Scientific Colloquium Series

EFSA Scientific Colloquium n°11

Acrylamide carcinogenicity – new evidence in relation to dietary exposure

Tabiano (province of Parma), Italy, 22-23 May 2008

BRIEFING NOTES FOR DISCUSSION GROUPS

In 2005, the Joint FAO/WHO expert committee on food additives (JECFA) carried out a risk assessment of acrylamide in food following a FAO/WHO expert consultation on this topic in 2002. The EFSA´s Scientific Panel on Contaminants in the Food Chain (CONTAM) concurred with the principle conclusions and recommendations made by the JECFA and did not see the need for an additional evaluation by EFSA at that time. Additional information has become available since 2005, including epidemiological studies of cancer risk associated with dietary exposure to acrylamide.

In relation to previous risk assessments carried out by international bodies and new scientific information published since, the objectives of the Colloquium are:

- **To discuss in an open scientific debate the state of the art, current issues and future challenges for the risk assessment of acrylamide in food in relation to its carcinogenicity and dietary exposure.**
- **To discuss the epidemiological evidence relating acrylamide exposure to cancer risk in humans including discussions on uncertainties.**
- **To discuss the applications of biomarkers for acrylamide and physiologically-based pharmacokinetic models in relation to exposure, metabolism and elimination (toxicokinetics) and the mode of action (toxicodynamics) of acrylamide in experimental animals and humans.**
- **To discuss the state of the art on genotoxic and non-genotoxic mechanisms for the carcinogenicity of acrylamide including new *in vitro/in vivo* evidence in experimental animals and humans.**
- **To discuss the current knowledge on dietary exposure to acrylamide across Europe and to explore if there are possibly new potential food sources contributing to the dietary intake**

- **To explore whether the new evidence in epidemiology, carcinogenicity and exposure would call for a revision of the previous risk assessment of acrylamide in food.**

These briefing notes have been prepared to stimulate an open interactive exchange of views and expertise on the scientific aspects of acrylamide carcinogenicity and the new evidence in relation to dietary exposure. Focus should be on the risk assessment methodology and, in particular, on quantitative and qualitative risk assessment.

GENERAL BACKGROUND DOCUMENTS

U.S. Environmental Protection Agency (2007) Toxicological review of acrylamide (CAS No. 79-06-1). Available at URL: http://oaspub.epa.gov/eims/eimsapi.dispdetail?deid=187729

Acrylamide. Monograph prepared for the 64th JECFA meeting. WHO Food Additives Series 55 (2006). Available at URL: http://whqlibdoc.who.int/publications/2006/9241660554_ACR_eng.pdf.

EFSA(2005)- Statement of the scientific panel on contaminants in the food chain to a summary report on acrylamide in food of the 64th meeting of the Joint FAO/WHO Commitee on Food Additives (JECFA). Available at URL: http://www.efsa.europa.eu/EFSA/Statement/contam_acrylamide_statement1,0.pdf

FAO/WHO (Food and Agricultural Organisation/World Health Organisation), 2005. Summary and conclusions of the sixty-forth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), pp. 7-17. Available at URL: http://www.who.int/ipcs/food/jecfa/summaries/en/summary_report_64_final.pdf

EC (European Commission), 2002. Opinion of the Scientific Committee on Food (SCF) on new findings regarding the presence of acrylamide in food. Available at URL : http://europa.eu.int/comm/food/fs/sc/scf/out131_en.pdf

Health Implications of Acrylamide in Food. Report of the FAO/WHO Consultation in 2002. Available at URL: http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPdf/Acrylamide.pdf

DISCUSSION GROUP 1 - *Epidemiological studies – evaluating evidence and addressing uncertainties*

INTRODUCTION

Over the last few years, a number of research groups from around the world have published data from epidemiological studies in relation to dietary exposure from acrylamide and human cancer risk in different target organs (kidney, bladder, endometrium, ovaries, breast). Key aspects in establishing such relationships are; the power of the studies to detect effects, how the exposure assessment was carried out, how well can food frequency questionnaires (and other diet assessment methods) capture dietary acrylamide intake (several studies comparing FFQ-assessed acrylamide intake with biomarkers of acrylamide exposure are now available); what kind of statistical tools have been applied and what are the confounding variables. It is important to review the evidence and to identify whether the methodologies applied in various epidemiological studies are comparable and consistent, and if uncertainties have been take into account.

