Risk Assessment — Folic Acid

General information

Chemistry

The term folate is used generically to describe the various derivatives of pteroylglutamic acid (PGA, folic acid), the common pharmaceutical and most stable form of the folate vitamins group, which is composed of three major subunits – pteridine, *p*-aminobenzoic acid, and glutamic acid.

Within this assessment, in accordance with the guidelines of the International Union of Pure and Applied Chemistry and International Union of Biochemistry and Molecular Biology (IUPAC-IUB) advisory panel, the term folic acid is used to indicate the parent compound, pteroylglutamic acid, and folate is used generically, to indicate one or a mixture of pteroylglutamates.

Natural occurrence

Folic acid (PGA) *per se* is not present in significant quantities in foods or in the human body. The derivatives of PGA which are predominantly present in the human body, and in plant- and animal-derived foods, are reduced folates, mostly 5,6,7,8-tetrahydrofolates (THF), and also 7,8-dihydrofolate (DHF). Other modifications also occur.

Occurrence in food, food supplements and medicines

Folates are present in the majority of natural foods. Liver, yeast extract, green leafy vegetables, legumes and some fruits are especially rich sources. Dietary forms are broken down to monoglutamates during storage, processing and cooking. The synthetic pharmaceutical form used for food fortification and in supplements is folic acid (PGA), as this compound is more stable in comparison to other forms of the vitamin. Folic acid is widely available as a nutritional supplement, either alone or as a component of B complex or multivitamin preparations, in daily doses of up to 0.80 mg. Preparations providing a daily dose of 5 mg are available on prescription only.

Recommended amounts

RNI for folate are 0.05, 0.07, 0.10 and 0.15 mg/day for infants and children $\leqslant$ 12 months, 1 – 3 years, 4 – 6 years and 7 – 10 years, respectively. The RNI for adults and children $\geqslant$ 11 years old is 0.200 mg/day. Recommendations to allow for increased requirements during pregnancy and lactation are increments of 0.10 and 0.060 mg/day, respectively, i.e. 0.30 mg/day for pregnant women and 0.26 mg/day for breastfeeding women. The UK Department of Health (COMA) also recommends that 'women who could become pregnant' should take 0.40 mg/day folic acid, in addition to normal dietary folate intake, and until the 12th week of pregnancy. An additional dose of 5 mg/day is recommended for women at high risk of a neural tube defect (NTD)-affected pregnancy.

Analysis of tissue levels and folate status

Folate status is usually measured by determination of serum and/or red cell folate levels, in which the predominant species is 5-methyl-THF. Serum folate is a short-term indicator of folate status; levels are normally within the range of 5-16 ng/mL (11-36 nmol/L folic acid activity). Red cell levels are more stable and reflect long term intake, levels < 140 ng/mL (317 nmol/L) indicate reduced body stores. Thus, negative folate balance is indicated by a serum folate concentration < 3 ng/mL, whilst folate deficiency is indicated by erythrocyte and liver folate levels < 120 ng/mL or 1.6 mg/kg (wet weight), respectively.

Brief overview of non-nutritional beneficial effects

Randomised controlled trials and observational studies indicate that peri-conceptional folic acid supplementation in women is associated with a significant reduction in the incidence of foetal NTDs. It has also been shown that folic acid supplementation is associated with a reduction of serum levels of homocysteine, but commensurate benefits for cardiovascular health are uncertain.

Function

Folate coenzymes within the cell are involved in one-carbon transfer reactions, including those involved in phases of amino acid metabolism, purine and pyrimidine synthesis, and the formation of the primary methylating agent, S-adenosylmethionine.

Deficiency

Folate deficiency results in reduced *de novo* DNA biosynthesis and thus, impairment of cell replication, with the most obvious effects relating to rapidly dividing cell-types, such as erythrocytes and other cells generated by the bone marrow, enterocytes and skin cells. The condition causes megaloblastic and macrocytic anaemia. Vitamin B_{12} deficiency also causes a macrocytic megaloblastic anaemia and should be excluded before folate treatment alone is given.

Nutritional folate deficiency may develop during pregnancy, infection, malignant disease, malabsorption syndromes (e.g. coeliac disease) or alcoholism, during some drug treatments and in the older people on restricted diets.

