissues after common administration

| Tissue | Amount of diazinon [ $\text{mg} \cdot \text{kg}^{-1}$ ] |                              |                                   |                              |
|--------|---|------------------------------|-----------------------------------|------------------------------|
|        | Control<br>$X \pm \text{SD}$                            | Variation coefficient<br>[%] | Experimental<br>$X \pm \text{SD}$ | Variation coefficient<br>[%] |
| Liver  | UDT   | —                            | $0.0006 \pm 0.002$                | 316.228                      |
| Kidney | UDT   | —                            | $0.063 \pm 0.135$                 | 212.385                      |
| Muscle | UDT   | —                            | $0.058 \pm 0.119$                 | 204.424                      |
| Fat    | UDT   | —                            | $1.339 \pm 1.039^*$               | 77.592                       |

UDT – under the detection limit, X – arithmetic mean, SD – standard deviation, \*  $p < 0.05$ .Fig. 5. Amount of diazinon in different tissues after common administration [ $\text{mg} \cdot \text{kg}^{-1}$ ]

## Conclusions

We detected significant increase of selenium amount in livers, kidneys and muscles after single selenium administration and also after common administration of selenium and diazinon. On the other hand we detected significant decrease of selenium amount in muscles and fat tissue after single diazinon administration. We also observed slight accumulation of diazinon amount in samples of kidneys and muscles and significant increase of diazinon in fat tissue after single diazinon administration and also after common administration of both substances.

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**DYSTRYBUCJA DIAZINONU I SELENU W WYBRANYCH TKANKACH  
PO POJEDYNCZYM PODANIU DOOTRZEWNOWYM**

**Abstrakt:** Celem pracy było określenie dystrybucji dianizonu i selenu w różnych tkankach szczurów laboratoryjnych po pojedynczym dootrzewnowym podaniu. Szczury w wieku 135 dni zostały losowo przyporządkowane do 4 grup. Każda grupa składała się z 10 samców. Zwierzętom z pierwszej grupy podano dootrzewnowo dianizon w dawce  $20 \text{ mg} \cdot \text{kg}^{-1}$  masy ciała. Zwierzętom drugiej grupy podano dootrzewnowo selen w roztworze fizjologicznym ( $\text{Na}_2\text{SeO}_3$ ) w dawce  $2 \text{ Se mg} \cdot \text{kg}^{-1}$  masy ciała. Zwierzęta trzeciej grupy otrzymały dootrzewnowo mieszaninę dianizonu ( $20 \text{ mg} \cdot \text{kg}^{-1}$ ) i selenu ( $\text{Na}_2\text{SeO}_3 - 2 \text{ Se mg} \cdot \text{kg}^{-1}$ ) w roztworze fizjologicznym. Czwartą grupę stanowiły zwierzęta kontrolne, którym podano wyłącznie roztwór fizjologiczny. Po 24 godzinach od podania dianizonu i selenu zwierzęta uśmiercono, po czym pobrano próbki wątroby, nerek, mięśni i tkanki tłuszczowej. Poziom dianizonu w tkankach został zbadany przy użyciu chromatografii gazowej oraz spektrofotometrii masowej. Selen oznaczono metodą spektrofotometrii absorpcji atomowej. Stwierdzono istotny statystycznie wzrost poziomu selenu w wątrobie, nerkach i mięśniach po jednorazowym podaniu selenu, a także po podaniu mieszaniny selenu i dianizonu. Z drugiej strony odnotowano również istotny statystycznie spadek zawartości selenu w mięśniach i tkance tłuszczowej po jednorazowym podaniu dianizonu. Zaobserwowano także niewielki wzrost poziomu dianizonu w nerkach i mięśniach oraz istotny statystycznie wzrost zawartości tego związku w tkance tłuszczowej zarówno po jednorazowym podaniu dianizonu, jak i po podaniu mieszaniny dianizonu i selenu.

**Słowa kluczowe:** dianizon, selen, wątroba, nerki, mięśnie, tłuszcz, podanie dootrzewnowe