DISCUSSION POINTS

1. Review the epidemiological evidence relating acrylamide dietary exposure and cancer risk.
2. Review the methodology used for the exposure assessment and if there is comparability between studies
3. Establish whether the statistical approaches are consistent between studies and review the sources of uncertainty (exposure, food consumption, confounding variables)
4. Review evidence on the validity of questionnaire-based acrylamide intake assessments
5. Discuss the power of the studies to detect effects
6. Discuss whether from the body of evidence conclusions can be drawn on the direct relationship between acrylamide dietary exposure and increased cancer risk in humans.

BACKGROUND DOCUMENTS

Acrylamide and Cancer: recent epidemiological evidence

Hogervorst,J.G., Schouten,L.J., Konings,E.J., Goldbohm,R.A., and van den Brandt,P.A. 2008. Dietary acrylamide intake and the risk of renal cell, bladder, and prostate cancer. Am.J.Clin.Nutr. 87, 1428-1438.

Olesen,P.T., Olsen,A., Frandsen,H., Frederiksen,K., Overvad,K., and Tjonneland,A. 2008. Acrylamide exposure and incidence of breast cancer among postmenopausal women in the Danish Diet, Cancer and Health Study. Int.J.Cancer 122, 2094-2100.

Kutting,B., Uter,W., and Drexler,H. 2008. The association between self-reported acrylamide intake and hemoglobin adducts as biomarkers of exposure. Cancer Causes Control 19, 273-281.

Wirfält, E., Paulsson, B., Törnqvist, M., Axmon, A., Hagmar, L.2008. Associations between estimated acrylamide intakes, and hemoglobin AA adducts in a sample from the Malmö Diet and Cancer cohort. Eur J Clin Nutr.62, 314-23.

Becher,G. 2007. Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. Toxicol.Sci. 98, 110-117.

Hogervorst,J.G., Schouten,L.J., Konings,E.J., Goldbohm,R.A., and van den Brandt,P.A. 2007. A prospective study of dietary acrylamide intake and the risk of endometrial, ovarian, and breast cancer. Cancer Epidemiol.Biomarkers Prev. 16, 2304-2313.

Pelucchi,C., Galeone,C., Dal,M.L., Talamini,R., Montella,M., Ramazzotti,V., Negri,E., Franceschi,S., and La,V.C. 2007. Dietary acrylamide and renal cell cancer. Int.J.Cancer 120, 1376-1377.

Pelucchi,C., Galeone,C., Levi,F., Negri,E., Franceschi,S., Talamini,R., Bosetti,C., Giacosa,A., and La,V.C. 2006. Dietary acrylamide and human cancer. Int.J.Cancer 118, 467-471.

Mucci,L.A., Adami,H.O., and Wolk,A. 2006. Prospective study of dietary acrylamide and risk of colorectal cancer among women. Int.J.Cancer 118, 169-173.

Mucci,L.A., Sandin,S., Balter,K., Adami,H.O., Magnusson,C., and Weiderpass,E. 2005. Acrylamide intake and breast cancer risk in Swedish women. JAMA 293, 1326-1327.

Mucci,L.A. and Adami,H.O. 2005. The role of epidemiology in understanding the relationship between dietary acrylamide and cancer risk in humans. Adv.Exp.Med.Biol. 561, 39-47.

Beer,M.U., Schlatter,J., Dudler,V., and Zoller,O. 2004. Fried potatoes and human cancer. Int.J.Cancer 108, 634-635.

Bjellaas,T., Olesen,P.T., Frandsen,H., Haugen,M., Stolen,L.H., Paulsen,J.E., Alexander,J., Lundanes,E., and Erdreich,L.S. and Friedman,M.A. 2004. Epidemiologic evidence for assessing the carcinogenicity of acrylamide. Regul.Toxicol.Pharmacol. 39, 150-157.

Konings,E.J., Baars,A.J., van Klaveren,J.D., Spanjer,M.C., Rensen,P.M., Hiemstra,M., van Kooij,J.A., and Peters,P.W. 2003. Acrylamide exposure from foods of the Dutch population and an assessment of the consequent risks. Food Chem.Toxicol. 41, 1569-1579.