Interactions

Folic acid supplementation can interfere with a number of drugs (anti-folate drugs, drugs used to treat epilepsy, anti-inflammatory drugs). Folate, vitamin B_6 and vitamin B_{12} metabolism are linked *via* the enzyme methionine synthase (which requires vitamin B_{12} as a cofactor). Some authors have reported a negative effect of folate supplementation on zinc status. Some animal studies have suggested that iron deficiency may cause folate depletion.

Absorption and bioavailability

The majority of dietary folate is absorbed within the proximal region of the small intestine by active, carrier-dependent mechanisms, and also by passive diffusion. Polyglutamate forms are first hydrolysed to monoglutamates by conjugase (hydrolase) enzymes within the enterocyte brush border. Ingested folic acid is enzymatically reduced and methylated within the intestinal lumen and enterocytes, although ingestion of high concentrations (> 0.20-0.30 mg/meal) result in the direct appearance of the compound, unmodified, in the plasma.

The absorption of natural folate from a typical North American diet has been suggested to be in the range of 50 to 75% of the amount ingested. Synthetic folic acid, as a food fortificant or supplement is more highly bioavailable than natural food folate.

Distribution and metabolism

Absorbed folate is carried *via* the portal blood to the liver, where a proportion (approximately 0.1 mg/day) is excreted into the bile and undergoes enterohepatic circulation and reabsorption. The liver is also the main storage site, containing approximately half of the total (5-10 mg) body folate. The majority of plasma folate is present as 5-methyl-THF-monoglutamate. Within cells, folate is retained in the cytoplasm by polyglutamation. 5 Methyl-THF is not a good substrate for polyglutamation, and must be first converted, *via* a vitamin B_{12}-dependent reaction, to THF. Alternatively, folic acid can be converted to polyglutamate (i.e. metabolically active) forms *via* a vitamin B_{12}-independent pathway.

Excretion

Folate is excreted in the urine, either as the metabolically active form or as breakdown products, and in the faeces.

Toxicity

Human data

Therapeutic use

Folic acid is generally considered safe for medicinal use, even at doses of about 10-20 mg/day. 'Indirect toxicity' may occur due to folic acid reversal of the haematological signs and symptoms of vitamin B_{12} deficiency; this masking effect could allow the neuropathy associated with vitamin B_{12} deficiency to develop untreated. Vitamin B_{12} deficiency is most prevalent in older people and, in general, in those with impaired absorption. Adverse effects may, potentially, occur in specific groups, such as individuals being treated with drugs that interact with folic acid metabolism.

A small number of case reports have described hypersensitivity reactions to oral folic acid therapy (generally ⩾ 1 mg/day). One short-term, uncontrolled supplementation trial reported adverse symptoms (mental changes, sleep disturbances and gastrointestinal symptoms) in healthy volunteers given very high doses of folic acid (15 mg/day) for 1 month, but other studies have not observed similar effects.

Supplementation trials

A substantial number of supplementation studies have been carried out or are ongoing to assess the effectiveness of folic acid therapy in disease prevention (generally, either for the prevention of foetal NTD-pregnancies or cardiovascular disease, in high-risk groups). Many of these trials have shown beneficial effects associated with folic acid supplementation at levels up to 10 mg/day for periods of several weeks or months. Very few adverse effects have been reported, although the majority of studies have not specifically addressed this issue.

Trials have shown that peri-conceptional supplementation with folic acid, or folic acid-containing multivitamin supplements, is associated with a significant reduction in the incidence of foetal NTDs. Consequently, COMA recommended that, for the prevention of foetal NTDs, women who could become pregnant should take a 0.40 mg folic acid supplement daily, in addition to normal dietary folate intake. Women in high risk groups may be advised to take up to 10 times this level of supplementation. Such therapy is generally considered to be without adverse reproductive or developmental effects, although it has been noted that human trials carried out to date were not sufficiently powerful to identify rare or possibly slight adverse effects.

One large study of peri-conceptional multivitamin supplementation (including 0.8 mg/day folic acid) in Hungary (1992) showed reduced incidence of NTDs, but with a significant increase in spontaneous abortions. Thus, it has been suggested that folic acid may prevent NTDs by causing spontaneous abortion of affected foetuses. However, this theory is not supported by recent studies using a genetically-predisposed NTD mouse model, in which the administration of folic acid to embryos *in vitro* normalised neural tube development without stimulating abortion.