Mucci,L.A., Dickman,P.W., Steineck,G., Adami,H.O., and Augustsson,K. 2003. Dietary acrylamide and cancer of the large bowel, kidney, and bladder: absence of an association in a population-based study in Sweden. Br.J.Cancer 88, 84-89.

Pelucchi,C., Franceschi,S., Levi,F., Trichopoulos,D., Bosetti,C., Negri,E., and La,V.C. 2003. Fried potatoes and human cancer. Int.J.Cancer 105, 558-560.

Ruden,C. 2004. Acrylamide and cancer risk--expert risk assessments and the public debate. Food Chem.Toxicol. 42, 335-349.

Mucci,L.A., Lindblad,P., Steineck,G., and Adami,H.O. 2004. Dietary acrylamide and risk of renal cell cancer. Int.J.Cancer 109, 774-776.

DISCUSSION GROUP 2 – *Biomarkers – new insights in exposure and mode of action*

INTRODUCTION

The characterisation of acrylamide metabolism has been the basis for the development of biomarkers. Acrylamide metabolism follows two basic routes: (1) CYP2E1- mediated epoxidation to glycidamide (GA) which is then conjugated by glutathione to *N*-acetyl-*S*-(2-(hydroxyl-ethyl-)carbamoylethyl) cystein (GAMA) and *N*-acetyl-*S*-(1-(hydroxyl-ethyl-)carbamoylethyl) or further metabolized by epoxide hydrolase to glyceramide; (2) Direct conjugation of acrylamide with glutathione to form the urinary metabolites *N*-acetyl-*S*-(3-amino-3-oxypropyl) cysteine and *N*-acetyl-*S*-(2-carbamoylethyl) cysteine (AAMA). However, only free unchanged glycidamide is thought to account for the genotoxicity of acrylamide by formation of DNA adducts and since the early 1990s, haemoglobin adducts of acrylamide have been used to reflect long term exposure to acrylamide since erythrocytes have a half life of 120 days. More recently, the mercapturic acid metabolites of acrylamide and GA have been quantified in human urine to reflect biomarkers of short term exposure with half-lives ranging from hours up to a few days. Physiologically based pharmacokinetic models (PB-PK) have also been developed for acrylamide, GA and the glutathione conjugates of acrylamide and liver GA-DNA adducts and hemoglobin adducts have been included as pharmacodynamic components of the model.

DISCUSSION POINTS

1. Discuss new insights into species differences in the kinetics of acrylamide
2. Review the current state of the art on the knowledge on acrylamide's biomarkers in relation to exposure and effects and whether some biomarkers provide better estimates than others.
   - Discuss the use of haemoglobin adducts and DNA adducts as biomarkers
   - Discuss the use of urinary metabolites (acrylamide and glycidamide) as biomarker
3. Review the available physiologically based pharmacokinetic models
4. Impact of these biomarkers on the risk assessment (both for exposure and the mode of action)

BACKGROUND DOCUMENTS

Settels,E., Bernauer,U., Palavinskas,R., Klaffke,H.S., Gundert-Remy,U., and Appel,K.E. 2008. Human CYP2E1 mediates the formation of glycidamide from acrylamide. Arch.Toxicol. [Epub ahead of print]

Kutting,B., Uter,W., and Drexler,H. 2008. The association between self-reported acrylamide intake and hemoglobin adducts as biomarkers of exposure. Cancer Causes Control 19, 273-281.

Bjellaas,T., Olesen,P.T., Frandsen,H., Haugen,M., Stolen,L.H., Paulsen,J.E., Alexander,J., Lundanes,E., and Becher,G. 2007. Comparison of estimated dietary intake of acrylamide with hemoglobin adducts of acrylamide and glycidamide. Toxicol.Sci. 98, 110-117.

Bjellaas,T., Olstorn,H.B., Becher,G., Alexander,J., Knutsen,S.H., and Paulsen,J.E. 2007. Urinary metabolites as biomarkers of acrylamide exposure in mice following dietary crisp bread administration or subcutaneous injection. Toxicol.Sci. 100, 374-380.