In two studies of the effects of folic acid supplementation in early pregnancy, statistically significantly increased numbers of multiple births were reported[14]. The effect appeared to be limited to dizygotic twinning and the theory proposed by the authors of one of the studies was that this effect is due to increased survival of multiple pregnancies, rather than increased ovulation, as multiple pregnancies have greater micronutrient requirements.

Animal data

Data from toxicological studies of folic acid in experimental animals are limited. A number of reports have described nephrotoxicity associated with the parenteral administration of extremely high doses ($\geqslant$ 75 mg/kg bw) of folic acid in rodents. Additionally, direct injection of high doses of folic acid or folates into the brain or spinal fluid has been shown to produce seizures in rats and mice. One study showed that high-dose dietary folic acid supplementation decreased the dose of pentylenetetrazol required to induce seizures in rats.

[14] However, a recent study was published after 31 December 2002, the EVM's cut-off date for studies to be included in the risk assessment, which showed no association between folic acid supplementation and risk of multiple births (Li *et al*., Lancet 2003; **361**: 380). The study was a population-based cohort study of 242,015 women, 127, 018 of whom who took 0.4 mg folic acid/day as a supplement before and/or during the first trimester of pregnancy. The relative risk for multiple births in the supplemented group was 0.91 (95% CI, 0.82-1.00).

Oral folic acid supplementation, alone has generally not shown reproductive or embryotoxic effects in animal models, although studies have shown that supplementation is associated with increased foetal folate levels. One group reported that the foetuses of rats fed diets containing approximately 2 mg/kg bw/day folic acid for 3 weeks during pregnancy showed reduced body weight and vertex-coccyx length compared with a control group of animals given a basal diet, although the validity of the reported statistical analysis is uncertain. Additionally, folic acid treatment has been reported to enhance the embryotoxic effects of certain drugs (pyrimethamine, valproic acid), and of zinc deficiency.

Carcinogenicity and genotoxicity

There are limited data to suggest that folic acid supplementation, in comparison to deficiency, may be associated with the promotion of tumours in animals that develop spontaneous tumours or are exposed to chemical carcinogens. However, this may be related to the role of folic acid in supporting cell replication. Data from *in vitro* and *in vivo* studies indicate that folic acid is not genotoxic.

Vulnerable groups

Groups vulnerable to adverse effects associated with folic acid supplementation include:

1] Individuals at risk of vitamin B_{12} deficiency (most prevalent in the older people), in whom folic acid supplementation may mask the haematological signs and symptoms of this deficiency, allowing the associated myeloneuropathy to develop.

2] Patients treated with drugs which interfere with folate metabolism, and in whom folic acid supplementation may be associated with reduced effectiveness of the therapy or increased incidence of side effects.

Genetic variations

Population groups with a genetically determined increase in susceptibility to folic acid toxicity have not been identified. In contrast, some individuals have a genetic predisposition to deficiency. Subjects homozygous for a variant of the enzyme 5,10-methylene tetrahydrofolate reductase (MTHFR) show reduced activity of this enzyme, resulting in altered cellular distribution of one-carbon units, and associated with low plasma folate status and hyperhomocysteinaemia. Defects of other enzymes involved in homocysteine metabolism (e.g. cystathionine ß-synthase, methionine synthase) may also be associated with pathology related to low folate intake/status. In addition, congenital errors of various enzymes involved in folate metabolism have been described, associated with functional folate-deficiency.

Mechanisms of toxicity

Folic acid is generally considered safe and no likely mechanisms for toxicity have been hypothesised. The metabolism of folate and vitamin B_{12} are linked by the enzyme methionine synthase, which is vitamin B_{12} dependent and results in polyglutamate synthesis. High levels of folate can result in the production of polyglutamates by a vitamin B_{12} independent mechanism, which may reverses the megaloblastic anaemia caused by vitamin B_{12} deficiency. This complicates the diagnosis of vitamin B_{12} deficiency and allows the neurological damage associated with it to continue.