Bjellaas,T., Stolen,L.H., Haugen,M., Paulsen,J.E., Alexander,J., Lundanes,E., and Becher,G. 2007. Urinary acrylamide metabolites as biomarkers for short-term dietary exposure to acrylamide. Food Chem.Toxicol. 45, 1020-1026.

Doerge,D.R., Twaddle,N.C., Boettcher,M.I., McDaniel,L.P., and Angerer,J. 2007. Urinary excretion of acrylamide and metabolites in Fischer 344 rats and B6C3F(1) mice administered a single dose of acrylamide. Toxicol.Lett. 169, 34-42.

Walker,K., Hattis,D., Russ,A., Sonawane,B., and Ginsberg,G. 2007. Approaches to acrylamide physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences. J.Toxicol.Environ.Health A 70, 2033-2055.

Young,J.F., Luecke,R.H., and Doerge,D.R. 2007. Physiologically based pharmacokinetic/pharmacodynamic model for acrylamide and its metabolites in mice, rats, and humans. Chem.Res.Toxicol. 20, 388-399.

Vesper,H.W., Bernert,J.T., Ospina,M., Meyers,T., Ingham,L., Smith,A., and Myers,G.L. 2007. Assessment of the relation between biomarkers for smoking and biomarkers for acrylamide exposure in humans. Cancer Epidemiol.Biomarkers Prev. 16, 2471-2478.

Boettcher,M.I., Bolt,H.M., and Angerer,J. 2006. Acrylamide exposure via the diet: influence of fasting on urinary mercapturic acid metabolite excretion in humans. Arch.Toxicol. 80, 817-819.

Boettcher,M.I., Bolt,H.M., Drexler,H., and Angerer,J. 2006. Excretion of mercapturic acids of acrylamide and glycidamide in human urine after single oral administration of deuterium-labelled acrylamide. Arch.Toxicol. 80, 55-61.

Fennell,T.R., Sumner,S.C., Snyder,R.W., Burgess,J., and Friedman,M.A. 2006. Kinetics of elimination of urinary metabolites of acrylamide in humans. Toxicol.Sci. 93, 256-267.

Fuhr,U., Boettcher,M.I., Kinzig-Schippers,M., Weyer,A., Jetter,A., Lazar,A., Taubert,D., Tomalik-Scharte,D., Pournara,P., Jakob,V., Harlfinger,S., Klaassen,T., Berkessel,A., Angerer,J., Sorgel,F., and Schomig,E. 2006. Toxicokinetics of acrylamide in humans after ingestion of a defined dose in a test meal to improve risk assessment for acrylamide carcinogenicity. Cancer Epidemiol.Biomarkers Prev. 15, 266-271.

Ghanayem, B.I., McDaniel, L.P., Churchwell, M.I., Twaddle, N.C., Snyder, R., Fennell, T.R., Doerge, D.R.2005 Role of CYP2E1 in the epoxidation of acrylamide to glycidamide and formation of DNA and hemoglobin adducts. Toxicol Sci. 88, 311-318.

DISCUSSION GROUP 3 – *Mechanism of carcinogenicity*

INTRODUCTION

Acrylamide exposure has been shown to increase incidences of thyroid, adrenal medulla, and testicular mesothelium neoplasia in male rats, and in thyroid, adrenal medulla, and mammary gland neoplasia in female rats. The rat thyroid follicular cell tumours and the mammary tumours from two studies were considered of possible relevance for human health and benchmark doses and benchmark dose lower confidence limits (BMDL) have been determined. Overall, genotoxic and non-genotoxic modes of action of acrylamide and its metabolites have been proposed: Although acrylamide is mostly negative for mutagenicity in prokaryotic cells, it increases chromosomal aberrations, sister chromatid exchanges, unscheduled DNA synthesis, DNA breaks and deletions, cell transformation, and mitotic disruption in mammalian cells. GA has been shown to be mutagenic and genotoxic in various *in vitro* and *in vivo* test systems. Conjugation of acrylamide with glutathione can result in depletion of cellular glutathione stores, thereby, changing the redox status of the cell which can increase cellular oxidative stress and potentially affect gene expression directly or through regulating various redox-dependent transcription factors. Consequently, cell transformation or proliferation and apoptosis might occur independently of acrylamide induced genotoxicity. Another non genotoxic mechanism of action of acrylamide refers to its hormonal mode of action in rat endocrine (thyroid) and mammary glands. Another recent report described the first evidence of acrylamide and GA inhibition of a mitotic/meiotic motor protein and was speculated as an alternative mechanism to DNA adduction in the production of cell division defects and potential carcinogenicity.