Dose response characterisation

Few systematic data exist regarding the level of folate intake required to mask vitamin B_{12} deficiency (Koehler *et al.*, 1997). The majority of available information relates to early case reports of folic acid therapy for the treatment of pernicious anaemia (mostly high doses, for example $\geqslant$ 5 mg/day) (Heinle *et al.*, 1947; Vilter *et al.*, 1947; Bethell *et al.*, 1948; Ross *et al.*, 1948; Vilter *et al.*, 1950; Will *et al.*, 1959; Schwartz *et al.*, 1950; Ellison and Curry, 1960; Marshall and Jandl, 1960; Baldwin *et al.*, 1961; Hansen & Weinfeld, 1962; Vilter *et al.*, 1963). In general, data taken from these reports suggest that supplementation with $\leqslant$ 1 mg/day folic acid does not mask vitamin B_{12}-associated anaemia in the majority of subjects. The effects of doses of between 1 and 5 mg/day are unclear (*cited by* Chanarin, 1994; Bower & Wald, 1995). Supplementation with $\geqslant$ 5 mg/day folic acid is reported to reverse the haematological signs of vitamin B_{12}-deficiency in at least 50% of subjects (discussed by Chanarin, 1994; Bower & Wald, 1995; Savage & Lindenbaum, 1995).

Studies of particular importance in the risk assessment

(For full review see http://www.food.gov.uk/science/ouradvisors/vitandmin/evmpapers or the enclosed CD).

Human data

Weissberg et al., 1950

This was a non-controlled study of the neurological effects of 20 mg/day folic acid supplementation for 6-12 months, in 26 normal volunteers and 22 patients with non-pernicious anaemia. Prior to therapy, 6 of the normal subjects and 7 of the anaemic subjects showed some abnormal neurological signs (but not those of subacute combined spinal cord degeneration), which were not significantly altered during the therapy. Four subjects (1 normal, 3 anaemic) developed central nervous system changes during the folic acid treatment, but these changes were not considered to be related to the therapy.

Harvey et al., 1950

In this open, uncontrolled study, oral folic acid supplementation (20 mg/day for 3 – 12 months) produced no indications of spinal cord or peripheral nerve damage in 40 healthy subjects without pernicious anaemia (13 subjects had mild hypochromic anaemia).

MRC Vitamin Study Research Group, 1991

This was a multicentre, randomised, double-blind, placebo-controlled trial carried out in the UK, in which a total of 1817 women with a previous NTD-affected pregnancy were assigned to 1 of 4 supplementation groups:

A] 4 mg/day folic acid

B] 4 mg/day folic acid + multivitamins (daily – 4000 IU vitamin A, 400 IU vitamin D, 1.5 mg each vitamins B_1 and B_2, 1.0 mg vitamin B_6, 40 mg vitamin C, 15 mg nicotinamide)

C] placebo

D] multivitamins (as B], without folic acid)

The duration of therapy was from the date of randomisation until the 12th week of pregnancy. Statistical analysis showed a significantly reduced relative risk for NTDs associated with folic acid supplementation compared with no folic acid supplementation, i.e. groups A and B compared with groups C and D (RR = 0.28; 95% CI, 0.12-0.71). The authors also reported that possible adverse effects of folic acid to the foetus and the mother were examined. The authors concluded that there was no demonstrable harm from the folic acid supplementation, although the ability of the study to detect rare or slight adverse effects was limited. The incidences of general side effects (e.g. infertility, irregular menses, vomiting in pregnancy, upper respiratory illness) reported by women taking part in the trial were similar in all 4 groups.

Animal data

Chung et al., 1993

In a study of the synergistic effects of folic acid and the anti-malarial drug, pyrimethamine (an inhibitor of dihydrofolate reductase), groups of 10 pregnant female rats were supplemented with combinations of the anti-malarial drug, pyrimethamine, and/or folic or folinic acid, from days 7 – 17 of gestation, by gavage. Folic acid treatment (50 mg/kg bw/day) alone showed no significant maternal or embryotoxicity, as compared with vehicle-only treatment.

Exposure assessment

Total exposure/intake:

Food	Mean: 0.26 mg/day 97.5th percentile: 0.49 mg/day (1986/87 NDNS)
Supplements	up to 0.50 mg in OTC supplements for males (OTC, 2001) up to 0.80 mg in OTC supplements for females (Annex 4)
Estimated maximum intake:	0.99 mg/day for males 1.29 mg/day for females

No potential high intake groups have been identified.

Implications of food fortification of wheat flour

During the 1990s, the public health policy with regard to preventing NTDs aimed to ensure that women of childbearing age were aware of the importance of acquiring sufficient folic acid in the diet at the time of conception to minimise the risk of NTDs in their offspring. In the UK, as in the US, there have been calls for more active public policy, including food fortification to address this. The Committee on Medical Aspects of Food and Nutrition Policy (COMA) reviewed the links between folates, including folic acid, and disease (COMA, 2000), and:

- confirmed the link between low folate status and the risk of NTDs
- concluded that there is insufficient evidence on which to establish a conclusive link with cardiovascular disease

- acknowledged that increasing the intake of folic acid might pose a risk to people with undiagnosed vitamin B_{12} deficiency, particularly older people, and advised clinical vigilance to avoid any delays in diagnosis
- recommended that the current policy of encouraging women who could become pregnant to take 0.40 mg folic acid as a supplement should continue

COMA further concluded that universal fortification of flour at 0.24 mg per 100 g in food products as consumed would result in:

- a significant effect in preventing NTD-affected births and pregnancies without resulting in unacceptably high intakes in any group of the population. It is estimated that this would reduce the incidence of NTD-affected pregnancies by 41% and would have, for example, prevented 38 of the 93 NTD-affected births in England and Wales in 1998, 30 of the 74 in Scotland in 1997, and 6 of the 14 in Northern Ireland in 1998
- The average intake of folic acid of women aged 16-45 years would increase by 0.201 mg/day, leading to a total folate intake of 0.405 mg/day
- Approximately 7% of women in this age group would have total folate intakes in excess of 0.600 mg/day
- Approximately 0.6% of people aged over 50 years would be exposed to levels of folic acid intake greater than 1 mg/day

Risk assessment

Folic acid is generally considered as safe in therapeutic use. Adverse effects may, potentially, occur in specific groups, such as individuals being treated with drugs that interact with folic acid metabolism. Women at risk of a NTD-affected pregnancy appear to be able to take folate supplements at up to 4 mg/day, without adverse reproductive or developmental effects.

Folic acid may lead to reversal of the symptoms of vitamin B_{12} deficiency, potentially allowing the neuropathy associated with vitamin B_{12} deficiency to develop untreated. Vitamin B_{12} deficiency is most prevalent in older people.

Few data are available from toxicological studies of folates in animals.

ESTABLISHMENT OF GUIDANCE LEVEL

There are insufficient data from animal or human studies to establish a Safe Upper Level for folic acid.

COMA recommended that, for the prevention of foetal NTDs, women who could become pregnant should take a 0.40 mg folic acid supplement daily, in addition to normal dietary folate intake, until the 12th week of pregnancy. Women in high risk groups may be advised to take up to 10 times this level of supplementation.

We are aware of evidence that increased folate acid intake lowers serum homocysteine levels and may potentially help prevent adverse cardiovascular events. However, consideration of such effects is not within the remit of the EVM. We are also aware of recent studies which have suggested that increased folate intake may increase the incidence of multiple births. We note that this is an area of potential concern, but that there is currently no substantive evidence for such an effect.

The main concern regarding ingestion of excess folic acid is the consequential masking of vitamin B_{12} deficiency. A general consistency of data indicates that supplementation with ⩽ 1 mg/day folic acid does not mask vitamin B_{12}-associated anaemia in the majority of subjects, whereas supplementation with ⩾ 5 mg/day folic acid does. The effects of doses of between 1 and 5 mg/day are unclear. No other significant adverse effects have been associated with ingestion of folic acid.

For guidance purposes only, in the general population a supplemental dose of 1 mg/day (equivalent to 0.017 mg/kg bw/day in a 60 kg adult) would not be expected to cause adverse effects. Because of the consistency of the data, from a large number of studies in humans, no uncertainty factors have been applied. Assuming a maximum intake from food of approximately 0.49 mg/day, a total dose of 1.5 mg/day (equivalent to 0.025 mg/kg bw/day in a 60 kg adult) would not be expected to have any adverse effects.