DISCUSSION POINTS

1. Review the recent evidence for the mutagenicity and genotoxicity of acrylamide and glycamide
2. Review the recent evidences for the non- genotoxic mechanism of acrylamide (and glycamide)
3. Weight the evidence as to whether AA acts via a non-genotoxic or genotoxic mechanism in contrast to its genotoxic metabolite.
4. Exploration of the consequences of changed conclusions about genotoxic versus non-genotoxic mechanism of carcinogenesis for human risk assessment.

BACKGROUND DOCUMENTS

Chatzizacharias,N.A., Kouraklis,G.P., and Theocharis,S.E. 2008. Disruption of FAK signaling: a side mechanism in cytotoxicity. Toxicology 245, 1-10.

Jin,L., Chico,G., V, Massart,C., Gervy,C., De,M., V, Friedman,M., and Van,S.J. 2008. Acrylamide does not induce tumorigenesis or major defects in mice in vivo. J.Endocrinol. [Epub ahead of print]

Lamy,E., Volkel,Y., Roos,P.H., Kassie,F., and Mersch-Sundermann,V. 2008. Ethanol enhanced the genotoxicity of acrylamide in human, metabolically competent HepG2 cells by CYP2E1 induction and glutathione depletion. Int.J.Hyg.Environ.Health 211, 74-81.

Sumizawa,T. and Igisu,H. 2008. Release of heat shock proteins from human neuroblastoma cells exposed to acrylamide. J.Toxicol.Sci. 33, 117-122.

Mei,N., Hu,J., Churchwell,M.I., Guo,L., Moore,M.M., Doerge,D.R., and Chen,T. 2008. Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells. Food Chem.Toxicol. 46, 628-636.

Besaratinia, A. and Pfeifer,G.P. 2007. A review of mechanisms of acrylamide carcinogenicity. Carcinogenesis 28, 519-528.

Olstorn,H.B., Paulsen,J.E., and Alexander,J. 2007. Effects of perinatal exposure to acrylamide and glycidamide on intestinal tumorigenesis in Min/+ mice and their wild-type litter mates. Anticancer Res. 27, 3855-3864.

Sickles,D.W., Sperry,A.O., Testino,A., and Friedman,M. 2007. Acrylamide effects on kinesin-related proteins of the mitotic/meiotic spindle. Toxicol.Appl.Pharmacol. 222, 111-121.

Carere,A. 2006. Genotoxicity and carcinogenicity of acrylamide: a critical review. Ann.Ist.Super.Sanita 42, 144-155.

Klaunig,J.E. and Kamendulis,L.M. 2005. Mechanisms of acrylamide induced rodent carcinogenesis. Adv.Exp.Med.Biol. 561, 49-62.

DISCUSSION GROUP 4 - *Dietary exposure across Europe - current situation*

INTRODUCTION

Since 2003, European Union Member States have been submitting occurrence data for acrylamide in food commodities to the Joint Research Centre (JRC) of the European Commission. The submission of the data to the JRC from member states, was done through official food control laboratories directly or via their Competent Authorities, and from the food industry on a voluntary basis. The database and the reliability of the data will be discussed with special regards to the analytical techniques, their sensitivity and specificity used to report acrylamide concentrations in food commodities and whether member states report the data consistently. In 2007, the Commission made recommendations to monitor levels of acrylamide in certain food categories. (2007/331/EC), these data will be reported to EFSA by member states on a yearly basis for the next three years (EC 2007). The content of acrylamide in different food commodities will also be discussed to establish food sources which contribute most to acrylamide exposure..

DISCUSSION POINTS

1. Data reliability with regards to sensitivity of the analytical techniques and consistency of the data reported by the member states, including new analytical techniques
2. Review the occurrence data for acrylamide in food commodities available in Europe. Is there a need to revisit the exposure assessment?
3. Which food commodities contribute most to acrylamide exposure – possibility and efficacy of mitigation measures?
4. Recommendations to improve data collection and data assessment in the future.

BACKGROUND DOCUMENTS

Alpmann,A. and Morlock,G. 2008. Rapid and sensitive determination of acrylamide in drinking water by planar chromatography and fluorescence detection after derivatization with dansulfinic acid. J.Sep.Sci. 31, 71-77.

Mizukami,Y., Sawai,Y., and Yamaguchi,Y. 2008. Changes in the concentrations of acrylamide, selected odorants, and catechins caused by roasting of green tea. J.Agric.Food Chem. 56, 2154-2159.

Palazoglu,T.K. and Gokmen,V. 2008. Development and experimental validation of a frying model to estimate acrylamide levels in French fries. J.Food Sci. 73, E109-E114.

Skog,K., Viklund,G., Olsson,K., and Sjoholm,I. 2008. Acrylamide in home-prepared roasted potatoes. Mol.Nutr.Food Res. 52, 307-312.

EC (2007) Commision Recommendation of 3 May 2007 on the monitoring of acrylamide levels in food http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:123:0033:0040:EN:PDF

Wenzl,T. and Anklam,E. 2007. European Union database of acrylamide levels in food: update and critical review of data collection. Food Addit.Contam 24 Suppl 1, 5-12.

Wenzl,T., Lachenmeier,D.W., and Gokmen,V. 2007. Analysis of heat-induced contaminants (acrylamide, chloropropanols and furan) in carbohydrate-rich food. Anal.Bioanal.Chem. 389, 119-137.

Zubeldia,L.L. and Gomar,F.J. 2007. [Acrylamide in potato crisps and snack foods produced in the autonomous Community of Valencia [Spain]]. Gac.Sanit. 21, 334-337.

Konings,E.J., Ashby,P., Hamlet,C.G., and Thompson,G.A. 2007. Acrylamide in cereal and cereal products: a review on progress in level reduction. Food Addit.Contam 24 Suppl 1, 47-59.

Lantz,I., Ternite,R., Wilkens,J., Hoenicke,K., Guenther,H., and van der Stegen,G.H. 2006. Studies on acrylamide levels in roasting, storage and brewing of coffee. Mol.Nutr.Food Res. 50, 1039-1046.

Rufian-Henares,J.A., gado-Andrade,C., and Morales,F.J. 2006. Relationship between acrylamide and thermal-processing indexes in commercial breakfast cereals: a survey of Spanish breakfast cereals. Mol.Nutr.Food Res. 50, 756-762.

Cook,D.J. and Taylor,A.J. 2005. On-line MS/MS monitoring of acrylamide generation in potato- and cereal-based systems. J.Agric.Food Chem. 53, 8926-8933.

Eriksson,S. and Karlsson,P. 2005. Some analytical factors affecting measured levels of acrylamide in food products. Adv.Exp.Med.Biol. 561, 285-291.

Lineback,D., Wenzl,T., Ostermann,O.P., de la,C.B., Anklam,E., and Taeymans,D. 2005. Overview of acrylamide monitoring databases. J.AOAC Int. 88, 246-252.

Matthys,C., Bilau,M., Govaert,Y., Moons,E., De,H.S., and Willems,J.L. 2005. Risk assessment of dietary acrylamide intake in Flemish adolescents. Food Chem.Toxicol. 43, 271-278.

Senyuva,H.Z. and Gokmen,V. 2005. Survey of acrylamide in Turkish foods by an in-house validated LC-MS method. Food Addit.Contam 22, 204-209.

Stadler,R.H. and Scholz,G. 2004. Acrylamide: an update on current knowledge in analysis, levels in food, mechanisms of formation, and potential strategies of control. Nutr.Rev. 62, 449-467.

Taeymans,D., Wood,J., Ashby,P., Blank,I., Studer,A., Stadler,R.H., Gonde,P., Van,E.P., Lalljie,S., Lingnert,H., Lindblom,M., Matissek,R., Muller,D., Tallmadge,D., O'Brien,J., Thompson,S., Silvani,D., and Whitmore,T. 2004. A review of acrylamide: an industry perspective on research, analysis, formation, and control. Crit Rev.Food Sci.Nutr. 44, 323-347.