References

Baldwin, J.N., Dalessio, D.J. (1961). Folic acid therapy and spinal-cord degeneration in pernicious anemia. *New England Journal of Medicine* **264**, 1339-1342

Bethell, F.H., Sturgis, C.C. (1948). The relation of therapy in pernicious anemia to changes in the nervous system: early and late results in a series of cases observed for a period of not less than 10 years and early results with folic acid. *Blood* **3**, 57-67

Bower, C., Wald, N.J. (1995). Vitamin B_{12} deficiency and the fortification of food with folic acid. *European Journal of Clinical Nutrition* **49**, 787-793

Chanarin, I. (1994). Adverse effects of increased dietary folate. Relation to measures to reduce the incidence of neural tube defects. *Clinical and Investigative Medicine* **17**, 244-252

Chung, M.K., Han, S.S., Roh, J.K. (1993). Synergistic embryotoxicity of combination pyrimethamine and folic acid in rats. *Reproductive Toxicology* **7**, 463-468

COMA (2000). Department of Health. Report on Health and Social Subjects **50**. *Folic Acid and the Prevention of Disease.* The Stationery Office. London

Ellison, A.B., Curry, A.B. (1960). Pernicious anemia masked by multivitamins containing folic acid. *Journal of the American Medical Association* **173**, 240-243

Hansen, H.A., Weinfeld, A. (1962). Metabolic effects and diagnostic value of small doses of folic acid and B_{12} in megaloblastic anemias. *Acta Medica Scandinavia* **172**, 427-443

Harvey, E.A., Howard, I., Murphy, W.P. (1950). Absence of a toxic effect of folic acid on the central nervous system of persons without pernicious anemia. *New England Journal of Medicine* **242**, 446-447

Heinle, R.W., Welch, A.D. (1947). Folic acid in pernicious anemia: failure to prevent neurologic relapse. *Journal of the American Medical Association* **133**, 739-741

Koehler, K.M., Pareo-Tubbeh, S.L., Romero, L.J., Baumgartner, R.N., Garry, P.J. (1997). Folate nutrition and older adults: challenges and opportunities. *Journal of the American Dietetics Association* **97**, 167-173

Marshall, R.A., Jandl, J.H. (1960). Responses to 'physiologic' doses of folic acid in the megaloblastic anemias. *Archives of Internal Medicine* **105**, 352-360

MRC Vitamin Study Research Group (1991). Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. *Lancet* **338**, 131-137

Ross, M.D., Belding, M.D., Paegel, B.L. (1948). The development and progression of subacute combined degeneration of the spinal cord in patients with pernicious anemia treated with synthetic pteroylglutamic (folic) acid. *Blood* **3**, 68-90

Savage, D.G., Lindenbaum, J. (1995). Folate-cobalamin interactions. In: Bailey, L.B. ed. *Folate in Health and Disease*. Marcel Dekker Inc, New York, pp. 237-285

Schwartz, S.O., Kaplan, S.R., Armstrong, B.E. (1950). The long-term evaluation of folic acid in the treatment of pernicious anemia. *Journal of Laboratory and Clinical Medicine* **35**, 894-898

Vilter, R.W., Vilter C.F., Hawkins, R. (1947). Combined system disease and hematologic relapse occurring in persons with pernicious anaemia treated with synthetic folic acid (pteroyl-glutamic acid) for a period of two years. *Journal of Laboratory and Clinical Medicine* **32**: 1426

Vilter, R.W., Horrigan, D., Mueller, J.F., Jarrold, T., Vilter, C.F., Hawkins, V., Seaman, A. (1950). Studies on the relationships of vitamin B_{12}, folic acid, thymine, uracil, and methyl group donors in persons with pernicious anemia and related megaloblastic anemias. *Blood* **5**, 695

Vilter, R.W., Will, J.J., Wright, T., Rullman, D. (1963). Interrelationships of vitamin B_{12}, folic acid, and ascorbic acid in the megaloblastic anemias. *American Journal Clinical Nutrition* **12**, 130-144.

Weissberg, J., McGavack, T.H., Vogel, M., Kenigsberg, S. (1950). The effect of folic acid on the central nervous system of normal subjects and subjects with anemia other than pernicious anemia. *Blood* **5**, 148-154.

Will, J.J., Mueller, J.F., Brodine, C., Kiely, C.E., Firedman, B., Hawkins, B.R., Dutra, J., Vilter, R.W. (1959). Folic acid and vitamin B_{12} in pernicious anemia: studies on patients treated with these substances over a ten-year period. *Journal of Laboratory and Clinical. Medicine* **53**, 22-38.